

TAXONOMY AND PHYLOGENY OF SELECTED HYPHOMYCETES WITH EMPHASIS ON *ALTERNARIA*

JUNFU LI

DOCTOR OF PHILOSOPHY
IN
BIOSCIENCES

SCHOOL OF SCIENCE
MAE FAH LUANG UNIVERSITY

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Junfu Li

Dissertation Title Taxonomy and Phylogeny of Selected Hyphomycetes with Emphasis on *Alternaria*

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Degree Doctor of Philosophy (Biosciences)

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ABSTRACT

Hyphomycetous fungi consisting of asexual morph of various species in Ascomycetes, partially in Basidiomycetes. Which characteristic as simple structures mostly consist of conidiophores, conidiogenous cell and conidia. Dothideomycetes to be the largest class in Ascomycetes, that contains vary of hyphomycetous fungi in high diversity. Hyphomycetous fungi also occur in other orders in Ascomycetes, such as Sordariomycetes and Eurotiomycetes. In addition are distributed worldwide, including pathogen, saprophytes and parasites on plants, animals, fungi, and some abiotic medium as air, water, dregs, soil and etc. As with most organisms, although hyphomycetes in tropical and subtropical area are researched, the future study on hyphomycetes in this area is still expected. However, recently, the research of hyphomycetous fungi in tropical and subtropical, such as in Great Mekong River Subregion (GMS) System is still lacking of reported. Following the the gradual development of GMS, fungi investigation in these regions is necessary.

This study aims to focus on taxanomy and phylogeny of hyphomycetes in ecological habitats of the temperate, subtropical and tropical environment. The hyphomyctous fungal species in ecosystem of this area are given main priority. To determine and investigate hyphomycetous fungi species on fresh specimens were collected from this area, were then morphological examined and cultural isolated, while their DNA

sequence data from rDNA (ITS, LSU and SSU) and protein cording genes (TEF-1α, RPB2 and GAPDH) were analyzed to convinced their identifications and to better evaluate their phylogenetic affinities and classifications. Consequently, a new family *Zygosporiaceae*, three new genus *Fusiconidium, Mariophialophora* and *Sporidesmioides*, and 24 new species (*Acremonium chiangraiensis, Alternaria doiliconidium, A. italica, Curvularia palmicola, Corynespora doipuiensis, Dendryphion hydei, Fusiconidium mackenziei, F. aquaticum, Helicomyces menglunicus, Lophium zalerioides, Marinophialophora garethjonesii, Myrothecium northernthailandicum, Periconia palmicola, Pseudocercospora maetaengensis, P. rosacearum, Pseudopithomyces palmicola, Torula chiangmaiensis, T. chiangmaiensis, T. hydei, T. mackenziei, T. pluriseptata, Seifertia shangrilaensis, Sporidesmioides thailandica, Vamsapriya breviconidiophora*), three new records *Hermatomyces sphaericus* (*H. chromolaenae*), *H. krabiensis* (*H. chiangmaiensis*), and *Nigrospora oryzae* were reported respectively in Dothideomycetes, Sordariomycetes and Eurotiomycetes.

The study also focus on revisiting of *Alternaria* sp. in *Pleosporaceae* (Dothideomycetes) and offer a better taxonomic relationship of this genus, in the aspects of taxonomy, phylogeny and preliminary evolution divergence. As a result, 24 new species viz. *Alternaria arctoseptata, A. arundinis, A. baoshanensis, A. breviconidiophora, A. brevirostra, A. ellipsoconidia, A. eupatoriicola, A. falcata, A. lathyri, A. macilenta, A. macroconidia, A. minimispora, A. muriformispora, A. nodulariconidiophora, A. oblongoellipsoidea, A. obpyriconidia, A. orobanches, A. ovoidea, A. phragmiticola, A. phytolaccae, A. pseudoinfectoriae, A. rostroconidia, A. salicicola* and *A torilis* spp. nov. are proposed and classified in sect. *Alternaria*, sect. *Infectoriae*, sect. *Porri* and sect. *Radicina*. *Alternaria alternata* and *A. doliconidium*, with new host and geographical records are also described. The further explores the utility of divergent time estimates to gain additional insights into the evolutionary relationships of *Alternaria*.

Moreover, the current study introduces new species and new records with DNA sequence data which can be used to understand the taxonomy and biodiversity of

hyphomycetes fungi in Ascomycetes. Moreover, The DNA sequence data from different gene regions from new collections were generated, properly annotated and upload to deposited in GenBank, and the alignments and phylogenetic trees were upload to deposited in TreeBASE for future phylogenetic studies. Indeed, the sample examinations and descriptions of each taxa were deposited in Faces of Fungi. New taxa were registered in Index Fungorum and Mycobank. This research provide comprehensive morphology as well as molecular sequences of mass hyphomycetes in diverse taxa, and outcrop significant species of hyphomycetes in GMS and Europe ecosystem habitats. The sequences data of different hyphomycetes species were provided while their taxonomy was established.

Keywords: *Alternaria*, Evolutionary Divergence, Eurotiomycetes, Dothideomycetes, Hypomycetes, Morphology, New Taxa, Phylogeny, Sordariomycetes

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CHAPTER 1

INTRODUCTION

Hyphomycetes are asexual morphs in life cycle of which mostly their sexual morph are Ascomycetes, and partially are basidiomycetous fungi. Hyphomycetous fungi make up the majority of what are commonly called as moulds and lacking of closed fruiting bodies, which play a role as pathogen and disintegrator in ecosystem, and some are regarded as the weeds in the fungal kingdom (Subramanian, 1982; Seifert et al., 1983). In addition to growing in a diversity of ecological habitats, such as on plant tissues, wood and bark, dung, insects and on many other natural substrates such as other fungi including lichens, hyphomycetous fungi (moulds) are related in food spoilage, cause the plant and animal disease, contaminate many manufactured materials such as wood, paper and textiles, while also be the disintegrator of biological remains and are frequent visitors to the human indoor environment (Eills, 1971, 1976; Seifert et al., 2011). The asexually or mitotically reproducing structures are called anamorphs, while the reproducing structures of sexual or meiotic, counterparts of the same life cycle are called teleomorphs. Totally, these forms of sporulation constitute a whole fungus, or holomorph (Hennebert & Weresub, 1977; Weresub & Hennebert, 1979). In some cases, anamorph and teleomorph develop side-by-side, however, as normally, they mature at different times or on different substrates. In history, due to lacking of modern technological method (such as DNA molecular technology), the discovery of anamorph–teleomorph connections was difficult. Even mass of connections are known, but the majority of hyphomycetes still remain solitary. It is certain that many anamorphs have lost the potential to mate or to develop a teleomorph constantly, and must be regarded as anamorphic holomorphs (Seifert et al., 2011).

As with most organisms, the first taxonomic research on hyphomycetes occurred in central and northern Europe, and was followed by explorations in North America. Although there were sporadic surveys in other parts of the globe, it was not until the mid-twentieth century that the moulds of the Indian subcontinent and then Japan and New

Zealand were studied with any intensity (Eills, 1976; Seifert et al., 2011). Although hyphomycetes in tropical and subtropical area are researched, the future study on hyphomycetes in this area is still expected (Subramanian, 1992). However, recently, the research of hyphomycetous fungi in Great Mekong River Subregion (GMS) System is still lacking of reported. Following the the gradual development of GMS, fungi investigation in this area is necessary.

Hyphomycetous fungi as well reported as many key plant pathogen, such as *Seifertia* spp. cause black withered on *Rhododendron* sp. (Partridge & Morgan-Jones, 2002; Glawe & Hummel, 2006; Li et al., 2016a) and *Alternaria* spp. are reported cause black spot disease on various plant hosts (Thambugala et al., 2017; Jayawardena et al., 2018; Wanasinghe et al., 2018). On the other hand, many food-borne fungi are hyphomycetes, such as *Penicillium* and *Aspergillus* are particularly common agents of food spoilage and also produce important mycotoxins that affect human health (Samson et al., 2010). Some species, such as *Penicillium digitatum* on citrus fruits, and *Penicillium expansum* on apples, are common on specific foods, while others are less specialized and grow on many different kinds of food. Indeed, Ajith et al. (2012) reported that a mycorrhizal hyphomycetous fungi, *Zygosporium masonii* has antifungal effect to a pathogen hyphomycetes, *Colletotrichum capsici* on bellpeper. Thus, working on pathogenic hyphomycetes is helpful in application of agriculture development to find out the potential assistant fungi species in bio-control.

In this study, I focus on taxanomy and phylogeny of hyphomycetes in ecological habitats of Great Mekong River Subregion (GMS), where contents the Temperate, Subtropical and Tropical continuously; Partically in Europe (typical Temperate area). The hyphomyctous fungal species in ecosystem of this area were given main priority. The taxonomy study of hyphomycetes in this region was revisited based on morphology add multi-gene phylogeny analysis and provide a better understanding and resolving of confused taxonomic about hyphomycetous fungi. This study also emphasis on revisiting of a key plant pathogenic fungi, *Alternaria* spp. in *Pleosporaceae* (Dothideomycetes) and offer a better taxonomic relationship of this genus. This research provide comprehensive morphology plus molecular sequences of mass hyphomycetes in diverse taxa and will outcrop significant species of hyphomycetes in GMS and Europe ecosystem habitats. The sequences data of different hyphomycetes species which previously lacking molecular data

will be provided, while their taxonomy will be established base on both methods of morphology and molecular phylogeny.

1.1 Hyphomycetes in Dothideomycetes

Dothideomycetes is the largest class of Ascomycota, which accommodates over 20,000 species (Kirk et al., 2008; Index Fungoruml, 2021). Species in the class are usually found in tropical to subtropical regions (Schoch et al., 2009; Zhang et al., 2011; Hyde et al., 2013), while widely distrbuted in terrestrial and aquatic habitats as endophy, epiphyte plant pathogen or possibilitical animal or human pathogen, fungicolous fungi, lichenicolous fungi and saprobe (Crous et al., 2009; Schoch et al., 2009; Chomnunti et al., 2011; Zhang et al., 2012; Hyde et al., 2013; Su et al., 2015). The class was updated by Hongsanan et al. (2020), which accepted 7 orders, 115 families in Dothideomycetidae and Pleosporomycetidae and provided a refined updated document on families of Dothideomycetes. Liu et al. (2017) provided guidelines of using divergence estimates for fungal ranks in the classification of Dothideomycetes.

In this study, the selected hyphomycetous species of Dothideomycetes that have associated with plant litter, such as dead stem, leave, branch, coat, bark and etc., which collected from China, Russia, Italy and Thailand were examined and phylogenetically analysed. In addition, *Alternaria* as a key genus of dematiaceous hyphomycete, was selected in the study to explain the phylogeny and taxonomy of hyphomycetous fungal species. Consequently, 103 specimens within 2 novel genera and 51 hyphomycetous species from in ten families of Dothideomycetes were introduced. The utility of divergent time estimates to gain additional insights into the evolutionary relationships of *Alternaria*, was also explored with approaching on section rank of this genus in this study.

1.1.1 Pleosporaceae

The family Pleosporaceae was introduced by Nitschke (1869) based on the immersed ascomata and presence of pseudoparaphyses, which is the largest family and is representative of the order Pleosporales (Zhang et al., 2012; Hyde et al., 2013; Wijayawardene et al., 2014; Ariyawansa et al., 2015). Pleosporaceae have been reported

as included numerous saprobic, opportunistic human, and plant pathogenic taxa (Sivanesan, 1984; Carter & Boudreaux, 2004). Morphologically, the classification in the Pleosporaceae has mainly been based on the *Pleospora* type by its centrum development (Dong et al., 1998) and asci are interspersed with pseudoparaphyses in the ascomata. The asexual morphs of Pleosporaceae can be coelomycetous or hyphomycetous (Zhang et al., 2012; Hyde et al., 2013; Ariyawansa et al., 2015) and some sexual genera in Pleosporaceae have been linked with asexual morphs (Zhang et al., 2012; Hyde et al., 2013, 2014; Ariyawansa et al., 2015). Ariyawansa et al. (2015) provided a backbone phylogenetic analysis of Pleosporaceae which included 18 genera.

Alternaria

Alternaria is a genus of ascomycete fungi and species are known as major plant pathogens as well as common allergens in humans (Woudenberg et al., 2015). Recently, this genus comprising over 590 legitimate species epithets, and approximately 368 species as accepted within 28 sections (Hongsanan et al., 2020; Wijayawadene et al., 2020, He et al., 2021; Species Fungorum, 2021), they are ubiquitous in the environment and are a natural part of fungal flora almost everywhere. They are normal agents of decay and decomposition. The spores are airborne and found in the soil and water, as well as indoors and on objects. Some species have shown promise as biocontrol agents against invasive plant species, and also been reported as endophytic microorganisms with highly bioactive metabolites (Nowicki et al., 2012). Among the taxonomy studies about *Alternarina*, the taxonomic study of the described *Alternarina* morphospecies base on the phylogenetic study is still need to investigate.

1.1.2 Melanommataceae

The family Melanommataceae was established by Winter (1885) for sexually reproducing ascomycetes, characterized by globose or sunken perithecial ascomata, bitunicate and fissitunicate asci, pigmented and phragmosporous ascospores (Barr, 1990; Sivanesan, 1984; Zhang et al., 2012; Hyde et al., 2013; Tian et al., 2015). Barr (1983) introduced Melanommatales to accommodate all taxa with trabeculate pseudoparaphyses which are morphologically distinct from cellular pseudoparaphyses in the Pleosporales. However, recent phylogenetic analyses have shown that Melanommatales should be assigned to Pleosporales (Berbee, 1996; Liew et al., 2000; Lumbsch & Lindemuth, 2001;

Schoch et al., 2006; Kruys et al., 2006; Wang et al., 2007; Tian et al., 2015) and Eriksson (2006) synonymized Melanommatales as Melanommataceae under Pleosporales. Currently Melanommataceae stands out as a distinct monophyletic family within the Pleosporales (Tian et al., 2015; Li et al., 2016a).

1.1.3 Torulaceae

Torulaceae Corda was introduced by Sturm (1829) and is typified by *Torula* Pers. Members in this family are known only by their asexual morphs which were characterized by forming micro- or macronematous conidiophores, with or without apical branches, and doliiform to ellipsoid or clavate, brown, smooth to verruculose, mono- to polyblastic, often cupulate, conidiogenous cells and subcylindrical, phragmosporous, acrogenous, brown, dry, smooth to verrucose conidia, characteristically produced in branched chains (Crous et al., 2015; Hyde et al., 2016; Su et al., 2016; Li et al., 2017; Su et al., 2018). Crous et al. (2015) investigated phylogenetic relationships of this family with the inclusion of *Torula* species and accepted *Dendryphion* and *Torula* within Torulaceae in Pleosporales. Su et al. (2016) introduced *Neotorula* and two new *Dendryphion* species in Torulaceae based on molecular data. Li et al. (2016b) established a novel genus, *Sporidesmioides* and Luo et al. (2017) accommodated *Rostriconidium* within Torulaceae. Currently, there are five genera *viz. Torula, Dendryphion, Neotorula, Rostriconidium* and *Sporidesmioides* in Torulaceae (Su et al., 2016; Hyde et al., 2016; Li et al., 2016a; Luo et al., 2017).

1.1.4 Hermatomycetaceae

The family Hermatomycetaceae (Pleosporales, Pleosporomycetidae) was validated by Hashimoto et al. (2017) to accommodated the solo and type genus *Hermatomyces*. Which morphologically known only by its asexual morph that is characterized by sporodochial conidiomata and dimorphic (lenticular or cylindrical) conidia of one or two types, the lenticular conidia are globose to subglobose, hyaline to pale brown peripheral cells with dark brown central cells, and the cylindrical conidia is hyaline, cylindrical to subcylindrical or turbinate and consisting of 1–4 columns of 2–12 cells (Spegazzini, 1911; Tibpromma et al., 2016; Hashimoto et al., 2017; Hyde et al., 2019; Pem et al., 2019; Phukhamsakda et al., 2020; Ren et al., 2021).

1.1.5 Corynesporascaceae

Corynesporascaceae established by Sivanesan (1996), is typified by genus *Corynesporasca*, with the type species *C. carotae* described for sexual morph of a species in *Corynespora* on topical plant host. Rossman et al. (2015) indicated that given the extensive use of the name *Corynespora* for plant pathogenic fungi and its priority, the name *Corynespora* is recommended for use to both *Corynesporasca* and *Corynespora*. Therefore, the family Corynesporascaceae recent accommodated the only genus *Corynespora* (Lumbsch & Huhndorf, 2007; Rossman et al., 2015). *Corynespora* has been widely used and includes approximately 200 species names, as well in the phylogenetic analysis, species of *Corynespora* form a distant clade together with the generic type, *C. cassiicola*, and is classified in *Corynesporascaceae* (Voglmayr & Jaklitsch, 2017). Its morphological characteristics are transverse distoseptate, cylindric, obclavate conidia with the thick walls, long, macronematous, mononematous, straight or flexuous, unbranched, brown or olivaceous brown, smooth conidophores, with monotretic, integrated, terminal, percurrent, cylindrical or doliiform conidiogenous cells (Güssow, 1906; Sivanesa, 1996; Hyde et al., 2020).

1.1.6 Periconiaceae

Periconiaceae has long been unused and placed as members of Massarinaceae until Tanaka et al. (2015) performed revision of the Massarineae and placed it as a distinct family based on their phylogenetic analysis (Tanaka et al., 2015; Hyde et al., 2017, 2018). This family morphological characteristics are pale to dark brown, branched or unbranched, smooth or verruculose, macronematous conidiophores. Conidiogenous cells are monoblastic or polyblastic, integrated or discrete, ovoid to clavate formed on the terminal or intercalary of the stipe. Conidia are spherical, aseptate, catenate or solitary, smooth or verruculose, pale to dark brown (Tanaka et al., 2015; Hyde et al., 2017, 2018; Liu et al., 2017; Thambugala et al., 2017).

1.1.7 Mycosphaerellaceae

The family *Mycosphaerellaceae* represent thousands of fungal species that are associated with diseases on a wide range of plant hosts. Understanding and stabilising the

taxonomy of genera and species of *Mycosphaerellaceae* is therefore of the utmost importance given their impact on agriculture, horticulture and forestry. This family includes many genera, such as *Cercospora*, *Cercosporella*, *Dothistroma, Lecanosticta*, *Phaeophleospora*, *Polythrincium*, *Pseudocercospora*, *Ramularia*, *Ramulispora*, *Septoria*, *Sonderhenia* and *Zasmidium*, which are under taxonomic and nomenclatural reassessment (Videira et al., 2017; Aguilera-Cogley et al., 2017).

1.1.8 Didymosphaeriaceae

The family Didymosphaeriaceae was first introduced by Munk (1953), which typified by the genus *Didymosphaeria*. This family has been revised by Ariyawansa et al. (2014) who accepted 16 genera. Species in this family occurred mainly in terrestrial habitats as parasitic or saprobic fungi on woody or herbaceous plant issue, and also are fungicolous fungi (Hyde et al., 2013; Ariyawansa et al., 2014). Some hyphomycetous genera were accommodated in this family via phylogenetic characters, such as *Spegazzinia* and *Pseudopithomyces* (Ariyawansa et al., 2015; Thambugala et al., 2017; Samarakoon et al., 2020).

1.1.9 Tubeufiaceae

Barr (1979) first introduced the family Tubeufiaceae and Boonmee et al. (2014) accommodated this family in the order Tubeufiales. Boonmee et al. (2011) comprehensively reviewed the family Tubeufiaceae based on both morphological re-examination and phylogenetic analysis, which included 20 genera in the family. Currently, the family comprises 24 genera (Wijayawardene et al., 2014; Doilom et al., 2017; Boonmee et al., 2014; Hyde et al., 2016; Chaiwan et al., 2017; Lu et al., 2017; Brahmanage et al., 2017; Luo et al., 2017; Tanney & Miller, 2017) with sexual and asexual morphs. The asexual morph of species in this family are commonly as hyphomycetes, morphologically charactorized helicosporous, phragmosporous and dictyosporous conidia (Barr, 1980; Kodsueb et al., 2006; Boonmee et al., 2011, 2014; Lu et al., 2017; Luo et al., 2017). Members of this family are saprobic, generally colonizing on dead woods or other decaying plant issue in terrestrial and aquatic habitat (Boonmee et al., 2011, 2014; Hyde et al., 2013, 2016; Lu et al., 2017; Chaiwan et al., 2017).

1.1.10 Mytilinidiaceae

The family Mytilinidiaceae is morphological characterised in having carbonaceous ascomata, fragile, globoid to obovoid, erect oyster or bivalve, shell-shaped or hatchet-shaped structures, a thin peridium enclosing a hamathecium and trabeculate pseudoparaphyses (Zogg, 1962; Boehm et al., 2009). Members in this family are widely distributed throughout temperate regions and often parasitic or saprobic on the plant litters, such as wood, bark, resin, cones, scales, needles, seeds, and roots of gymnosperms (Hyde et al., 2013, 2017).

1.2 Hyphomycetes in Sordariomycetes

Sordariomycetes is one of the largest classes of Ascomycota which comprises 7 subclasses, 150 families with more than 1300 genera and over 20,000 species (Kirk et al., 2008; Lumbsch & Huhndorf, 2010; Maharachchikumbura et al., 2015; Hyde et al., 2020; Wijayawardene et al., 2020; Huang et al., 2021). Species of Sordariomycetes have a wide range of ecological roles in nature, including saprobes on dead organic matter, endophytes, or pathogens on living plants, animal and arthropods in terrestrial and aquatic hobitats (Samuels & Blackwell, 2001; Zhang et al., 2006; Hyde et al., 2014, 2015; Maharachchikumbura et al., 2015, 2016).

In this study, the selected hyphomycetous taxa of Sodariomycetes, as one new family and three new species collected from Thailand and China were morphological examined and phylogenetical analysed, which belonging to Xylariales (Apiosporaceae, Zygosporiaceae fam. nov., Xylariaceae) and Hypocreales (Bionectriaceae, Stachybotryaceae) were introduced.

1.2.1 Zygosporiaceae fam. nov.

Zygosporiaceae was invalidly published as 'Zygosporaceae' (Nom. inval., Art. 39.1, Melbourne) by Locquin (1984) to accommodate the genus Zygosporium (Index Fungorum, 2021; MycoBank, 2021). However, the lacking of molecular data to clarify phylogenetic

position for *Zygosporium* species, as well having unique morphological characters of setae/setiform or vesicular conidiophores, with a sphaerical apex and distinct vesicles which are different from other hyphomycetous taxa. In this study, a new family Zygosporiaceae was established to accommodate the genus *Zygosporium* based on the morphological and phylogenetic support.

1.2.2 Xylariaceae

The family Xylariaceae is one of the largest families in Sordariomycetes, was first introduced by Tulasne and Tulasne (1863) (Maharachchikumbura et al., 2015, 2016; Tibpromma et al., 2017; Hongsanan et al., 2017). Species in family Xylariaceae are high diversity in tropical regions and comprises the highest number of bioactive secondary metabolite producers (Stadler & Hellwig, 2005; Kuhnert et al., 2014; Senanayake et al., 2015). The majority members in the family are saprobes and endophytes and small number of species are plant pathogens (Stadler et al., 2013; Pažoutová et al., 2013).

1.2.3 Apiosporaceae

Hyde et al. (1998) reexamined the type (*Apiospora montagnei* Sacc.) and introduced the family Apiosporaceae to accommodate *Apiospora* (Sexual morph of *Arthrinium*) and *Appendicospora*. Subsequently this family has been recognized in order Xylariales by several authors (Huhndorf et al., 2004; Zhang et al., 2006; Jaklitsch & Voglmayr, 2012; Senanayake et al., 2015; Dai et al. 2016).

1.2.4 Bionectriaceae

Rossman et al. (1999) introduced Bionectriaceae based on Bionectria Speg. (current name: Clonostachys Corda) as the type genus. Currently, there are 40 accepted genera in the family (Wijayawardene et al., 2018; Voglmayr & Jaklitsch, 2019). Species of Bionectriaceae are associated with fresh or dead plant material, lichens, dung and, are parasitic on other fungi (Maharachchikumbura et al., 2016). Bionectriaceae species are characterised by variously coloured, perithecial or rarely cleistothecial ascomata, aseptate to multi-septate or rarely muriform ascospores and acremonium-like, gliocladium-like, gyrostroma-like or penicillium-like asexual morphs (Rossman et al., 1999; Maharachchikumbura et al., 2016; Voglmayr & Jaklitsch, 2019).

1.2.5 Stachybotryaceae

Stachybotryaceae was introduced with its type genus *Stachybotrys* (Corda, 1837) which *S. chartarum* (Ehrenb.) S. Hughes as the type species (Wang et al., 2015). Maharachchikumbura et al. (2015) provide outline of Sordariomycetes and accommodate the genera *Myrothecium*, *Peethambara* and *Stachybotrys* in the family. Recently, 39 genera were accepted in the family Stachybotryaceae (Hyde et al., 2020; Wijayawardene et al., 2020).

1.3 Hyphomycetes in Eurotiomycetes

The class Eurotiomycetes (Ascomycota, Pezizomycotina) is a monophyletic ascomycetous fungi group consisting of two subclasses, Eurotiomycetidae and Chaetothyriomycetidae. Members in Eurotiomycetidae contains most of the fungi previously recognized as Plectomycetes with their characters in having mostly enclosed ascomata and prototunicate asci; and Members in Chaetothyriomycetidae produce ascomata with an opening reminiscent, while these characters as well occurred in species of Dothideomycetes or Sordariomycetes (Geiser et al., 2006). Asexual morphs of species in this class are often fund as sapbrobic hyphomycetes in terrestrial and aquatic habitats (Liu et al., 2015c; Dong et al., 2020; Wang et al., 2020). Liu et al. (2015c) first provided the molecular data of species in Eurotiomycetes.

In this study, a novel genus with one new fungicolous taxa in family Herpotrichellaceae (Chaetothyriales, Eurotiomycetes), which collected from marine habitat in southern Thailand was introduced.

Herpotrichellaceae

The family Herpotrichellaceae was introduced by Munk (1953), which typical with the genus *Herpotrichiella*. About one-third of the species of this family were described from pathogenic strains isolated from human diseases, and occasionally infections can be fatal (Li et al., 2011). With various Eurotiomycetous species belong to this family were

examined, the family Herpotrichellaceae is the best known family in the order Chaetothyriales (Nascimento et al., 2016; Dong et al., 2020).

1.4 Importance of this Research

1.4.1 The taxonomy of hyphomycetes fungi in Ascomycetes are still confused. Many hyphomycetous species are lacking of molecular data and confused their phylogenetic position, while various unclarify taxa are expected to discover especially in the Great Mekong Subregion (GMS) area (Hyde et al., 2018). Therefore, this study investigated hyphomycetous fungi from GMS area. It is aimed to provide illustrations and descriptions of the 27 new discovered hyphomycetes in this area, and determine their phylogentic position and classification based on morphology and available molecular data.

1.4.2 The genus *Alternaria* is well known as dematiaceous hyphomycete. Although this genus was previously well studied, the taxonomic study of the described *Alternarina* morphospecies base on the phylogenetic study is still need to investigate and update. Thus, the samples of *Alternaria* species on diverse plant hosts from the Temperate (Italy and Russia), Subtropical (China) and Tropical (Thailand) zone, were fully examined in this study. It is provided updated phylogenetic relationships for the sections and 24 novel taxa in *Alternaria*, based on phylogenetic analyses of a concatenated dataset from combined multi-gene regions and estimated evolutionary divergence time of *Alternaria*.

1.5 Research Objectives

1.5.1 To collect, examine the morphology and isolate strains of hyphomycetes species mainly in Southeast Asia and partly in Europe, study on the diversity of hyphomycetous fungi. Living cultures of the isolated strain are depositing in Mae Fah Luang University (MFLU) culture collection (Thailand) and the Culture Collection in Kunming Institute of Botany, Chinese Science Academy (China) for conservation.

1.5.2 To generate molecular sequence data from the strains of novel taxa, and construct phylogenetic trees of hyphomycetous species to analyses the taxonomy with the phylogenetic method.

1.5.3 To study the taxonomy of hyphomycetes species in genus *Alternaria* (Pleosporaceae, Pleosporales) in Dothediomycetes, use phylogenetic method to clarity and justify the taxa, provide updated phylogenetic classification and discuss the estimate evolutionary divergence time of the genus *Alternaria*.

1.5.4 To provide update phylogenetic analyses of hyphomycetes in Ascomycetes, and photo-plates, descriptions and notes of re-examined and novel hyphomycetous species in this study.

1.6 Research Contents

This thesis is divided into nine chapters.

Chapter 1 is the general introduction, which provides a general background about hyphomycetes in Ascomycetes. The brief notes of the selected families belongs to ascomycetous classes, Dothideomycetes, Sordariomycetes and Eurotiomycetes in this study are provided. The research objectives and contents are provided as well.

Chapter 2 introduces a new hyphomycetous species, *Seifertia shangrilaensis*, collected from southwest China and a new genus *Fusiconidium*, which to accommodate two new species *Fusiconidium mackenziei* and *F. aquaticum*. This chapter clarifies the affinities of *Seifertia shangrilaensis*, *Fusiconidium mackenziei* and *F. aquaticum* in morphology and multigene phylogenetic analysis, while placed the genus *Seifertia* and accommodated genus *Fusiconidium* in family Melanommataceae (Pleosporales, Dothideomycetes), based on molecular evidence.

Chapter 3 introduces a new genus *Sporidesmioides*, which typical a new species *Sporidesmioiodes thailandica*, and six novel species *Torula chiangmaiensis*, *T. pluriseptata*, *T. chromolaenae*, *T. hydei*, *T. mackenziei* and *Dendryphion hydei*, and two new record *T. ficus* and *T. masonii* respectively collected from Italy, China and Thailand in the family Torulaceae (Pleosporales, Dothideomycetes) based on morphology and phylogenetic analyses. Indeed, this chapter discussed the polyphyletic affinities of sporidemium-like taxa, some updated morphological characters are indicated. Clarifies the update classification of Torulaceae in phylogenetic analysis, and discussed the similarity of hosts in some species in *Torula* and *Dendryphion* in this chapter.

Chapter 4 provides morphological and phylogenetic studies on species in genus *Alternaria* in family Pleosporaceae (Pleosporales, Dothideomycetes) from China, Italy, Russia and Thailand. The genus *Alternaria* was reviewed, and 24 new species and two new records are introduced and justified based on multi-genes combined phylogenetic analysis. On the other hand, the estimate evolutionary divergence time of *Alternaria* sections is provided, and diversity of hosts species for specific taxa and multi-species parasite on specific hosts are discussed in this chapter.

Chapter 5 introduces a new family Zygosporiaceae to accommodated and typified the genus *Zygosporium* based on multi-gene analysis. This chapter discussed the unique morphological characters of *Zygosporium* species, and historical invalidly establishing of "Zygosporaceae". Consequently, based on both morphology and molecular data, the family Zygosporiaceae is validly established and placed in Xylariaceae (Xylariales, Sordariomycetes).

Chapter 6 introduces a new genus *Marinophialophora*, typified with a new species *Marinophialophora garethjonesii* parasite on *Halocyphina* sp., from marine habitat in souther Thailand. The genus *Marinophialophora* phylogenetically posited in family Herpotrichellaceae (Chaetothyriales, Eurotiomycetes) that poorly reported as marine fungi, the morphological characterisation and phylogenetic position of this new taxon, *Marinophialophora garethjonesii*, collected from a tropical marine habitat is investigated in this chapter.

Chapter 7 introduces fifteen new species *Acremonium chiangraiensis, Alternaria doiliconidium, A. italica, Curvularia palmicola, Corynespora doipuiensis, Helicomyces menglunicus, Lophium zalerioides, Myrothecium septentrionale, Periconia palmicola, Pseudocercospora maetaengensis, Pse. rosacearum, Pseudopithomyces palmicola, Pse. chartarum, Pse. maydicus, Vamsapriya breviconidiophora*, and three new records *Hermatomyces sphaericus* (*H. chromolaenae*), *H. krabiensis* (*H. chiangmaiensis*), and *Nigrospora oryzae* respectively within Corynesporascaceae, Didymosphaeriaceae, Hermatomycetaceae, Periconiaceae, Pleosporaceae (Pleosporales), Tubeufiaceae (Tubeufiales), Mycosphaerellaceae (Mycosphaerellales), Mytilinidiaceae (Mytilinidiales) in Dothideomycetes and Bionectriaceae, Stachybotryaceae (Hypocreales), Apiosporaceae, Xylariaceae (Xylariales) in Sordariomycetes based on morphological examination of

specimens, coupled with phylogenetic sequence data. Those taxa were collected from China, Italy and Thailand.

Chapter 8 provides the overall conclusion of the above-mentioned studies and suggestions for future works.

CHAPTER 2

SEIFERTIA SHANGRILAENSIS SP. *NOV*. AND *FUSICONIDIUM* GEN. NOV. WITH TWO NOVEL TAXA WITHIN MELANOMMATACEAE (PLEOSPORALES)[1]

Abstract

Seifertia shangrilaensis sp. nov. was isolated from hanging rachides of *Rhododendron decorum* in Yunnan Province, Southwest China. The new taxon was compared with the type species, *S. azalea* and differs in having wider conidiophores, with hyaline to subhyaline and smaller conidia, while *S. azalea* has olive-brown to brown, rarely branched conidiophores, and pale brown or olive-brown, very rarely septate conidia.

Fusiconidium mackenziei sp. nov. and *Fusiconidium aquaticum* sp. nov., isolated from a dead branch of *Clematis vitalba* in Italy and a submerged decaying wood in China respectively, are described and illustrated. The new taxa are characterized by having rhexolytic conidial secession, percurrently proliferating conidiophores, with enteroblastic conidiogenous cells and acrogenous, pale brown to dark brown, verruculose, fusiform, euseptate conidia. *Fusiconidium* is introduced as a novel genus to accommodate these novel species base on morphologically distinct from related asexual genera within the Dothideomycetes and multigene phylogenetic analyses.

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Based on multigene phylogenetic analyses, the genera *Fusiconidium* and *Seifertia* are both referred to the family Melanommataceae (Pleosporales).

2.1 Introduction

Many asexual genera were isolated from dead plant materials and have been referred to the order Pleosporales, Dothideomycetes (Wang et al., 2007; Zhang et al., 2009). They are associated with diverse habitats and associated with various hosts worldwide (Hyde et al., 2013; Wijayawardene et al., 2014). Pleosporales is the largest order in the class Dothideomycetes (Kirk et al., 2008; Zhang et al., 2012; Hyde et al., 2013; Wijayawardene et al., 2014; Phookamsak et al., 2015; Tian et al., 2015). Over 40 families, including Melanommataceae, were included in the order Pleosporales based on morphological and DNA based phylogenetic studies (Liew et al., 2000; Lumbsch & Lindemuth, 2001; Zhang et al., 2012; Hyde et al., 2013; Wijayawardene et al., 2014; Phookamsak et al., 2015; Tian et al., 2015; Ariyawansa et al., 2015; Li et al., 2016).

The family Melanommataceae was established by Winter (1885) for sexually reproducing ascomycetes, characterized by globose or sunken perithecial ascomata, bitunicate and fissitunicate asci, pigmented and phragmosporous ascospores (Barr, 1990; Sivanesan, 1984; Zhang et al., 2012; Hyde et al., 2013; Tian et al., 2015). Barr (1983) introduced Melanommatales to accommodate all taxa with trabeculate pseudoparaphyses which are morphologically distinct from cellular pseudoparaphyses in the Pleosporales. However, recent phylogenetic analyses have shown that Melanommatales should be assigned to Pleosporales (Berbee, 1996; Liew et al., 2000; Lumbsch & Lindemuth, 2001; Schoch et al., 2006; Kruys et al.; 2006; Wang et al., 2007; Tian et al., 2015) and thus Eriksson (2006) transferred Melanommatales to Pleosporales. Currently Melanommataceae stands out as a distinct monophyletic family within the Pleosporales (Tian et al., 2015; Li et al., 2016).

Many asexual genera have been reported as asexual morphs of melanommataceous genera, but all of these need to be substantiated with updated taxonomic descriptions coupled with phylogenetic data. The asexual morphs of melanommataceous genera have been reported

as coelomycetes and hyphomycetes (Sivanesan, 1983, 1984; Wijayawardene et al., 2012, 2014; Tian et al. 2015). *Aposphaeria* and phoma-like asexual morphs have been reported for *Melanomma*, while pyrenochaeta-like (pycnidia with or without pycnidial setae) has been reported as asexual morph of *Herpotrichia* and *Byssosphaeria* (Chesters, 1938; Barr, 1984; Sivanesan, 1984; Zhang et al., 2009). *Anomalemma* was reported as asexual morph of *Exosporiella* (Sivanesan, 1983; Wijayawardene et al., 2012; Tian et al., 2015). Samuels (1980) linked *Ohleria brasiliensis* Starbäck with *Monodictys putredinis* (Wallr.) S. Hughes. Despite difficulties in classifying asexual morphs, molecular data has been useful resolving their phylogeny (Wang et al., 2007; Li & Zhuang, 2008; Mugambi & Huhndorf, 2009). Hyde et al. 2013 and Wijayawardene et al. (2014) included 18 sexual and asexual genera in Melanommataceae. Later, Tian et al. (2015) accepted seven asexual genera and 13 sexual genera viz. *Apospheria*, *Bertiella*, *Byssosphaeria*, *Herpotrichia*, *Melanomma*, *Monotosporella*, *Muriformistrickeria*, *Mycopappus*, *Phragmocephala*, *Pseudostrickeria*, *Sarimanas*, *Seifertia*, *Xenostigmina*.

Seifertia was introduced as a monotypic genus by Partridge and Morgan-Jones (2002) and is typified by *S. azalea* (Peck) Partridge & Morgan-Jones which was originally described as *Periconia azaleae* Peck. with the names *Briosia azaleae* (Peck) Dearn, *Cephalotrichum azaleae* (Peck) Kuntze, *Pycnostysanus azalea* (Peck) E.W. and *Sporocybe azaleae* (Peck) Sacc. treated as synonyms (Partridge & Morgan-Jones, 2002; Glawe & Hummel, 2006; Index Fungorum, 2016).

Seifertia azalea has been reported as a cosmopolitan taxon causing bud blast and twig blight of azaleas and rhododendrons in Japan, Europe and North America (Mason, 1941; Ellis, 1976; Farr et al., 1996; Partridge & Morgan-Jones, 2002; Glawe & Hummel, 2006). However, *Seifertia* species are relatively poorly studied worldwide, and have not been studied in the southwest of China. Based on phylogenetic analysis of LSU sequence data, Seifert et al. (2007) treated *Seifertia azalea* in Dothideomycetes, as closely related to *Mycosphaerella mycopappi* A. Funk & Dorworth, but unrelated to *Mycosphaerella sensu stricto*. Crous et al. (2009, 2013) placed *Seifertia* in Pleosporales which was shown to be allied to *Xenostigmina*, a synanamorph

of *Mycopappus*, based on combined ITS and LSU phylogenetic analyses. There is little molecular data to resolve the natural placement of *Seifertia* in Dothideomycetes.

In this Chapter, I describe and illustrate three new taxa, *Fusiconidium mackenziei*, *F. aquaticum*, and *Seifertia shangrilaensis* isolated from a dead branch of *Clematis vitalba* Linn. from Italy, on hanging rachides of *Rhododendron decorum* and submerged decaying wood in China respectively, and discuss their differences with other similar genera such as *Sporidesmiella*, *Nigrolentilocus*, *Phragmocephala* and *Distophragmia*. A new genus *Fusiconidium* is proposed to accommodate the new species *Fusiconidium mackenziei* and *F. aquaticum*, and a new species *Seifertia shangrilaensis* in genus *Seifertia* are accommodated into Melanommataceae (Dothideomycetes).

2.2 Materials and Methods

2.2.1 **Isolation and Identification**

The specimens were collected from a dead hanging branch of *Clematis vitalba* Linn. in Italy, on a living branch of *Rhododendron decorum* Franch. (Ericaceae) and submerged decaying wood in Yunnan Province, southwest China during the year 2014–2015. Samples were returned to the laboratories for examination and description of morphological characters. The specimens were observed under a Motic SMZ 168 series dissecting stereo-microscope. The conidial structures were picked up by a surgical needle and transferred into 10% lacto-glycerol on a clean slide and examined under a Nikon Eclipse 80i compound microscope and photo-captured with a Canon 600D digital camera using DIC microscopy. The morphological structures were photographed with a Discovery V.8 stereo-microscope fitted with a CARL ZEISS Axio Cam ERc5S microscope camera. Tarosoft (R) Image Frame Work program and Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems Inc., The United States) were used for measurements and drawing photographic plate. Single spore isolation was carried out to obtain pure cultures as described in Chomnunti et al. (2014). Germinating conidia were transferred aseptically to potato dextrose agar (PDA) and malt extract agar

(MEA) plates and grown at 25°C in alternating day and night light. Colony characters were observed and recorded after 1 week and at weekly intervals.

The type specimens are deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and the Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (HKAS), Yunnan, China. Ex-type living cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Institute of Botany Culture Collection (KUMCC). Faces of Fungi number and MycoBank number are registered (Jayasiri et al., 2015; MycoBank, 2016), and establishment of new species was followed as described in Jeewon and Hyde (2016).

2.2.2 **DNA Extraction, PCR Amplification and Sequencing**

Fungal mycelium was scraped off and transferred to 1.5 ml microcentrifuge tube using a sterilized lancet for genomic DNA extraction. Individual fungal conidia were picked up from sample substrate under a Motic SMZ 168 series dissecting stereo-microscope, using a surgical needle and transferred to 1.5 ml micro centrifuge tube for direct DNA extraction. The Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China) was used to extract fungal genomic DNA from mycelium and the Omega DNA Extraction Kit (OMEGA® E.Z.N.A.) was used to extract fungal genomic DNA from conidia, following the protocols in the manufacturer's instructions.

2.2.3 **Sequence Alignment and Phylogenetic Analyses**

The DNA amplification was performed by Polymerase Chain Reaction (PCR) using the nuclear large subunit gene (28S, LSU), the nuclear small subunit gene (18S, SSU), the partial RNA polymerase second largest subunit (RPB2) and the translation elongation factor 1-alpha gene (TEF1-α). Respective primers LROR and LR5 (Vilgalys & Hester, 1990) were used to amplify the partial ribosomal RNA for the LSU gene; NS1 and NS4 (White et al., 1990) were used to amplify the partial ribosomal RNA for SSU gene; fRPB2-5F and fRPB2-7cR (Liu et al., 1999) were used to amplify the second largest subunit of RNA polymerase II and EF1-983F and EF1-2218R (Rehner, 2001) were used to amplify the protein coding region for TEF1-α gene. The final volume of the PCR reaction were 25 µl, containing 1 µl of DNA

template, 1 µl of each forward and reward primer, 12.5 µl of 2xMaster Mix and 9.5 µl of ddH_2O. The PCR thermal cycle program of LSU, SSU and TEF1-α gene was processed as initially 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 50 seconds, elongation at 72°C for 1 minute and a final extension at 72°C for 10 minutes, and finally kept at 4°C. The PCR thermal cycle program for the RPB2 gene was followed as initially 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 1 minute, annealing at 52°C for 2 minutes, elongation at 72°C for 90 seconds, and final extension at 72°C for 10 minutes. Purification and sequencing of PCR products were carried out at Shanghai Majorbio Biopharm Technology Co., Ltd, China.

2.2.4 **Sequence Alignment and Phylogenetic Analyses**

Phylogenetic analyses were performed from a combined LSU, SSU, TEF1-α and RPB2 sequence data. Sequences generated from this study were analyzed with related taxa in the family Melanommataceae which were obtained from GenBank and derived from Tian et al. (2015), Ariyawansa et al. (2015) and Li et al. (2016) (Table 2.1). Consensus sequences were obtained from both forward and reverse directions using Geneious. Pro.v4.8.5. Sequence alignments were automatically aligned with MAFFT v.6.864b (Katoh & Standley 2013: http://mafft.cbrc.jp/alignment/server/) and manually aligned wherever necessary in MEGA version 6.0 (Tamura et al., 2013). The sequence datasets were combined using BioEdit v.7.2.3 (Hall, 1999).

The phylogenetic analyses were obtained from Randomized Accelerated Maximum Likelihood (RAxML) and Bayesian analyses. The evolutionary model of nucleotide substitution for Bayesian analysis and Maximum-likelihood were selected independently for each locus using MrModeltest 2.2 (Nylander, 2004). Randomized Accelerated Maximum Likelihood (RAxML) was performed by using RAxMLGUI v. 0.9b2 (Stamatakis, 2006, 2014; Stamatakis et al., 2008; Silvestro & Michalak, 2010) with 1,000 rapid bootstrap replicates using the GAMMAI model of nucleotide substitution. Best scoring is the Final ML Optimization Likelihood: -12784.689821.

Bayesian Inference analysis (BI) (Huelsenbeck et al., 2001) was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001) to evaluate posterior probabilities (PP)

(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 and 10,000 trees were obtained. The first 2000 trees, representing the burn-in phase of the analyses, were discarded, the remaining 8000 trees were used for calculating posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic is 0.01) (Cai et al., 2006). Bayesian Posterior Probabilities (BYPP) equal or greater than 0.90 is given below or above each node (Figure 2.1 and 2.2).

The phylograms were represented in Treeview X (Page, 1996) and drawn in Microsoft power point and converted to jpeg file in Adobe Photoshop version CS5 (Adobe Systems Inc., The United States). The new sequences were submitted in GenBank (Table 2.1 and 2.2). The alignment was deposited in TreeBASE (2016) under the accession number 20406 and 21944.

2.3 Table of Contents

Table 2.1 Taxa used in the phylogenetic analyses of *Seifertia* and their corresponding GenBank numbers

Species	Culture collection	GenBank accession numbers		
		LSU	SSU	EF1-α
Aposphaeria populina	CBS 543.70	EU754130	EU754031	
Aposphaeria populina	CBS 350.82	JF740265		
Byssosphaeria jamaicana	SMH1403	GU385152	/	GU327746
Byssosphaeria jamaicana	SMH3085	GU385154	/	/
Byssosphaeria rhodomphala	SMH3086	GU385155	/	/
Byssosphaeria rhodomphala	GKM L153N	GU385157	/	GU327747
Byssosphaeria salebrosa	SMH2387	GU385162	/	GU327748
Byssosphaeria schiedermayeriana	SMH3157	GU385163	/	GU327745
Byssosphaeria schiedermayeriana	GKM 152N	GU385168	/	GU327749
Byssosphaeria villosa	GKM 204N	GU385151	/	GU327751
Byssosphaeria musae	MFLUCC 11-0146	KP744477	KP753947	/
Byssosphaeria siamensis	MFLUCC 10-0099	KT289895	KT289897	KT962059
Byssosphaeria schiedermayeriana	MFLUCC 10-0100	KT289894	KT289896	KT962060
Herpotrichia diffusa	CBS 250.62	DQ678071	DQ678019	DQ677915

Table 2.1 (continued)

Species	Culture collection	GenBank accession numbers		
		LSU	SSU	EF1-α
Herpotrichia juniper	CBS 200.31	DQ678080	DQ678029	DQ677925
Herpotrichia juniperi	CBS 468.64	DQ384093	DQ384077	/
Herpotrichia macrotricha	GKM 196N	GU385176	/	GU327755
Hysterium angustatum	CBS 123334	FJ161207	FJ161167	FJ161111
Hysterium angustatum	CBS 236.34	FJ161180.	GU397359	FJ161096
Melanomma pulvis-pyrius	CBS 124080	GU456323	GU456302	GU456265
Melanomma pulvis-pyrius	CBS 109.77	FJ201986	FJ201987	GU456274
Melanomma pulvis-pyrius	CBS 371.75	FJ201988	FJ201989	GU349019
Melanomma rhododendri	ANM 73	GU385198	/	/
Monotosporella tuberculata	CBS 256.84	GU301851	/	GU349006
Mycopappus aceris	CBS 124109	FJ839660	/	/
Pleomassaria siparia	CBS 279.74	DQ678078	DQ678027	DQ677923
Prosthemium betulinum	CBS 127468	AB553754	AB553644	/
Prosthemium canba	JCM 16966	AB553760	AB553646	/
Prosthemium orientale	JCM 12841	AB553748	AB553641	/
Prosthemium stellar	CBS 126964	AB553781	AB553650	/
Seifertia azalea	DAOM 239136	EU030276	/	/

Table 2.1 (continued)

Species	Culture collection	GenBank accession numbers		
		LSU	**SSU**	**EF1-α**
Seifertia shangrilanesis	MFLUCC 16-0238	NG_059680	NG_063608	KU954102
Xenostigmina zilleri	CBS 115686	GU253858	/	/
Xenostigmina zilleri	CBS 115685	GU253857	/	/
Xenostigmina zilleri	CBS 124108	FJ839675	/	/

Note The newly generated sequences are indicated in red bold and the type strains are in bold. ANM: A.N. Miller; CBS Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; GKM: G.K. Mugambi; IL:I. Lopez; JK: J. Kohlmeyer; KT: K. Tanaka; MAFF: Ministry of Agriculture, Forestry and Fisheries, Japan; MFLUCC Mae Fah Luang University Culture Collection, ChiangRai, Thailand; SMH: S.M. Huhndorf. LSU: large subunit (28S) of the nrRNA gene operon; ITS: internal transcribed spacers and intervening 5.8S nrDNA.

Table 2.2 Taxa used in the phylogenetic analysis of *Fusiconidium* and their corresponding GenBank numbers

Species	Culture collection	GenBank accession numbers			
		LSU	SSU	TEF1-α	RPB2
Aposphaeria corallinolutea	CBS 131287	JF740330	–	–	–
Aposphaeria corallinolutea	CBS 131286	JF740329	–	–	–
Aposphaeria populina	CBS 543.70	EU754130	EU754031	–	–
Aposphaeria populina	CBS 350.82	JF740265	–	–	–
Bertiella macrospora	IL 5005	GU385150	–	–	GU371768
Byssosphaeria jamaicana	SMH1403	GU385152	–	GU327746	–
Byssosphaeria jamaicana	SMH3085	GU385154	–	–	–
Byssosphaeria rhodomphala	SMH3086	GU385155	–	–	–
Byssosphaeria rhodomphala	GKM L153N	GU385157	–	GU327747	–
Byssosphaeria salebrosa	SMH2387	GU385162	–	GU327748	–
Byssosphaeria schiedermayeriana	SMH3157	GU385163	–	GU327745	–
Byssosphaeria schiedermayeriana	GKM 152 N	GU385168	–	GU327749	–
Byssosphaeria schiedermayeriana	MFLUCC 10–0100	KT289894	KT289896	–	–
Byssosphaeria villosa	GKM 204 N	GU385151	–	GU327751	–
Byssosphaeria musae	MFLUCC 11–0146	KP744477	KP753947	–	–
Byssosphaeria siamensis	MFLUCC 10–0099	KT289895	KT289897	–	–
Fusiconidium aquaticum	MFLUCC 16–0991	KX641894	KX641895	KX641896	–
Fusiconidium mackenziei	MFLUCC 14–0434	KX611112	KX611114	KX611118	KX611116
Fusiconidium mackenziei	HKAS 95019	KX611113	KX611115	KX611119	KX611117

Table 2.2 (continued)

Species	Culture collection	GenBank accession numbers			
		LSU	SSU	TEF1-α	RPB2
Herpotrichia diffusa	CBS 250.62	DQ678071	DQ678019	DQ677915	DQ677968
Herpotrichia juniper	CBS 200.31	DQ678080	DQ678029	DQ677925	DQ677978
Herpotrichia macrotricha	GKM 196 N	GU385176	–	GU327755	–
Herpotrichia macrotricha	SMH 269	GU385177	–	GU327756	–
Herpotrichia vaginatispora	MFLUCC 13–0865	KT934252	KT934256	KT934260	–
Roussoella nitidula	MFLUCC 11-0634	KJ474842	–	KJ474851	KJ474858
Roussoella nitidula	MFLUCC 11-0182	KJ474843	–	KJ474852	KJ474859
Melanomma pulvis-pyrius	CBS 124080	GU456323	GU456302	GU456265	GU456350
Melanomma pulvis-pyrius	CBS 109.77	FJ201986	FJ201987	GU456274	GU456359
Melanomma pulvis-pyrius	CBS 371.75	FJ201988	FJ201989	GU349019	GU371798
Melanomma rhododendri	ANM 73	GU385198	–	–	–
Monotosporella tuberculata	CBS 256.84	GU301851	–	GU349006	–
Muriformistrickeria rubi	MFLUCC 15–0681	KT934253	KT934257	KT934261	–
Mycopappus aceris	CBS 124109	FJ839660	–	–	–
Phragmocephala atra	MFLUCC 15–0021	KP698729	KP698725	–	–
Phragmocephala garethjonesii	MFLUCC 15–0018	KP698730	KP698726	–	–
Pseudostrickeria muriformis	MFLUCC 13–0764	KT934254	KT934258	KT934262	–
Pseudostrickeria ononidis	MFLUCC 14–0949	KT934255	KT934259	KT934263	KT934264
Sarimanas shirakamiense	MAFF 242969	LC001716	LC001713	–	–

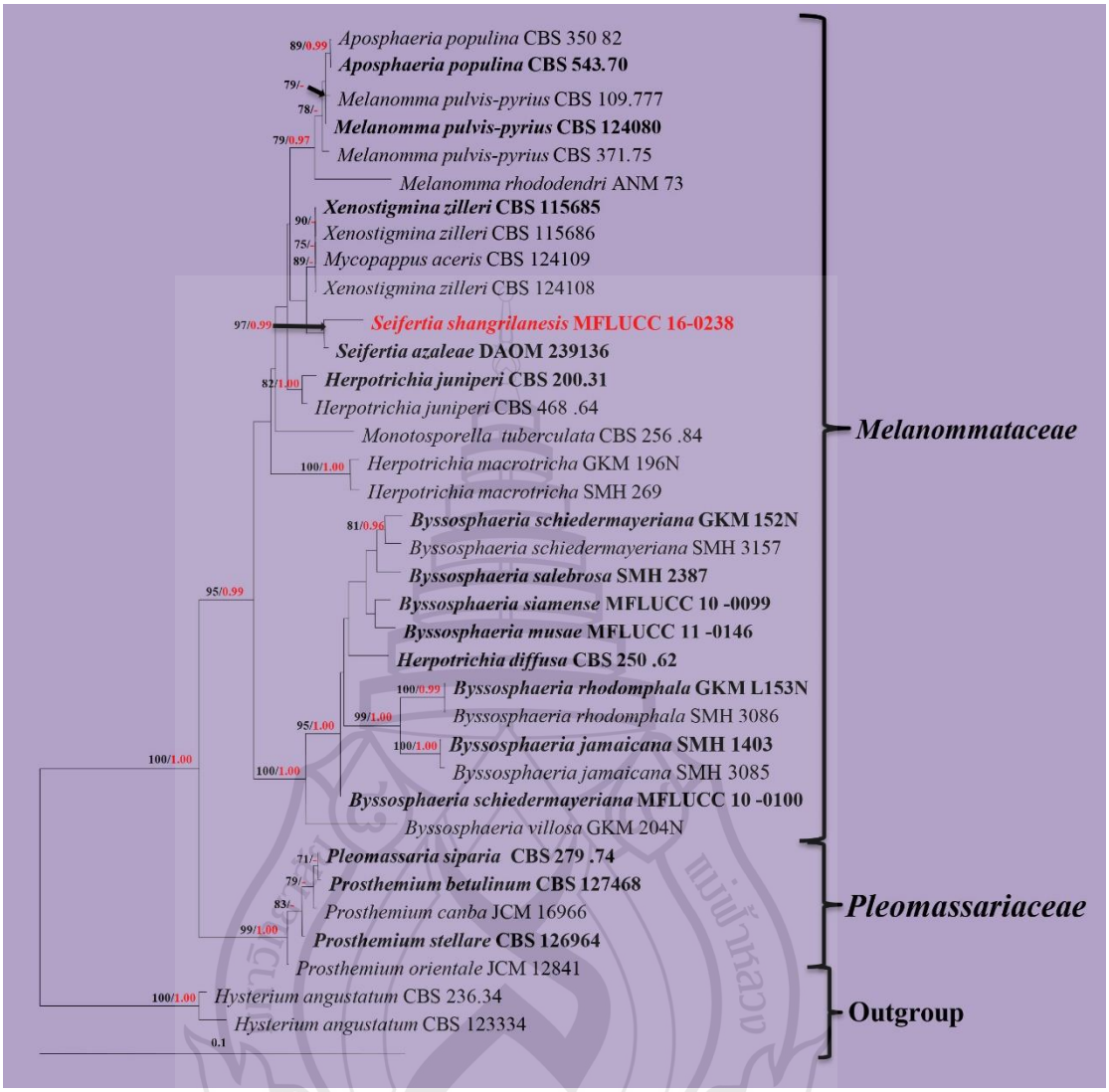
Table 2.2 (continued)

Species	Culture collection	GenBank accession numbers			
		LSU	SSU	TEF1-α	RPB2
Sarimanas shirakamiense	MAFF 244768	LC001715	LC001712	–	–
Sarimanas pseudofluviatile	MAFF 239465	LC001714	LC001711	–	–
Seifertia azalea	DAOM 239136	EU030276	–	–	–
Seifertia shangrilaensis	MFLUCC 16-0238	KU954100	KU954102	KU954101	–
Xenostigmina zilleri	CBS 115686	GU253858	–	–	–
Xenostigmina zilleri	CBS 115685	GU253857	–	–	–
Xenostigmina zilleri	CBS 124108	FJ839675	–	–	–

Note The newly generated sequences are indicated in red bold, while the type strains are in black bold. ANM: A.N. Miller; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; GKM: G.K. Mugambi; HKAS: The Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica, Kunming, China. IL: I. Lopez; JK: J. Kohlmeyer; KT: K. Tanaka; MAFF: Ministry of Agriculture, Forestry and Fisheries, Japan; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; SMH: S.M. Huhndof

2.4 Results

The best scoring tree from RAxML analysis is chosen to represent the phylogenetic relationships among a newly generated taxon with other genera in Melanommataceae and Pleomassariaceae in Dothideomycetes (Figure 2.1). The phylogenetic analyses obtained from maximum likelihood and Bayesian analyses gave similar topologies of the relationships in Melanommataceae and Pleomassariaceae and were not significantly different. Phylogenetically shows that Seifertia shangrilaensis (MFLUCC 16-0238) belongs to thefamily Melanommataceae (Pleosporales, Dothideomycetes) which forms a robust clade to S. azalea (97% ML, 0.99 PP) and formed a sister group with *Xenostigmina zilleri* (A. Funk) Crous (Figure 2.1). Therefore, a new species, *Seifertia shangrilaensis* is established. While the best scoring tree of Fusiconidium (Final ML Optimization Likelihood: -12784.689821) from RAxML analysis is chosen to represent the phylogenetic relationships of the new taxa with other genera in Melanommataceae (Pleosporales). The phylogenetic trees obtained from maximum likelihood and Bayesian analyses gave essentially similar topologies with regards to the position of our new taxa within the Melanommataceae. The maximum likelihood and Bayesian analyses trees were similar in topology and did not differ significantly. Both *Fusiconidium mackenziei* (MFLUCC 14–0434, HKAS 95019) and *F. aquaticum* (MFLUCC 16-0991) isolates sequenced and analysed constitute a strongly supported distinct clade basal to *Bertiella* and *Byssosphaeria* (92% ML, 1.00 PP; Figure 2.3). Single gene dataset analysis also yielded trees of identical topology where *Fusiconidium mackenziei* and *F. aquaticum* formed an independent distinct lineage within the Melanommataceae (result not shown). Thus, establishment of the new genus, *Fusiconidium*, is supported as outlined by Jeewon and Hyde (2016).



Note Phylogenetic construction using RAxML based analysis of a combined LSU, SSU and TEF1-α dataset. Bootstrap support values for maximum likelihood (ML, black), equal or greater than 70% and Bayesian posterior probabilities (PP, red) equal to or greater than 0.95 are shown above the nodes. The tree is rooted to *Hysterium angustatum* (CBS 236.34 and CBS 123334). The type strains are in black bold and the newly generated sequences are indicated in red bold.

Figure 2.1 Phylogram generated from maximum likelihood analysis of sequences in *Seifertia* (Melanommataceae)

2.4.1 *Seifertia shangrilaensis* J.F. Li, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF552010; Facesoffungi number: FoF 01885

Etymology: Refers to the location, where the holotype was collected.

Diagnosis: Differs from *Seifertia azalea* in its shorter and hyaline to subhyaline conidia.

Holotype: MFLU 16-0267

Epiphytic on living rachides of *Rhododendron decorum* Franch. Sexual morph: Undetermined. Asexual morph: Synnemata 1000–2300 µm high, 120–200 µm wide, erect, simple, unbranched, dark brown to black, with bubble-like, tightly interwoven, branched hyphae, compacted into an elongate bundle. *Mycelium* superficial, partly immersed on the substrate, composed of septate, branched, smooth, thin-walled, pale white to white hyphae. *Conidiophores* (1000–) 1300–2100 (–2150) × (9.5–) 10–11 (–13) µm, ($\bar{x}$ = 1804.7 × 10.3 µm, n = 40), synnematous, closely packed into a bundle, hyaline to pale brown, thin-walled, smooth, septate, unbranched, straight or flexuous, cylindrical. *Conidiogenous cells* (5–) 7–11 (–12.5) × (4.5–) 5.5–6.5 (–7) µm, ($\bar{x}$ = 7.9 × 5.8 µm, n = 100), holoblastic, phialidic, with not more than two conidiogenous loci, integrated, terminal, determinate, subhyaline, cylindrical to subclavate, smooth. *Conidia* (2.5–) 3.5–5 (–6) × 2.5–3.5 µm ($\bar{x}$ = 4.2 × 3.1 µm, n = 100), phialoconidia, hyaline to subhyaline, fusiform to subglobose, or ellipsoidal, aseptate, borne in basipetally, developing pseudo-chains, clavate, smooth, thin-walled, sometimes aggregated into slimy masses at the apex of the synnema.

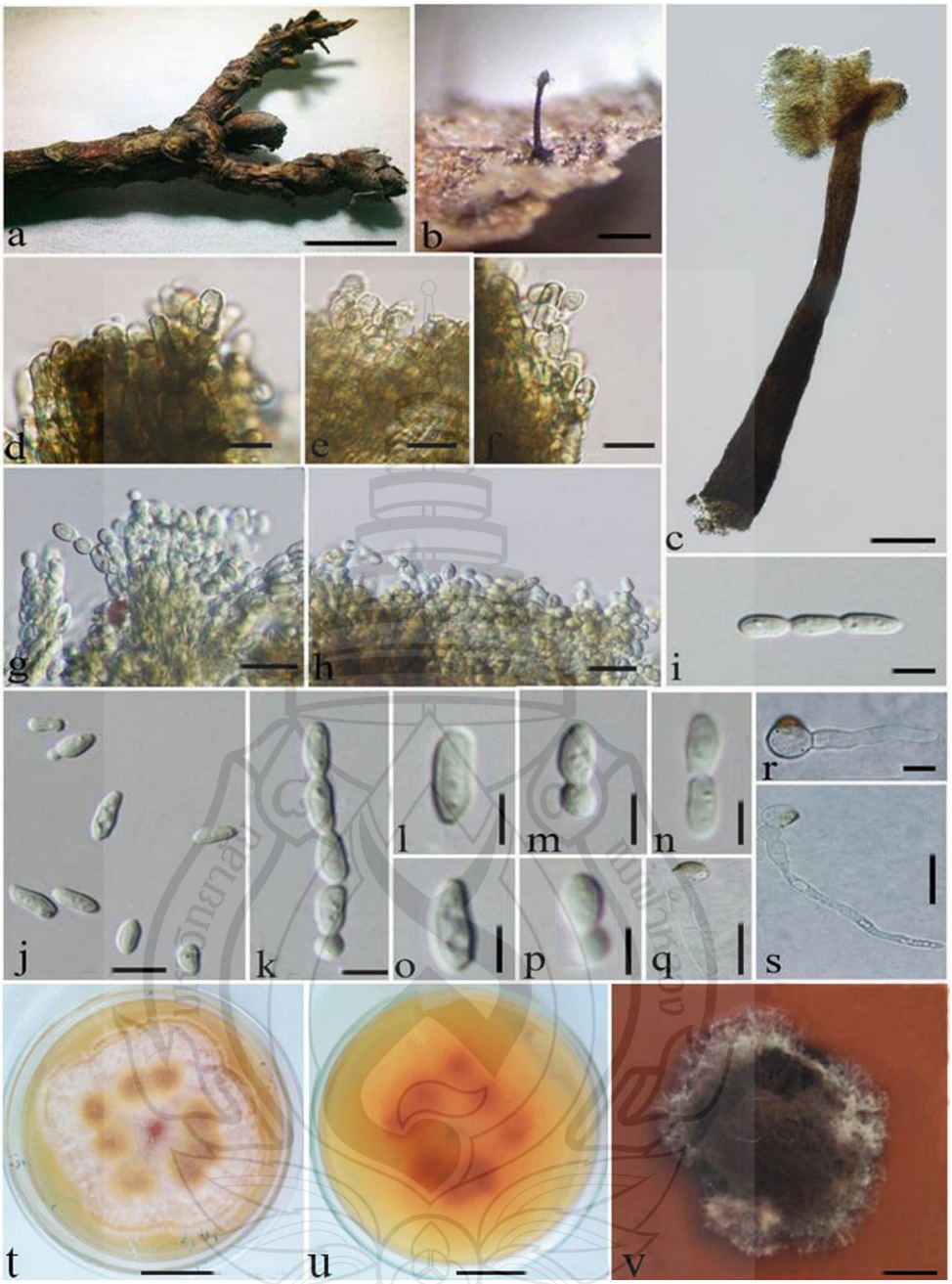
Cultural characteristics: Conidia germinating on PDA within 14 hours at 15ºC, germ tubes produced from apex. Colonies growing on PDA, reaching 5 mm diam. after 4 weeks at 25ºC, mycelium semi-immersed to superficial, irregular in shape, flat, slightly raised, with undulate edge, slightly rough on surface, cottony to fairy fluffy, colony from above, initially write to cream at the margin, yellowish-brown in the centre, becoming white at the margin, dark brown at the centre after 4 weeks; from below, initially, cream at the margin, orangish-brown to reddish-brown at the centre, becoming dark brown to black after 4 weeks, producing brown pigmentation in agar.

Material examined: CHINA, Yunnan Province, Shangrila, on living rachides of *Rhododendron decorum* (Ericaceae), 20 October 2014, R. Phookamsak, SgL027 (MFLU

16-0267, holotype!; KUN-HKAS 93733, isotype!), ex-type living culture, MFLUCC 16-0238!, KUMCC 16-0002.

Known distribution: widespread in temperate and boreal regions.

Notes: *Seifertia shangrilaensis* is similar to the type species, *Seifertia azalea* in having narrow and pale conidia, with one or two conidiogenous loci of conidiogenous cells, dark conidiophores and erect, synnemata (Partridge & Morgan-Jones 2002, Glawe *et al.* 2006, Seifert *et al.* 2007). However, *S. shangrilaensis* differs from *S. azalea* in having hyaline to subhyaline, and wider conidiophores (9.5–12.6 µm vs. 4–7 µm), and smaller conidia (2.5–6 × 2.5–3.6 µm vs. 4–12 × 4–8 µm).



Note a. Living rachides of *Rhododendron decorum*. b. Appearance of synnema on rachides. c. A synnema visualized under the compound microscope. d–h. Conidiogenous cells. i–p. Conidia. q–s. Germinating conidia. t. Multiple culture colonies from above. u. Multiple culture colonies. v. Single colony on PDA after 4 weeks. Scale bars: t–u = 2 cm, v = 1 cm, a = 0.5 cm, b = 500 µm, c = 200 µm, g–h, j–k, q–s = 10 µm, d–f, i, l–n = 5 µm.

Figure 2.2 Morphology of *Seifertia shangrilaensis* (MFLU 16-0267)

2.4.2 *Fusiconidium* J.F. Li, Phookamsak & K.D. Hyde, gen. nov.

MycoBank number: MB 817936; Facesoffungi number: FoF02516

Etymology: Named after its fusiform conidia.

Saprobic on woody litter or herbs. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. *Colonies* effuse, superficial, brown. *Conidiophores* macronematous, mononematous, erect, flexuous, septate, unbranched, brown to dark brown, single, smooth, thick-walled, cylindrical, percurrently proliferating. *Conidiogenous cells* enteroblastic, integrated, terminal, often light brown and subhyaline at apex, cylindrical or doliiform. *Conidia* acrogenous, solitary, dry, rostrate, straight or curved, fusiform to ellipsoidal, euseptate, pale brown to brown, smooth or verruculose, thick-walled. *Conidial secession* rhexolytic.

Type species: *Fusiconidium mackenziei* J.F. Li, Phookamsak, Camporesi & K.D. Hyde, sp. nov.

Notes: *Fusiconidium* forms a separate clade basal to *Bertiella* (Melanommataceae) in the phylogeny from the combined sequence data (Figure 2.3). Based on the sequence data and the distinct morphology, we treat *Fusiconidium* as a new genus in Melanommataceae (Pleosporales, Dothideomycetes). Morphologically, *Fusiconidium* resembles *Sporidesmiella*, *Nigrolentilocus*, *Phragmocephala* and *Distophragmia* in conidial shape. *Sporidesmiella*, *Nigrolentilocus* and *Phragmocephala* are positioned within the Melanommataceae (Tian et al., 2015), while *Distophragmia* has been referred to family *incertae sedis* (Castañeda-Ruiz et al., 2015). In addition, *Sporidesmiella* and *Distophragmia* have distoseptate conidia with schizolytic conidial secession (Kirk, 1982; Castañeda-Ruiz et al., 2001). *Phragmocephala* has synnematous conidiophores and holoblastic conidiogenous cells without percurrent extensions (Mason & Hughes, 1951; Whitton et al., 2012; Su et al., 2015). *Nigrolentilocus* has polyblastic or holoblastic conidiogenous cells with schizolytic conidial secession (Castañeda-Ruiz et al., 2001). *Fusiconidium* can be distinguished from these genera in having rhexolytic conidial secession, percurrently proliferating conidiophores, with enteroblastic conidiogenous cells and acrogenous, pale brown to dark brown, verruculose, fusiform, euseptate conidia. Therefore, we introduced a new genus *Fusiconidium* to accommodate two new taxa; *Fusiconidium mackenziei* and *F. aquaticum* in Melanommataceae.

2.4.3 *Fusiconidium mackenziei* J.F. Li, Phookamsak, Camporesi & K.D. Hyde, sp. nov.

MycoBank number: MB 817937; Facesoffungi number: FoF 02484

Etymology: Named in honour of the mycologist Dr. E.H.C. McKenzie, in recognition of his contribution to the study of hyphomycetes.

Holotype: MFLU 16–2139

Saprobic on branch of *Clematis vitalba* (Ranunculaceae). Sexual morph: Undetermined. Asexual morph: Hyphomycetes. *Colonies* on the natural substratum effuse, hairy, black. *Mycelium* immersed, composed of septate, branched, smooth hyphae. *Conidiophores* 91–114 µm long × 8.5–11 µm wide ($\bar{x}$ = 111 µm × 9.8 µm, n = 30), macronematous, mononematous, dark brown, septate, unbranched, thick-walled, smooth, erect, paler towards the apex, cylindrical, with 7–9 transverse eusepta. *Conidiogenous cells* enteroblastic, percurrently proliferating, cylindrical, pale brown to dark brown, thin-walled, smooth. *Conidia* 38–40 µm long × 12–15 µm wide ($\bar{x}$ = 40 µm × 14 µm, n = 30) acrogenous, straight or curved, fusiform to ellipsoidal, with 7 transverse eusepta, constricted at the septum, pale brown to dark brown, light colored and obtuse at the apex, echinulate, with broadly truncate at the base. *Conidial secession* rhexolytic.

Cultural characteristics: Conidia germinating on PDA within 14 hours at 16ºC, with germ tubes produced from both ends. Colonies reaching 75 mm diam. after 6 week at 25ºC, with mycelium semi-immersed to superficial, regular, flat, slightly raised, with undulate edge, slightly rough on surface, cottony, grayish brown, cream at the margin; reverse, orange-brown to reddish brown, medium dense, circular, umbonate.

Material examined: ITALY, Forlì-Cesena Province, Passo la Calla - Santa Sofia, on dead branch of *Clematis vitalba* (Ranunculaceae), 25 January 2014, E. Camporesi, IT1669 (MFLU 16-2139, holotype!), ex-type living culture, MFLUCC 14-0434, KUMCC 16-0031 (isotype in HKAS, as HKAS 95019).

Notes: *Fusiconidium mackenziei* is described based on a collection on dead branch of *Clematis vitalba* from Italy and represents the type species of genus *Fusiconidium. Fusiconidium. mackenziei* is characterized by thick-walled, fusiform 7-euseptate, echinulate conidia, enteroblastic conidiogenous cells with percurrent extensions, subhyaline at the apex and rhexolytic conidial secession. In the phylogenetic analysis based on combined LSU,

SSU, TEF1-α and RPB2 gene, *Fusiconidium mackenziei* and *Fusiconidium aquaticum* formed an independent and distinct lineage within the Melanommataceae.

2.4.4 *Fusiconidium aquaticum* Z.L. Luo & K.D. Hyde, sp. nov.

MycoBank number: MB 817938; Facesoffungi number: FoF 02216

Etymology: With reference to the aquatic habitat.

Holotype: HKAS 92705

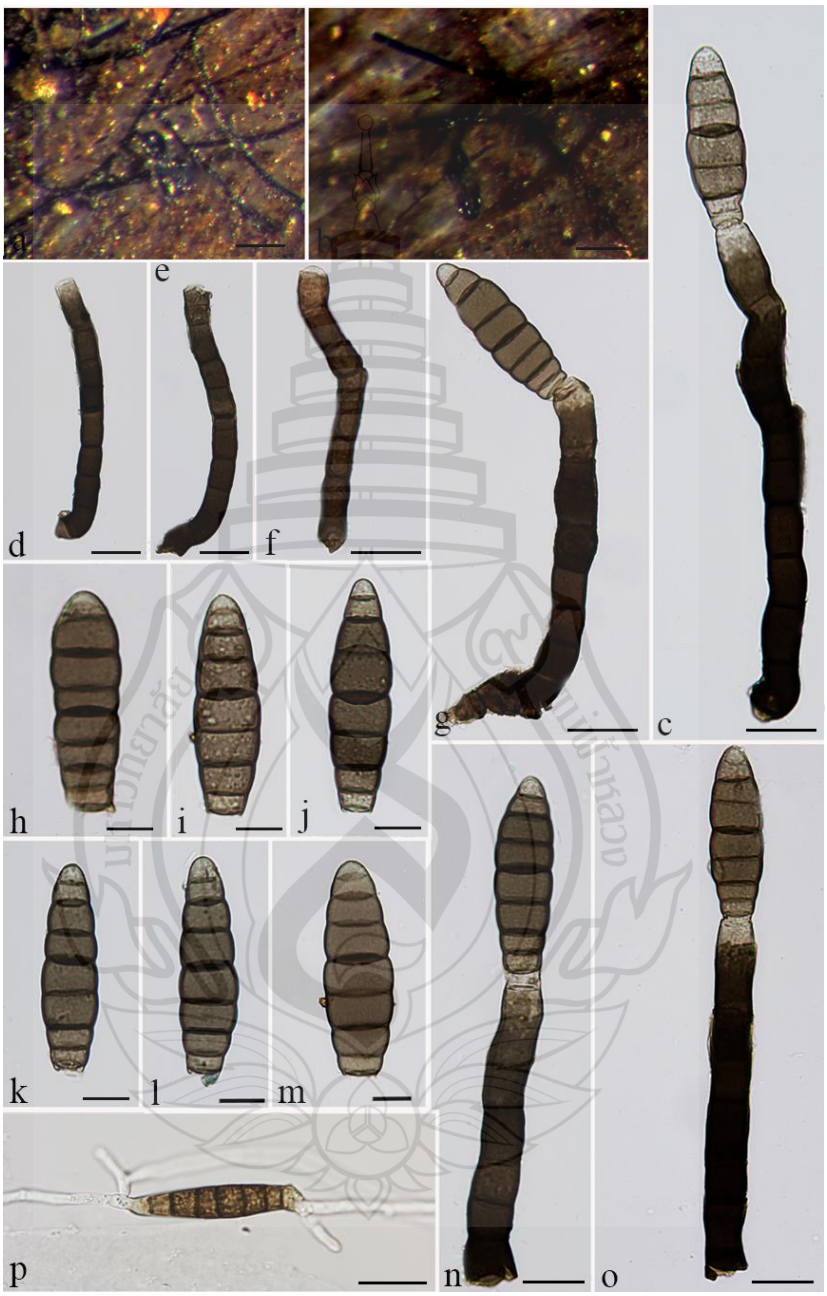
Saprobic on submerged decaying wood. *Sexual morph*: Undetermined. *Asexual morph*: *Colonies* on the substratum superficial, effuse, gregarious, dark brown to black. *Mycelium* immersed, composed of septate, branched, smooth, pale brown hyphae. *Conidiophores* 49–65 μm long × 5.5–7.5 μm wide ($\bar{x}$ = 57 μm × 6.5 μm, n = 10), mononematous, macronematous, pale brown to brown, paler towards the apex, unbranched, straight or flexuous, smooth, erect, cylindrical, 1–3-septate. *Conidiogenous cells* enteroblastic, percurrent, sub-cylindrical to geniculate, slightly recurved, subhyaline to pale brown, thin-walled, smooth. *Conidia* 59–66 μm long × 15–18 μm wide ($\bar{x}$ = 62.5 μm × 16.5 μm, n = 25), acrogenous, fusiform to ellipsoidal with round at the apex and truncate base, mostly straight, infrequently slightly curved, with 8–10 transverse eusepta, rarely longitudinal septa, sometimes with guttules, initially subhyaline to pale brown, brown at maturity, with central cells brown to dark brown and terminal cells light brown, slightly rounded at the apex and truncate base, distinctly verruculose. *Conidial secession* rhexolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours at 23ºC, with germ tubes produced from the second cell of ends. Colonies reaching 90 mm diam. after six weeks at room temperature, with mycelium semi-immersed to superficial, regular, flat, slightly raised, with undulate edge, slightly rough on surface, cottony, golden yellow; reverse saffron yellow, medium dense.

Material examined: CHINA, Yunnan Province, saprobic on decaying wood submerged in Dulong River, 6 May 2015, Z.L. Luo, HD 3–3–4 (HKAS 92705, holotype!), ex-type living culture, KUMCC 15–0300, MFLUCC 16-0991. (isotype in MFLU, as MFLU 15-0300).

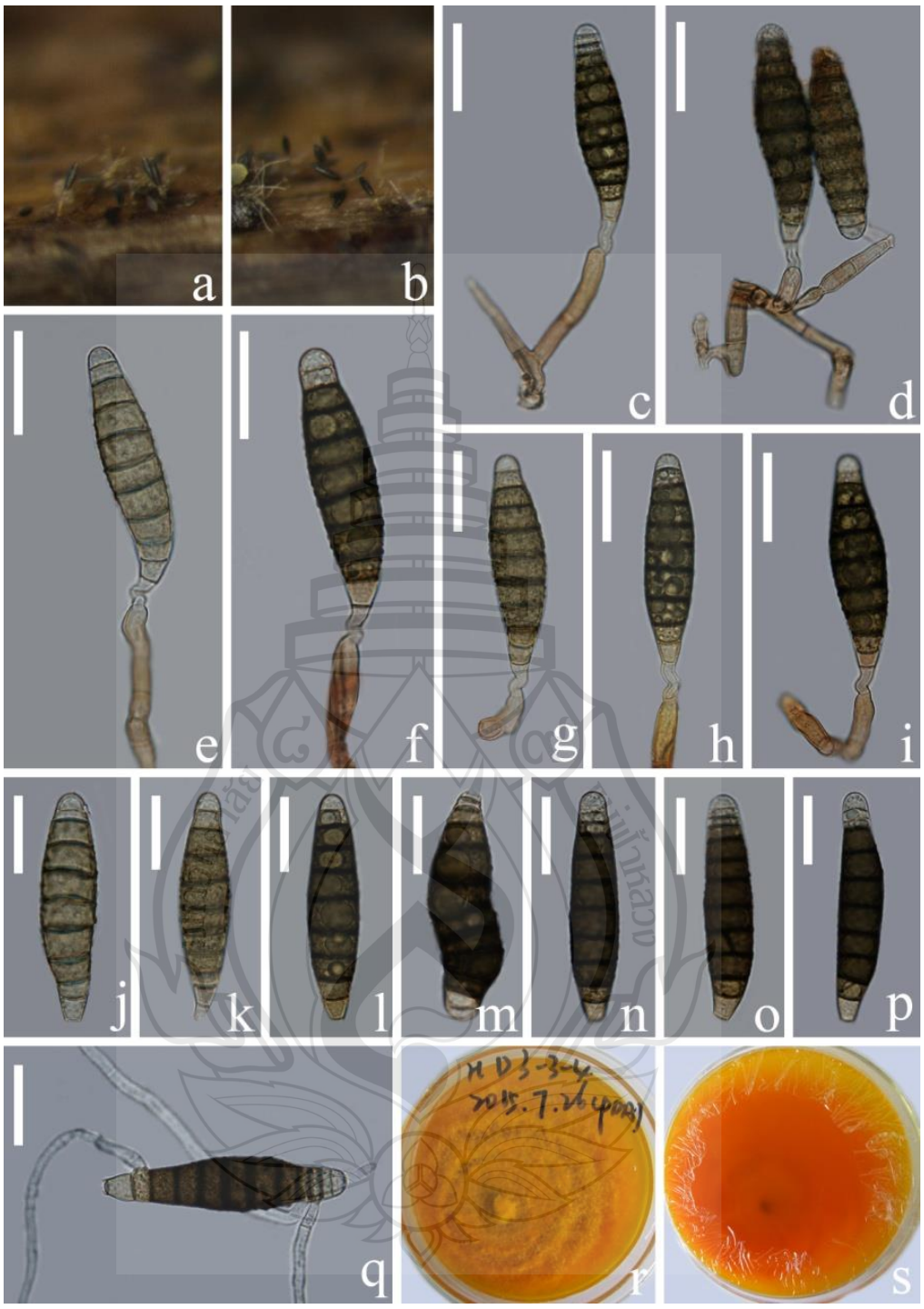
Notes: *Fusiconidium aquaticum* is different from *F. mackenziei* in having paler, narrow, short condiophore (49–65 μm × 5.5–7.5 μm vs. 91–114 μm × 8.5–11 μm), big conidia (59–66 μm × 15–18 μm vs. 38–40 μm× 12–15 μm), with rarely longitudinal septa,

distinctly verruculose and guttulate conidia (Figure 2.3), *Fusiconidium aquaticum* clusters with *F. mackenziei* (Figure 2.4).



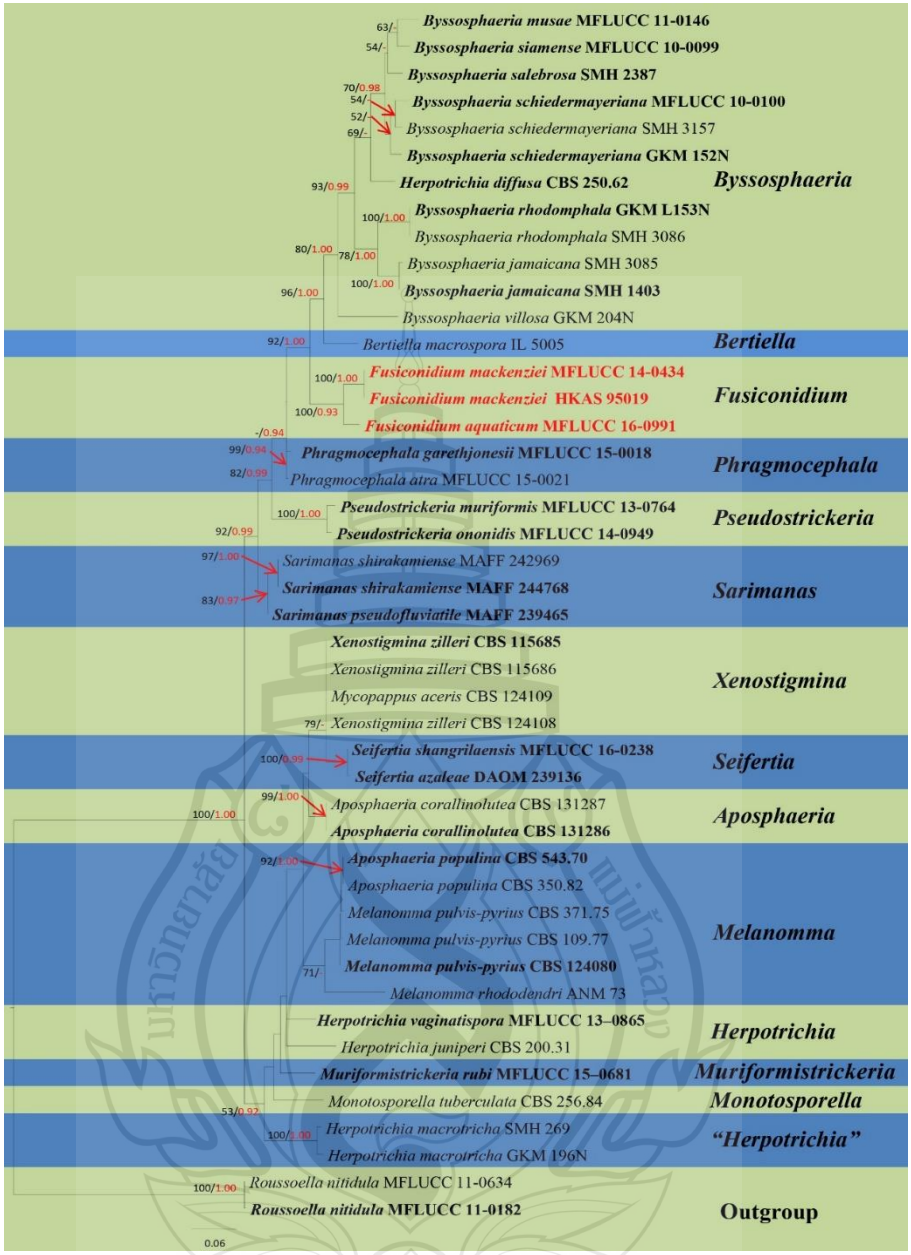
Notes a–b. Colonies on dead branch of *Clematis vitalba*. c, g, n–o. Conidiophores bearing conidia. d–f. Conidiophores. h–m. Conidia. p. Germinating conidia. Scale bars: a = 200 µm, b = 50 µm, c–g = 20 µm, n–p = 10 µm, h–m = 5 µm.

Fugure 2.3 Morphology of *Fusiconidium mackenziei* (MFLU 16–2139)



Notes a–b. Colonies on the substratum. c–i. Conidiophores with conidia. j–p. Conidia. q. Germinating conidium. r. Surface view of culture on PDA. s. Reverse view of culture on PDA. Scale bars: c–i = 30 μm, j–l, n–q = 25 μm, m = 20 μm.

Fugure 2.4 Morphology of *Fusiconidium aquaticum* (HKAS 92705)



Note Phylogenetic construction using RAxML based analysis of a combined LSU, SSU, TEF1-α and RPB2 dataset. Bootstrap support values for Maximum Likelihood (ML, black), equal or greater than 50% and Bayesian posterior probabilities (PP, red) equal to or greater than 0.90 are shown above the nodes. The tree is rooted to *Roussoella nitidula* (MFLUCC 11-0634, MFLUCC 11-0182). The type strains are in black bold and the newly generated sequences are indicated in red bold.

Figure 2.5 Phylogram generated from maximum likelihood analysis of sequences in *Fusiconidium*

2.5 Discussion

The genus *Seifertia* is relatively poorly studied and was introduced to accommodate a pathogen species occurring on *Rhododendron* in the UK. Based on the little sequence data available in GenBank, the placement of *Seifertia* is uncertain. According to its morphological characters, *Seifertia* was previously treated in various genera such as *Periconia* (Peck, 1873), *Pycnostysanus* (Mason, 1941) and also considered a taxonomic synonymy of *Sorocybe* (Ellis, 1976; Carmichael et al., 1980). The type species, *Seifertia azalea* is characterized by erect, simple, and dark synnemata, unicellular or very rarely septate, narrow, paler conidia laterally thick-walled, macronematous and synnematous conidiophores and holoblastic, integrated, terminal and determinate conidiogenous cells (Partridge & Morgan-Jones, 2002).

Seifertia is known to occur on azaleas and rhododendrons and is a cosmopolitan species causing bud blight or bud blast disease in Japan, Europe and North America (Mason, 1941; Ellis, 1976; Farr et al., 1996; Partridge & Morgan-Jones, 2002; Glawe & Hummel, 2006). Insects were thought to play a role of pathogenic distribution (Howell et al., 1962; Pirone, 1978; Viennot-Bourgin, 1981). Disease symptoms were as initially, infecting dead flower buds, and later becoming blackened bearing numerous synnemata (Glawe & Hummel, 2006).

Pirone (1978) reported that 90% death of *Rhododendron* flowers were caused by *S. azalea*, with a perennial infection (Chant & Gbaja, 1984; Glawe & Hummel, 2006). Farr et al. (2007) listed eight *Rhododendron* species infected by *Seifertia azalea* in the USA viz. *R. arborescens* (Pursh) Torr., *R. catawbiense* Michx., *R. macrophyllum* D. Don ex G. Don, *R. maximum* L., *R. minus* Michx., *R. nudiflorum* (L.) Torr., *R. vaseyi* A. Gray and *R. viscosum* (L.) Torr. Recently, Farr and Rossman (2016) listed only two *Rhododendron* species, *R. hemsleyanum* and *R. ponticum* caused by *S. azalea* in the USA. However, further occurrence, incidence, host and geographical range were relatively poorly known (Glawe & Hummel, 2006). Glawe and Hummel (2006) reported a new host, *R. hemsleyanum* infected by *S. azalea* in North America. They mentioned that the disease incidence was limited by a low number of vulnerable genotypes in collection, critical environmental conditions and some other factors.

Seifertia species has so far not been reported to occur on *Rhododendron decorum*. *Seifertia shangrilaensis* was collected from living rachides of *R. decorum* in South-west China. The species occurs on both living rachides and dead hanging rachides of *R. decorum*. *Seifertia shangrilaensis* appears to be a pathogen causing death of flower buds of *R. decorum*. The development of disease infection might be initially limited where the synnemata occurred, and later, infected widely in other host cells. However, pathogenicity test is not done in this study.

Seifert et al. (2007) treated *Seifertia* in Dothideomycetes based on the large subunit LSU gene sequence data and it clustered with *Mycosphaerella mycopappi*, but was unrelated to *Mycosphaerella sensu stricto*. Crous *et al*. (2009) re-examined *Xenostigmina* and confirmed that *Xenostigmina* is a synanamorph of *Mycopappus* and showed to be allied with *Seifertia* in Pleosporales. Furthermore, Crous et al. (2013) revealed that *Xenostigmina* and *Mycopappus* clustered in the Phaeosphaeriaceae. Phookamsak et al. (2014), however, they excluded *Mycopappus* and *Xenostigmina* from Phaeosphaeriaceae due to these genera forming a single clade close to Melanommataceae. Tian et al. (2015) accepted *Xenostigmina* and *Mycopappus* in Melanommataceae based on multiple gene phylogenetic analyses. Hyde et al. (2013) and Wijayawardene et al. (2014) did not treat *Seifertia*, while Tian et al. (2015) discussed its relationship with *Xenostigmina* and *Mycopappus*, but did not place it in Melanommataceae.

Several asexual morphs of melanommataceous genera have been reported. *Pyrenochaeta* is the asexual morph of *Herpotrichia* (Sivanesan, 1984). *Aposphaeria* and *Phoma*-like asexual morphs have been reported for *Melanomma* species (Sivanesan, 1984; Tian et al., 2015). These links however, need to be confirmed in the light of modern taxonomic treatments and molecular data (Tian et al., 2015). In this study, phylogenetic analyses of combined LSU, SSU and TEF1-α sequence data provide a support that *Sefertia* belongs in the family Melanommataceae, Dothideomycetes (*sensu* Hyde et al., 2013; Wijayawardene et al., 2014) and is distinct from *Mycopappus* and *Xenostigmina*.

The natural classification of many hyphomycetous fungi is highly subjective and controversial. In several studies, it has been shown that their morphologies have been subjected to convergent evolution and taxa are polyphyletic (Shenoy et al., 2006, 2010; Wang et al., 2007; Tian et al., 2015). The plant genus *Clematis* is known to harbour fungi, especially hyphomycetes such as *Cercospora* and *Pseudocercospora* (Braun & Urtiaga,

2013). But no mycological studies have so far reported taxa possessing euseptate, enteroblastic and rhexolytic conidial fungi from *Clematis* species. In addition, there has been a lack of multigene phylogenetic studies on these fungal groups. In our survey, apart from morphological characterization, DNA sequence data from several genes are analysed to accommodate our new taxa into a precise taxonomic system along with their sexual morphs.

In this study, the new taxa, *Fusiconidium mackenziei* and *F. aquaticum*, collected on a dead branch of *Clematis vitalba* from Italy and on a submerged decaying wood in China, are recognized as morphologically distinct species in Melanommataceae. *Fusiconidium* has unique morphological characters are distinct from superficially similar genera such as *Sporidesmiella* (distoseptate conidia, schizolytic conidial secession), *Distophragmia* (distoseptate conidia) and *Phragmocephala* (holoblastic conidiogenous cells) (Shenoy, 2006, 2010). Based on phylogeny of the concatenated dataset, *Fusiconidium mackenziei* and *F. aquaticum* isolates did not group with any existing species of the Melanommataceae. Instead they grouped together with high support in an independent distinct clade in between *Pseudostrickeria*, *Bertiella* and *Byssosphaeria* species (Figure 2.3). There is no phylogenetic discordance in combined gene datasets, and the high support obtained here provides substantial evidence to support the establishment of our new genus. Therefore, we propose *Fusiconidium* as a new genus in Melanommataceae (order Pleosporales).

It is worth mentioning herein that an additional precaution taken in this study is that we extracted DNA separately from single-spore cultures as well as from conidia picked up directly from substrates to ascertain that no contaminated cultures are used for phylogenetic inferences. The phylogenies confirm that DNA sequences obtained from both conidia (HKAS 95019) and cultures (MFLUCC 14–0434) are identical. It is advisable that future studies on novel taxa among hyphomycetes use a similar strategy wherever possible to avoid use and deposit of contaminated cultures as well as allow valid sequences to be deposited in databases for future reference.

CHAPTER 3

MOLECULAR TAXONOMY AND MORPHOLOGICAL CHARACTERIZATION REVEAL NEW SPECIES AND NEW HOST RECORDS OF SPECIES IN TORULACEAE, PLEOSPORALES[2]

Abstract

Seven new species and two new host records, with one new genus in Torulaceae, are described and illustrated from herbaceous litter collected in Italy and Thailand. The new species possess colony, conidiophore and conidial characteristics that fit within the generic concept of Torulaceae. Detail observations of morphologies clearly demarcate seven of them from extant species and are hence described as new (*Dendryphion hydei* sp. nov., *Sporidesmioides thailandica* gen. et sp. nov., *Torula chiangmaiensis* sp. nov., *Torula pluriseptata* sp. nov., *Torula chromolaenae* sp. nov., *Torula hydei* sp. nov., *Torula mackenziei* sp. nov.). Details of asexual morphs are described and justifications for establishing these new species are provided. In addition, we sequenced the nuclear ribosomal RNA genes as well as protein coding genes to infer phylogenetic relationships and discuss phylogenetic affinities with morphologically similar species. The morphological distinction is further supported by

[2] This paper has been published in Mycological Progress, 2016, 15(10), 1169–1178. doi:10.1007/s11557-016-1238-0; in Mycological Progress, 2017, 16(4), 447–461. doi:10.1007/s11557-017-1292-2; in PloS one, 2020, 15(2), e0228067. doi: 10.1371/journal.pone.0228067

phylogenetic discrimination. In particular phylogenies depict a close relationship and updated host record of new taxa in Torulaceae are discussed.

3.1 Introduction

The family Torulaceae was first introduced by Corda (Sturm, 1829). Torulaceae is known only by asexual characters of its erect, micro- or macronematous conidiophores, with or without apical branches, and doliiform to ellipsoid or clavate, brown, smooth to verruculose, mono- to polyblastic, often cupulate conidiogenous cells and subcylindrical, phragmosporous, acrogenous, brown, dry, smooth to verrucose conidia, characteristically produced in branched chains (Crous et al., 2015; Su et al., 2016; Hyde et al., 2016). Catenate conidia or branched conidial chains are formed mostly by the terminal cell and sometimes even other cells of the conidia becoming fertile and producing newer conidia in chains (Ellis, 1971; Crous et al., 2015). Crous et al. (2015) investigated phylogenetic relationships of the Torulaceae and provided DNA sequence data for four *Torula* species and accepted *Dendryphion* and *Torula* in Torulaceae within the order Pleosporales. Further taxonomic work by Su et al. (2016) introduced *Neotorula* and two new *Dendryphion* species and Li et al. (2016) reported *Sporidesmioides* within Torulaceae. Currently *Torula*, *Dendryphion*, *Neotorula* and *Sporidesmioides* are accommodated in Torulaceae (Su et al., 2016; Hyde et al., 2016; Li et al., 2016).

Torula, typified by *T. herbarum* (Pers.) Link is characterized by terminal or lateral, monoblastic or polyblastic conidiogenous cells which have a basally thickened and heavily melanized wall, with the apex thin-walled and frequently collapsing and becoming coronate (Crane & Miller, 2016). Additionally, Crane and Schoknecht (1977) reported conidiogenesis aspects among *Torula* species from light and transmission electron microscopy. These morphological characters used to segregate *Torula* were also observed by Mason (1941), Hughes (1953), Subramanian (1971), Ellis (1971, 1976) and Bhat (2010). However, there is little information regarding the phylogenetic relationships of *Torula* until the study of Crous et al. (2015) which reported two new species.

Sporidesmium-like taxa are a fungal group that include several dematiaceous hyphomycetous genera, taxonomically segregated under *Sporidesmium sensu lato* (Shenoy et al., 2006), which produce holoblastic phragmoconidia on proliferating or non-proliferating conidiophores, with schizolytic conidial secession (Ellis, 1958, 1971, 1976; Kirk, 1982; Subramanian, 1992; Hernández-Guitiérrez & Sutton, 1997; Shoemaker & Hambleton, 2001; Wu & Zhuang, 2005). In their asexual phase, they occur predominantly as saprobes on rotten wood, dead branches, and decaying leaves of various plant species. Some species are also plant pathogens (Ellis, 1971, 1976; Guo, 1989; Wu & Zhuang, 2005). Su et al. (2016) introduced three new species in *Sporidesmiaceae* and a novel genus *Pseudosporidesmium*, typified by *Pseudosporidesmium knawiae* (Crous) K.D. Hyde & McKenzie, in Sordariomycetes. Morphologically, members of *Sporidesmiaceae* and *Pseudosporidesmium* have holoblastic conidial development. *Acroconidiellina arecae* (Berk. & Broome) M.B. Ellis (Ellis, 1971) also produces sporidesmium-like conidiophores and conidia, but conidial development of this taxon is polytretic (Bhat, 2010).

Réblová and Winka (2001) provided a phylogenetic analysis based on LSU nu-rDNA sequence data to show that *Sporidesmium folliculatum* (Corda) E.W. Mason & S. Hughes and *S. hormiscioides* Corda are linked to *Chaetosphaeriaceae* and the study revealed that sporidesmium-like genera may have sexual associations with several ascomycete families in both Dothideomycetes and Sordariomycetes (Shenoy et al., 2006). Thus, taxonomic analysis using morphological characters alone was found to be unreliable in interpreting natural relationships (Réblová, 1999). Shenoy et al. (2006) investigated phylogenetic affinities and delimited the genera of sporidesmium-like taxa based on the LSU and RPB2 sequence data (without combination). In their study, the LSU nu-rDNA sequence phylogenetic analysis showed that sporidesmium-like species can be separated in different families in Dothideomycetes and Sordariomycetes, with *Sporidesmium australiense* M.B. Ellis in a clade grouped with *Arthopyrenia salicis* A. Massal (CBS 368.94) in *Arthopyreniaceae* (Dothideomycetes) (Shenoy et al., 2006). Subsequently, Schoch et al. (2009) and Tanaka et al. (2009) presented the first molecular data to show that *Roussoella* and *Roussoellopsis* clustered together with *Arthopyreniaceae* in Dothideomycetes. Liu et al. (2014)

accommodated *Roussoella* and *Roussoellopsis* in *Roussoellaceae*, but this study did not include *Sporidesmium australiense* and *Arthopyrenia salicis.* Su et al. (2016) provided phylogenetic analysis based of LSU sequence data and showed that *Sporidesmiaceae* and *Pseudosporidesmium* are taxonomically distinct, but remain within Sordariomycetes (Su et al., 2016).

Dendryphion Wallr. was introduced by Wallroth (Pratibha & Prabhugaonkar, 2017) to accommodate hyphomycetous species, *D. comosum* Wallr. The genus is commonly known to be saprobic on dead stems of herbaceous plants and decaying wood, and is characterized by having erect, solitary, branched in upper part, polytretic conidiophores, forming septate,pigmented, thick-walled, finely roughened stipe and a distinct conidiogenous apparatus, with darkscars andcatenate, in simple or branched chains of brown, septate (didymo- or cheiro) conidia (Crous et al., 2015; Shaw et al., 2015). Crous et al. (2015) introduced *D. europaeum* Crous & R. K. Schumacher based on morphological characteristics and molecular data and later Crous et al. (2015) accommodated the species in Torulaceaeand further accepted *Dendryphion* in Torulaceae. Su et al. (2016) circumscribed genera of Torulaceae from freshwater habitats and introduced two *Dendryphion* species, *D. aquaticum* Hong Y. Su & K. D. Hyde and *D. submersum* Hong Y. Su & K. D. Hyde and designated a reference specimen of *D. nanum* (Nees) S. Hughes based on molecular phylogeny. Su et al. (2018) also introduced *D. fluminicola* Z. L. Luo, D. J. Bhat & K. D. Hyde. Only seven *Dendryphion* species have DNA sequence data and their phylogenetic affinities to members of the Torulaceae have been investigated.

This chapter introduces seven new species and two new host records, with a new genus collected from Italy and Thailand within the family Torulaceae and investigate their similarity of host species, and phylogenetic relationships based on DNA sequence analyses from nucleotides and protein genes.

3.2 Material and Methods

3.2.1 Isolation and Identification

The specimens were collected from herbaceous litters from Italy and Thailand during the year 2013 to 2016. Samples were returned to the laboratory (Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand) for examination and description of morphological characteristics. The specimens were observed under a Motic SMZ 168 series dissecting stereo-microscope. The conidial structures were picked up by a sterilized surgical needle and transferred into 10% lacto-glycerol on a clean slide and examined under a Nikon Eclipse 80i compound microscope and photo-captured with a Canon 600D digital camera using DIC microscopy. Macro- morphological structures were photographed with a Discovery V.8 stereo microscope fitted with a CARL ZEISS Axio Cam ERc5S microscope camera. Tarosoft® Image Frame Work program v.0.9.0.7 and Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems Inc., The United States) were used for measurements and drawing photographic plates.

Single conidia isolation was carried out to obtain pure cultures as described in Dai et al. (2017). Germinating conidia were transferred aseptically to potato dextrose agar (PDA) andmalt extract agar (MEA) plates and grown at 16–30°C in alternating day and night light. Colony characters were observed and recorded after one week and at weekly intervals (Boehm et al., 2009; Chomnunti et al., 2014).

The type specimens were deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and the Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (KUN-HKAS), Yunnan, China. Ex-type living cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC 18-0250 and MFUCC 18-0236) and Kunming Institute of Botany Culture Collection (KUMCC 16-0037 and KUMCC 18-0009). Faces of Fungi and Index Fungorum numbers are registered as outlined in Jayasiri et al. (2015) and Index Fungorum (2019). New species are established based on guidelines of Jeewon and Hyde (2016).

3.2.2 DNA Extraction, PCR Amplification and Sequencing

Fungal mycelium was scraped off and transferred to a 1.5 ml micro-centrifuge tube using a sterilized lancet for genomic DNA extraction. The Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China) was used to extract fungal genomic DNA, following the protocols in the manufacturer's instructions.

DNA amplification was performed by polymerase chain reaction (PCR) using the following genes (ITS, LSU, SSU, RPB2 and TEF1-α). The primers ITS5 and ITS4 primer pairs were used to amplify the ITS and 5.8S regions of the rDNA gene (White et al., 1990); The primers LR0R and LR5 were used to amplify the partial ribosomal RNA for the 28S nuclear large subunit (LSU) (Vilgalys et al., 1990); NS1 and NS4 were used to amplify the partial ribosomal RNA for the 18S nuclear small subunit (SSU) (White et al., 1990); fRPB2-5F and fRPB2-7cR were used to amplify the partial RNA polymerase second largest subunit (RPB2) (Liu et al., 1999) and EF1-983F and EF1-2218R were used to amplify the translation elongation factor 1-alpha gene (TEF1-α) (Rehner et al., 2001).

The final volume of the PCR reaction was 25 µl, containing 1 µl of DNA template, 1 µl of each forward and reward primer, 12.5 µl of 2×Easy TaqPCR SuperMix (mixture of *EasyTaq*TM DNA Polymerase, dNTPs, and optimized buffer, Beijing TransGen Biotech Co., Ltd., Beijing, P.R. China) and 9.5 µl of ddH_2O. The PCR thermal cycling conditions of ITS, LSU, SSU and TEF1-α were as follows: 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 50 seconds, elongation at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. The PCR thermal cycle program for RPB2 was as follows: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 1 minute, annealing at 52°C for 2 minutes, elongation at 72°C for 90 seconds, and final extension at 72°C for 10 minutes. Purification and sequencing of PCR fragments with PCR primers mentioned above were carried out at Shanghai Majorbio Biopharm Technology Co., Ltd, China.

3.2.3 Sequence Alignment and Phylogenetic Analyses

Phylogenetic analyses were performed from single gene (LSU dataset) as well as based on a combined LSU, SSU, TEF1-α, RPB2 and ITS sequence dataset. Sequences generated from this study were analyzed with other similar sequences obtained from GenBank and those derived from recent publications (Ariyawansa et al., 2015; Crous et al., 2020; Hyde et al., 2019; Li et al., 2016; Li et al., 2017; Su et al., 2016; Su et al., 2018) (Table 3.1, 3.2, 3.3). The single gene alignment was performed by using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley, 2013) and manually aligned wherever necessary in MEGA version 7.0 (Kumar et al., 2016). Further analyses for the combined dataset were analyzed by maximum likelihood (ML) implemented in RAxML GUI v.0.9b2 (Silvestro & Michalak, 2010; Stamatakis, 2006, 2008, 2014) and Bayesian Inference (BI) criteria (Huelsenbeck & Ronquist, 2001; Huelsenbeck et al., 2001) following the methodology in Li et al. (2017).

The phylogram was represented in Treeview (Page, 1996) and drawn in Microsoft Power Point and converted to jpeg file in Adobe Photoshop version CS5 (Adobe Systems Inc., the United States). The new sequences were submitted in GenBank (Table 3.1, 3.2, 3.3). The alignment was deposited in TreeBASE (53) under the accession number 19927, 20535 and 25100.

3.3 Table of Contents

Table 3.1 Taxa used in the phylogenetic analysis of *Sporidesmioides* and their corresponding GenBank numbers

Species	Culture collection	GenBank accession numbers				References
		LSU	SSU	TEF1-α	RPB2	
Arthopyrenia salicis	CBS 368.94	AY779288	AY538333	KF443404	KF443397	Ahmed et al. (2014)
Arthopyrenia salicis	CBMAI1330	JN903536	–	–	–	Passarini et al. (2013)
Biatriospora mackinnonii	CBS 110022	GQ387614	GQ387553	–	–	Ahmed et al. (2014)
Biatriospora mackinnonii	CBS 674.75	GQ387613	GQ387552	–	–	
Biatriospora marina	CY 1228	GQ925848	GQ925835	GU479848	GU479823	Suetrong et al. (2009)
Byssosphaeria salebrosa	SMH 2387	GU385162	–	GU327748	–	Mugambi and Huhndorf (2009)
Dendryphion europaeum	CPC 23231	KJ869202	–	–	–	Crous et al. (2014)
Dendryphion europaeum	CPC 22943	KJ869203	–	–	–	
Elongatopedicellata lignicola	MFLUCC 15-0642	KX421368	KX421369	–	–	Ariyawansa et al. (2015)
Herpotrichia macrotricha	GKM 196N	GU385176	–	GU327755	–	Mugambi and Huhndorf (2009)
Hysterium angustatum	CBS 236.34	FJ161180	GU397359	FJ161096	–	Boehm et al. (2009)
Melanomma pulvis-pyrius	CBS 124080	GU456323	GU456302	GU456265	GU456350	Zhang et al. (2009)
Monotosporella tuberculata	CBS 256.84	GU301851	–	GU349006	–	Schoch et al. (2009)
Neooccultibambusa chiangraiensis	MFLUCC 12-0584	KU764699	KU712458	–	–	Doilom et al. (2016)

Table 3.1 (continued)

Species	Culture collection	GenBank accession numbers				References
		LSU	SSU	TEF1-α	RPB2	
Neoroussoella bambusae	MFLUCC 11-0124	KJ474839	–	KJ474848	KJ474856	Liu et al. (2014)
Nigrograna mackinnonii	E5202H	KJ605422	–	–	–	Shaw et al. (2015)
Occultibambusa bambusae	MFLUCC 13-0855	KU863112	KU872116	–	KU940170	Dai et al. (2016)
Occultibambusa bambusae	MFLUCC 11-0394	KU863113	KU872117	–	KU940171	
Occultibambusa fusispora	MFLUCC 11-0127	KU863114	–	–	KU940172	
Occultibambusa pustule	MFLUCC 11-0502	KU863115	KU872118	–	–	
Paradictyoarthrinium diffractum	MFLUCC12-0557	KP744497	–	–	KX437765	Liu et al. (2015)
Paradictyoarthrinium diffractum	MFLUCC 13-0466	KP744498	KP753960	–	KX437764	
Paradictyoarthrinium tectonicola	MFLUCC 12-0556	KP744499	–	–	–	
Paradictyoarthrinium tectonicola	MFLUCC 13-0465	KP744500	KP753961	–	KX437763	
Pleomassaria siparia	CBS 279.74	DQ678078	DQ678027	DQ677923	DQ677976	Tanaka et al. (2010)
Prosthemium stellar	CBS 126964	AB553781	AB553650	–	–	Wohlbach et al. (2011)
Roussoella angustior	MFLUCC 15-0186	KT281979	–	–	–	Ariyawansa et al. (2015)
Roussoella chiangraina	MFLUCC 10-0556	KJ474840	–	KJ474849	KJ474857	Liu et al. (2014)
Roussoella hysterioides	CBS 125434	AB524622	AB524481	AB539115	AB539102	Ahmed et al. (2014)
Roussoella intermedia	CBS170.96	KF443382	KF443390	–	–	
Roussoella magnatum	MFLUCC 15-0185	KT281980	–	–	–	Ariyawansa et al. (2015)
Roussoella nitidula	MFLUCC 11-0182	KJ474843	–	KJ474852	KJ474859	Liu et al. (2014)
Roussoella nitidula	MFLUCC 11-0634	KJ474842	–	KJ474851	KJ474858	

Table 3.1 (continued)

Species	Culture collection	GenBank accession numbers				References
		LSU	SSU	TEF1-α	RPB2	
Roussoella scabrispora	MFLUCC 11-0624	KJ474844	–	KJ474853	KJ474860	
Roussoella siamensis	MFLUCC 11-0149	KJ474845	KU872125	KJ474854	KJ474861	
Roussoella thailandica	MFLUCC 11-0621	KJ474846	–	–	–	
Roussoella pustulans	KT 1709	AB524623	AB524482	AB539116	AB539103	Tanaka et al. (2009)
Roussoellopsis macrospora	MFLUCC 12-0005	KJ474847	KJ739608	KJ474855	KJ474862	Liu et al. (2014)
Roussoellopsis tosaensis	KT1659	AB524625	AB524484	AB539117	AB539104	Tanaka et al. (2009)
Seriascoma didymospora	MFLUCC 11-0179	KU863116	KU872119	–	KU940173	Dai et al. (2016)
Seriascoma didymospora	MFLUCC 11-0194	KU863117	KU872120	–	KU940174	
Sporidesmium australiense	HKUCC 10833	DQ408554	–	–	DQ435080	Shenoy et al. (2006)
Sporidesmioides thailandica	MFLUCC 13-0840	KX437757	KX437759	KX437766	KX437761	In this study
Sporidesmioides thailandica	KUMCC 16-0012	KX437758	KX437760	KX437767	KX437762	
Torula herbarum	CBS 111855	KF443386	KF443391	KF443403	KF443396	Crous et al. (2015)
Torula herbarum	CBS 379.58	KF443383	KF443388	KF443400	GU456362	

Table 3.1 (continued)

Species	Culture collection	GenBank accession numbers				References
		LSU	SSU	TEF1-α	RPB2	
Torula hollandica	CBS 220.69	KF443384	KF443389	KF443401	KF443393	
Versicolorisporium triseptatum	JCM 14775	AB330081	–	–	–	Hatakeyama et al. (2008)

Note The newly generated sequences are indicated in red bold, while the type strains are in black bold. *CBS* Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; *CBMAI* Brazilian Collection of Environmental and Industrial Microorganisms, Campinas State University, Brazil; *CMW* Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; *CPC* Collection of Pedro Crous housed at CBS; *HKUCC* University of Hong Kong Culture Collection, Department of Ecology and Biodiversity, Hong Kong, China; *JCM* the Japan Collection of Microorganisms, Japan; *KUMCC* Kunming Institute of Botany Culture Collection, Chinese Science Academy, Kunming, China; *MFLUCC* Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; *GKM* G.K. Mugambi; *JK* J. Kohlmeyer; *KT* K. Tanaka; *SMH* S.M. Huhndorf

Table 3.2 Taxa used in the phylogenetic analysis of *Torula* and their corresponding GenBank numbers

Species	Culture collection	GenBank accession numbers				References
		LSU	SSU	TEF1-α	RPB2	
Arthopyrenia salicis	CBS 368.94	AY779288	AY538333	KF443404	KF443397	Ahmed et al. (2014)
Arthopyrenia salicis	CBMAI1330	JN903536	–	–	–	Passarini et al. (2013)
Biatriospora mackinnonii	CBS 110022	GQ387614	GQ387553	–	–	Ahmed et al. (2014)
Biatriospora mackinnonii	CBS 674.75	GQ387613	GQ387552	–	–	
Biatriospora marina	CY 1228	GQ925848	GQ925835	GU479848	GU479823	Suetrong et al. (2009)
Byssosphaeria salebrosa	SMH 2387	GU385162	–	GU327748	–	Mugambi and Huhndorf (2009)
Dendryphion aquatic	MFLUCC 15-0271	KU500573	KU500580	–	–	Su et al. (2016)
Dendryphion europaeum	CPC 23231	KJ869202	–	–	–	Crous et al. (2014)
Dendryphion europaeum	CPC 22943	KJ869203	–	–	–	
Dendryphion namum	HKAS 84010	KU500575	KU500582	AY383601	–	Su et al. (2016)
Dendryphion namum	HKAS 84012	KU500574	KU500581	–	–	
Dendryphion submersum	MFLUCC15-0271	KU500572	KU500579	–	–	
Elongatopedicellata lignicola	MFLUCC 15-0642	KX421368	KX421369	–	–	Ariyawansa et al. (2015)
Herpotrichia macrotricha	GKM 196N	GU385176	–	GU327755	–	Mugambi and Huhndorf (2009)
Hysterium angustatum	CBS 236.34	FJ161180	GU397359	FJ161096	–	Boehm et al. (2009)
Melanomma pulvis-pyrius	CBS 124080	GU456323	GU456302	GU456265	GU456350	Zhang et al. (2009)
Monotosporella tuberculata	CBS 256.84	GU301851	–	GU349006	–	Schoch et al. (2009)
Neooccultibambusa chiangraiensis	MFLUCC 12-0584	KU764699	KU712458	–	–	Doilom et al. (2016)
Neoroussoella bambusae	MFLUCC 11-0124	KJ474839	–	KJ474848	KJ474856	Liu et al. (2014)
Neotorula aquatic	MFLUCC 15-0342	KU500576	KU500583	–	–	Su et al. (2016)
Neotorula submerse	HKAS 92660	KX789217	–	–	–	Hyde et al. (2016)

Table 3.2 (continued)

Species	Culture collection	GenBank accession numbers				References
		LSU	SSU	TEF1-α	RPB2	
Nigrograna mackinnonii	E5202H	KJ605422	–	–	–	Shaw et al. (2015)
Occultibambusa bambusae	MFLUCC 13-0855	KU863112	KU872116	–	KU940170	Dai et al. (2016)
Occultibambusa bambusae	MFLUCC 11-0394	KU863113	KU872117	–	KU940171	
Occultibambusa fusispora	MFLUCC 11-0127	KU863114	–	–	KU940172	
Occultibambusa pustule	MFLUCC 11-0502	KU863115	KU872118	–	–	
Paradictyoarthrinium diffractum	MFLUCC12-0557	KP744497	–	–	KX437765	Liu et al. (2015)
Paradictyoarthrinium diffractum	MFLUCC 13-0466	KP744498	KP753960	–	KX437764	
Paradictyoarthrinium tectonicola	MFLUCC 12-0556	KP744499	–	–	–	
Paradictyoarthrinium tectonicola	MFLUCC 13-0465	KP744500	KP753961	–	KX437763	
Pleomassaria siparia	CBS 279.74	DQ678078	DQ678027	DQ677923	DQ677976	Tanaka et al. (2010)
Prosthemium stellar	CBS 126964	AB553781	AB553650	–	–	Wohlbach et al. (2011)
Roussoella angustior	MFLUCC 15-0186	KT281979	–	–	–	Ariyawansa et al. (2015)
Roussoella chiangraina	MFLUCC 10-0556	KJ474840	–	KJ474849	KJ474857	Liu et al. (2014)
Roussoella hysterioides	CBS 125434	AB524622	AB524481	AB539115	AB539102	Ahmed et al. (2014)
Roussoella intermedia	CBS170.96	KF443382	KF443390	–	–	
Roussoella magnatum	MFLUCC 15-0185	KT281980	–	–	–	Ariyawansa et al. (2015)
Roussoella neopustulans	MFLUCC 11-0609	KJ474841	KU872122	KJ474850	–	Liu et al. (2014)
Roussoella nitidula	MFLUCC 11-0182	KJ474843	–	KJ474852	KJ474859	
Roussoella nitidula	MFLUCC 11-0634	KJ474842	–	KJ474851	KJ474858	
Roussoella scabrispora	MFLUCC 11-0624	KJ474844	–	KJ474853	KJ474860	
Roussoella siamensis	MFLUCC 11-0149	KJ474845	KU872125	KJ474854	KJ474861	

Table 3.2 (continued)

Species	Culture collection	GenBank accession numbers				References
		LSU	SSU	TEF1-α	RPB2	
Roussoella thailandica	MFLUCC 11-0621	KJ474846	–	–	–	
Roussoella pustulans	KT 1709	AB524623	AB524482	AB539116	AB539103	Tanaka et al. (2009)
Roussoellopsis macrospora	MFLUCC 12-0005	KJ474847	KJ739608	KJ474855	KJ474862	Liu et al. (2014)
Roussoellopsis tosaensis	KT1659	AB524625	AB524484	AB539117	AB539104	Tanaka et al. (2009)
Seriascoma didymospora	MFLUCC 11-0179	KU863116	KU872119	–	KU940173	Dai et al. (2016)
Seriascoma didymospora	MFLUCC 11-0194	KU863117	KU872120	–	KU940174	
Sporidesmium australiense	HKUCC 10833	DQ408554	–	–	DQ435080	Shenoy et al. (2006)
Sporidesmioides thailandica	MFLUCC 13-0840	KX437757	KX437759	KX437766	KX437761	Li et al. (2016)
Sporidesmioides thailandica	KUMCC 16-0012	KX437758	KX437760	KX437767	KX437762	
Torula chiangmaiensis	KUMCC 16-0039	KY197856	KY197863	KY197876	–	In this study
Torula chromolaenae	KUMCC 16-0036	KY197860	KY197867	KY197880	KY197873	
Torula ficus	KUMCC 16-0035	KY197858	KY197865	KY197878	KY197871	
Torula ficus	KUMCC 16-0038	KY197859	KY197866	KY197879	KY197872	
Torula ficus	CBS 595.96	KF443385	KF443387	KF443402	KF443395	Crous et al. (2015)
Torula herbarum	CBS 111855	KF443386	KF443391	KF443403	KF443396	
Torula herbarum	CBS 379.58	KF443383	KF443388	KF443400	GU456362	
Torula hollandica	CBS 220.69	KF443384	KF443389	KF443401	KF443393	
Torula mackenziei	MFLUCC 13-0839	KY197861	KY197868	KY197881	KY197874	In this study
Torula masonii	KUMCC 16-0033	KY197857	KY197864	KY197877	KY197870	
Torula pluriseptata	MFLUCC 14-0437	KY197855	KY197862	KY197875	KY197869	
Torula masonii	CBS245.57	KR873289	–	–	–	Crous et al. (2015)

Table 3.2 (continued)

Species	Culture collection	GenBank accession numbers				References
		LSU	SSU	TEF1-α	RPB2	
Torula sp.	CBS245.57	KR873290	–	–	–	
Versicolorisporium triseptatum	JCM 14775	AB330081	–	–	–	Hatakeyama et al. (2008)

Note The newly generated sequences are indicated in underlined bold font, while the type strains are in black bold font. *CBS* Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; *CBMAI* Brazilian Collection of Environmental and Industrial Microorganisms, Campinas State University, Brazil; *CMW* Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; *CPC* Collection of Pedro Crous housed at CBS; *HKAS* Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (HKAS), Yunnan, China; *HKUCC* University of Hong Kong Culture Collection, Department of Ecology and Biodiversity, Hong Kong, China; *JCM* the Japan Collection of Microorganisms, Japan; *KUMCC* Kunming Institute of Botany Culture Collection, Chinese Science Academy, Kunming, China; *MFLUCC* Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; *GKM* G.K. Mugambi; *JK* J. Kohlmeyer; *KT* K. Tanaka; *SMH* S.M. Huhndorf.

Table 3.3 Taxa used in the phylogenetic analysis of *Torula hydei* and *Dendryphion hydei* and their corresponding GenBank numbers

Species	Culture collection/ Voucher no.	GenBank accession numbers					References
		ITS	LSU	SSU	RPB2	TEF1-α	
Arthopyrenia salicis	CBS 368.94	KF443410	AY779288	AY538333	KF443397	KF443404	[1]
Cycasicola goaensis	MFLUCC 17-0754	MG828885	MG829001	MG829112	–	MG829198	[57]
Dendryphion aquaticum	MFLUCC 15-0257	KU500566	KU500573	KU500580	–	–	[47]
Dendryphion comosum	CBS 208.69	MH859293	MH871026	–	–	–	[55]
Dendryphion europaeum	CPC 22943	KJ869146	KJ869203	–	–	–	[9]
Dendryphion europaeum	CPC 23231	KJ869145	KJ869202	–	–	–	
Dendryphion fluminicola	KUMCC15-0321	MG208160	MG208139	–	MG207971	MG207990	[48]
Dendryphion fluminicola	DLUCC 0849	MG208161	MG208140	–	MG207972	MG207991	
Dendryphion fluminicola	MFLUCC17-1689	NR_157490	MG208141	–	–	MG207992	
Dendryphion hydei	KUMCC 18-0009	MN061343	MH253927	MH253929	–	MH253931	**This study**
Dendryphion nanum	HKAS84010	KU500568	KU500575	KU500582	–	–	[47]
Dendryphion nanum	HKAS84012	KU500567	KU500574	KU500581	–	–	
Dendryphion nanum	MFLUCC 16-0987	MG208156	MG208135	–	MG207967	MG207986	[48]
Dendryphion submersum	MFLUCC15-0271	KU500565	KU500572	KU500579	–	–	[47]
Dendryphion submersum	KUMCC15-0455	MG208159	MG208138	–	MG207970	MG207989	[48]
Hobus wogradensis	CBS 141484	NR_147652	KX650546	NG_061253	KX650575	KX650521	[25]
Liua muriformis	KUMCC 18-0177	MK433599	MK433598	MK433595	MK426799	MK426798	[36]
Neooccultibambusa chiangraiensis	MFLUCC 12-0584	NR_154238	KU764699	KU712458	–	–	[12]
Neoroussoella bambusae	MFLUCC 11-0124	KJ474827	KJ474839	–	KJ474856	KJ474848	[31]
Neotorula aquatica	MFLUCC 15-0342	KU500569	KU500576	KU500583	–	–	[47]

Table 3.3 (continued)

Species	Culture collection/ Voucher no.	GenBank accession numbers					References
		ITS	LSU	SSU	RPB2	TEF1-α	
Neotorula submersa	HKAS 92660	NR_154247	KX789217	–	–	–	[20]
Nigrograna mackinnonii	E5202H	JK26415	KJ605422	JK264155	JK264156	JK264154	[40]
Nigrograna mackinnonii	CBS 110022	KF015653	KF015609	GQ387553	KF015704	KF407985	[1]
Nigrograna mackinnonii	CBS 674.75	NR_132037	GQ387613	GQ387552	–	–	
Nigrograna marina	CY 1228	–	GQ925848	GQ925835	GU479823	GU479848	[50]
Occultibambusa bambusae	MFLUCC 13-0855	KU940123	KU863112	KU872116	KU940170	KU940193	[11]
Ohleria modesta	WU36870	KX650562	–	–	KX650582	KX650533	[25]
Ohleria modesta	CBS141480	KX650563	–	KX650513	KX650583	KX650534	
Parathyridaria ramulicola	CBS 141479	NR_147657	KX650565	KX650514	KX650584	KX650536	[25]
Parathyridaria percutanea	CBS 868.95	NR_147631	NG_058022	NG_062999	KF366452	KF407987	[1]
Parathyridaria robiniae	MFLUCC 14-1119	KY511142	KY511141	–	–	KY549682	[52]
Roussoella chiangraina	MFLUCC 10-0556	NR_155712	KJ474840	–	KJ474857	KJ474849	[31]
Roussoella nitidula	MFLUCC 11-0182	KJ474835	KJ474843	–	KJ474859	KJ474852	[31]
Roussoella scabrispora	MFLUCC 11-0624	KJ474836	KJ474844	–	KJ474860	KJ474853	[31]
Rostriconidium aquaticum	KUMCC 15-0297	MG208165	MG208144	–	MG207975	MG207995	[48]
Rostriconidium aquaticum	MFLUCC 16-1113	MG208164	MG208143	–	MG207974	MG207994	
Roussoellopsis macrospora	MFLUCC 12-0005	KJ739604	KJ474847	KJ739608	KJ474862	KJ474855	[31]
Roussoellopsis tosaensis	KT1659	–	AB524625	AB524484	AB539104	AB539117	[51]
Sporidesmium australiense	HKUCC 10833	–	DQ408554	–	DQ435080	–	[41]
Sporidesmioides thailandica	MFLUCC 13-0840	MN061347	NG_059703	NG_061242	KX437761	KX437766	[29]
Sporidesmioides thailandica	KUMCC 16-0012	MN061348	KX437758	KX437760	KX437762	KX437767	

Table 3.3 (continued)

Species	Culture collection/ Voucher no.	GenBank accession numbers					References
		ITS	LSU	SSU	RPB2	TEF1-α	
Thyridaria broussonetiae	CBS 141481	NR_147658	KX650568	NG_063067	KX650586	KX650539	[25]
Thyridaria broussonetiae	CBS 121895	KX650567	KX650567	–	KX650585	KX650538	
Thyridariella mahakashae	NFCCl 4215	MG020435	MG020438	MG020441	MG020446	MG023140	[13]
Thyridariella mangrovei	NFCCl 4213	MG020434	MG020437	MG020440	MG020445	MG020443	[13]
Torula acaciae	CPC 29737	NR_155944	NG_059764	–	KY173594	–	[10]
Torula aquatica	DLUCC 0550	MG208166	MG208145	–	MG207976	MG207996	[48]
Torula aquatica	MFLUCC16-1115	MG208167	MG208146	–	MG207977	–	
Torula breviconidiophora	KUMCC 18-0130	MK071670	MK071672	MK071697	–	MK077673	[22]
Torula chiangmaiensis	KUMCC 16-0039	MN061342	KY197856	KY197863	–	KY197876	[30]
Torula chromolaenae	KUMCC 16-0036	MN061345	KY197860	KY197867	KY197873	KY197880	[30]
Torula fici	CBS 595.96	KF443408	KF443385	KF443387	KF443395	KF443402	[8]
Torula fici	KUMCC 15-0428	MG208172	MG208151	–	MG207981	MG207999	[48]
Torula fici	KUMCC 16-0038	MN061341	KY197859	KY197866	KY197872	KY197879	[30]
Torula gaodangensis	MFLUCC 17-0234	MF034135	NG_059827	NG_063641	–	–	[21]
Torula goaensis	NFCCL 4040	NR_159045	NG_060016	–	–	–	[37]
Torula herbarum	CPC 24414	KR873260	KR873288	–	–	–	[8]
Torula hollandica	CBS 220.69	NR_132893	NG_064274	KF443389	KF443393	KF443401	[8]
Torula hydei	KUMCC 16-0037	MN061346	MH253926	MH253928	–	MH253930	This study
Torula mackenziei	MFLUCC 13-0839	MN061344	KY197861	KY197868	KY197874	KY197881	[30]
Torula masonii	CBS245.57	NR_145193	NG_058185	–	–	–	[8]
Torula masonii	DLUCC 0588	MG208173	MG208152	–	MG207982	MG208000	[47]

Table 3.3 (continued)

Species	Culture collection/ Voucher no.	GenBank accession numbers					References
		ITS	LSU	SSU	RPB2	TEF1-α	
Torula masonii	KUMCC 16-0033	MN061339	KY197857	KY197864	KY197870	KY197877	[30]
Torula pluriseptata	MFLUCC 14-0437	MN061338	KY197855	KY197862	KY197869	KY197875	[30]
Torula polyseptata	KUMCC 18-0131	MK071671	MK071673	MK071698	–	MK077674	[22]
Torula sp.	CBS246.57	KF443411	KR873290	–	–	–	[8]
Torula sp.	KUMCC 19-0112	******	****	****	*****	******	In prep.

Note The newly generated sequences are indicated in bluebold font, while the type strains are in black bold font. Abbreviations: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CPC: Collection of Pedro Crous housed at CBS; DLUCC: Dali University Culture Collecting Center, Dali, Yunnan, China. HKAS: Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (HKAS), Yunnan, China; HKUCC: University of Hong Kong Culture Collection, Department of Ecology and Biodiversity, Hong Kong, China; KUMCC: Kunming Institute of Botany Culture Collection, Chinese Science Academy, Kunming, China; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; NFCCI: National Fungal Culture Collection of India; KT: K. Tanaka.

Table 3.4 Morphological differences among taxa in *Torula*

Species name	Conidiophores	Conidiogenous cells	Conidia	Reference
Torula chiangmaiensis	8–12.6(–16.1) × 4.5–5.2 µm, macronematous, mononematous, consisting of 2–3 ampulliform cells, subhyaline to paler brown.	3.4–6.5 × 4.8–7.6 µm, polyblastic, smooth to minutely verruculose, ellipsoid to coronal, dark brown to black.	(25.5–)43.6–70(–86.5) × 5.6–7.8 µm, 4–12-septate, composed of moniliform cells, light brown to greyish-brown.	In this study
Torula chromolaenae	5–6.3 × 3.5–4.6 µm, macronematous, mononematous, consisting of an ellipsoid cell, light brown.	(3.5–)4.5–5.1 × 4.8–5.6 µm, polyblastic, smooth to minutely verruculose, doliiform, light brown to brown, paler at apex.	12.1–16.5 × (3.6–)4.1–5 µm, 2–3-septate, rounded at both ends, catenated, light brown to brown, often paler at apex,	In this study
Torula ficus	9.4–12.5 × 3.7–4.5 µm, macronematous, mononematous, consisting of 1–2 ampulliform cells, paler brown to brown.	5.4–8.5 × 5–7.4 µm, polyblastic, smooth to minutely verruculose, ellipsoid to coronal, dark brown to black.	12–20 × 4.6–6.6 µm, 2–4-septate, rounded and paler coloured at apex, light brown to greyish-brown.	Crous et al. (2015) In this study
Torula herbarum	Reduced to conidiogenous cells, or with one supporting cell, mononematous, brown.	5–8 × 6–8 µm, mono- to polyblastic, verruculose at apex, doliiform to ellipsoid, brown	(15–)16–24 × 6–7 µm, 3–4-septate, composed of subglobose cells, brown, apex pale brown.	Crous et al. (2015)
Torula hollandica	Reduced to conidiogenous cells.	6–7 × 6–7 µm, mono- to polyblastic, verruculose, doliiform, brown.	13–26 × 6–7 µm, predominantly 4-septate, in branched chains, composed of subglobose cells, brown, apex pale brown, branching cell in conidial chain darker brown than other cells.	Crous et al. (2015)

Table 3.4 (continued)

Species name	Conidiophores	Conidiogenous cells	Conidia	Reference
Torula mackenziei	3–4.3 × 3.4–3.7 µm, macronematous, mononematous, consisting of 1–2 ellipsoid cells or reduced to conidiogenous cells, light brown.	(3.6–)4.3–4.7 × 4–4.8 µm, polyblastic, smooth to minutely verruculose, globose to ellipsoid, dark brown to black.	9.4–18.5 × (3.8–)4.4–4.7 µm, 2–3-septate, composed moniliform cells, rounded at both ends, catenated, light brown to greyish-brown, often paler at apex.	In this study
Torula masonii	7.6–20 × 3.5–5.4 µm macronematous, mononematous, consisting of 2–3 cells, subcylindrical, subhyaline to paler brown.	6.4–7.2× 4.8–6.6 µm, polyblastic, dark, smooth to minutely verruculose, ellipsoid to coronate, brown to black.	15.6–25.4 µm long × 6.8–11.5 µm wide, 2–5-septate, often with a dark coronal conidiogenous cell at apex, greyish-brown.	Crous et al. (2015) In this study
Torula pluriseptata	(2.8–)3–4.3 × 2.5–3 µm, macronematous, mononematous, consisting of 1–2 subglobose cells or reduced to 1-celled conidiogenous cells, pale brown	(3–)3.2–3.5 × 3.8–4.6 µm, polyblastic, smooth to minutely verruculose, doliiform to ellipsoid, dark brown to black.	(14–)23.5–36(–55) × (3.3–)3.6–4.4 µm, 3–10-septate, composed of subglobose cells, dark brown.	In this study

Table 3.5 Synopsis of morphological features of *Dendryphion* species discussed in this study

Species	Size (µm)			Conidial septation	Host/substrate and habitat	Distribution	Reference
	Conidiophores	Conidiogenous cells	Conidia				
Dendryphion aquaticum	250–285 × 7.5–11.5	5–9×4–6	22–33 × 6.5–7.5	3–6	Decaying wood submerged in stream	China (Yunnan)	Su et al. (2016)
Dendryphion comosum	Up to 400 × 9–14	Up to16 × 5–8	9–65 × 5–9	1–5(−9)	Various hosts and substrates	Cosmopolitan distribution	Farr and Rossaman (2019), Seifert et al. (2011)
Dendryphion europaeum	10–15 × 5–7	6–10 × 5–7	(15–)20–28(–33) × (6 –)7	(2–)3(–5)	*Hedera helix*, *Heracleum sphondylium*	Germany, Netherlands	Crous et al. (2014)
Dendryphion fluminicola	114–176 × 7–10	N/A	31–46 × 8–9	2–6	Decaying wood submerged in a stream in Cangshan Mountain,Lancang River and Jinsha River	China (Yunnan)	Shaw et al. (2015)
Dendryphion hydei	260–395.4 × 7.8–13.8	4.5–5 × 5.2–5.5	4.5–5 × 5.2–5.5	4.8–5.1	Branch litter of of *Bidens pilosa*	Thailand	This study
Dendryphion nanum	52–64 × 6.5–8.5	13–19 × 6–8	56.7–74.5 × 10–12	3–11	Various hosts and substrates	Cosmopolitan distribution	Su et al. (2018)
Dendryphion submersum	210–335 × 3.5–4.5	11–15 × 4.5–6.5	15–25 × 5–7	2–5	Decaying wood submerged in stream	China (Yunnan)	Su et al. (2018)

Table 3.6 Synopsis of morphological features of the genera in Torulaceae

Genus	Morphological features			Reference
	Conidia	Conidiophores	Conidiogenous cells	
Dendryphion	Acropleurogenous, catenate or solitary,simple or branched, cylindrical to obclavate, or cheiroid, paleto mid brown or olivaceous brown, multi-septate, smooth or verrucose	Macronematous, mononematous, branched at the apex, brown to black, smooth or with verruculose at the upper part, with paler branches	Mono- or polytretic, integrated, terminal and intercalary on branches, sympodial, clavate, cylindrical or doliiform, cicatrized, with large and dark scars.	Su et al. (2016, 2018)
Neotorula	Acrogenous, in chains, clavate to subcylindrical, septate, dark bands at the septa, pale green when young, brown when mature, verruculose	Macronematous, mononematous, cylindrical, 3-6-septate, with one or several short branches nea the apex, smooth, dark brown, paler towards the apex,	Tretic, with a distinct pore, integrated, terminal, pale brown or subhyaline, doliiform or lageniform	Su et al. (2016)
Rostriconidium	Solitary, pyriform to rostrate, dark brown to black, with a thick, black truncate scar at the base and pale pigment cell above the scar, narrowly cylindrical and obtuse at the apex	Macronematous, mononematous, single or caespitose, septate, smooth, brown or dark brown, unbranched, thick-walled, cylindrical, arising from a stromatic base.	Monotretic or polytretic, integrated, terminal, cylindrical, dark brown	Su et al. (2018)
Sporidesmioides	Acrogenous, solitary, pyriform to rostrate, ampulliform to obclavate, truncate at the base, septate, brown to dark brown, with paler at the upper end cells, smooth or verruculose to echinulate	Macronematous, mononematous, scattered,unbranched, straight to curved, sometimes percurrently proliferating	Polyblastic, integrated, indeterminate or percurrent, terminal, sometimes intercalary sympodial, dark and prominent, cylindrical or doliiform.	Li et al. (2016)
Torula	Acrogenous, in branched chains, subcylindrical to cylindrical, brown, constricted at septa, smooth to verrucose, conidial cells subglobose	Micronematous, reduced to conidiogenous cells, or with a brown supporting cell	Mono- to polyblastic, solitary on mycelium, doliiform to ellipsoid or clavate, cupulate, brown, smooth to verruculose,	Crous et al. (2015), Su et al. (2016), Li et al. (2017)

Table 3.7 Taxonomy of *Sporidesmium* and some sporidesmium-like taxa in Ascomycota

Species	Family	Order	Class	References
Acroconidiellina arecae	*Incertae sedis*	*Incertae sedis*	Dothideomycetes	Ellis (1971, 1976), Bhat (2010)
Eupelte amicta	*Asterinaceae*	Asterotexiales		Sivanesan (1984), Shenoy et al. (2006)
Eupelte rapaneae				
Taeniolella punctata	*Incertae sedis*			Ertz et al. (2016)
Taeniolella hawksworthiana				
Taeniolella pyrenulae				
Taeniolella toruloides				
Taeniolella exilis	*Kirschsteiniotheliaceae*	*Incertae sedis*		
Sporidesmium pachyanthicola	*Capnodiaceae*	Capnodiales		Shenoy et al. (2006)
Sporidesmium australiense	*Roussoellaceae*	Pleosporales		
Sporidesmium obclavatulum	*Incertae sedis*			
Sporidesmium tengii				
Sporidesmiella fusiformis	*Melanomataceae*			
Sporidesmioides thailandica	*Torulaceae*			This study
Dendryphion aquaticum				Crous et al. (2015), Su et al. (2016)
Dendryphion europaeum				
Dendryphion nanum				
Dendryphion submersum				
Neotorula aquatica				
Ellisembia abscendens	*Chaetosphaeriaceae*	Chaetosphaeriales	Sordariomycetes	Shoemaker and White (1985),
Ellisembia bambusicola				Wu and Zhang (2005)

Table 3.7 (continued)

Species	Family	Order	Class	References
Pseudosporidesmium knawiae	*Incertae sedis*	*Incertae sedis*		Su et al. (2016)
Sporidesmium aquaticum	*Sporidesmiaceae*			
Sporidesmium fluminicola				
Sporidesmium macrurum				
Sporidesmium malabarica				
Sporidesmium minigelatinosa				
Sporidesmium parvum				
Sporidesmium submersum				
Sporidesmium aquaticum	*Sporidesmiaceae*	*Incertae sedis*	Sordariomycetes	Su et al. 2016
Taeniolella alta	*Incertae sedis*	Diaporthales		Ertz et al. 2016
Taeniolella phialosperma		Sordariales		
Sterigmatobotrys rudis		*Incertae sedis*	*Incertae sedis* Ascomycota	

3.4 Results

Bayesian Inference (BI) and maximum likelihood (ML) analyses of the combined dataset were performed to determine the placement of our new taxa and infer relationships at the intrageneric level as well as resolving the phylogenetic relationships of the core families in Pleosporales. The phylogenetic trees obtained from BI and ML analyses resulted in trees with largely similar topologies and also similar to those generate from previous studies based on maximum likelihood analysis, The best scoring RAxML tree are shown

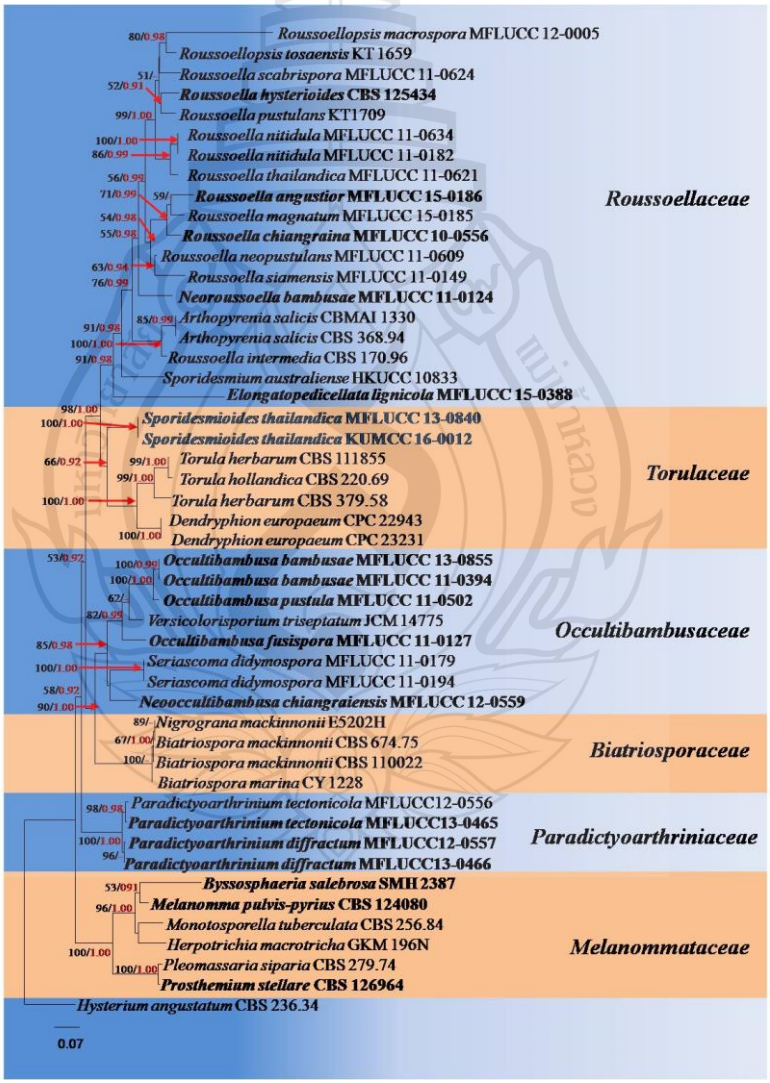


Figure 3.1 Phylogram generated from maximum likelihood analysis of sequences in *Sporidesmioides*

Figure 3.1 Phylogenetic construction using RAxML based analysis of a combined LSU, SSU, TEF1-α and RPB2 dataset. Bootstrap support values for maximum likelihood (ML, black), equal or greater than 50% and Bayesian posterior probabilities (PP, red) equal to or greater than 0.90 are shown above the nodes. The tree is rooted to *Hysterium angustatum.* The type strains are in black bold and the newly generated sequences are indicated in blue bold.

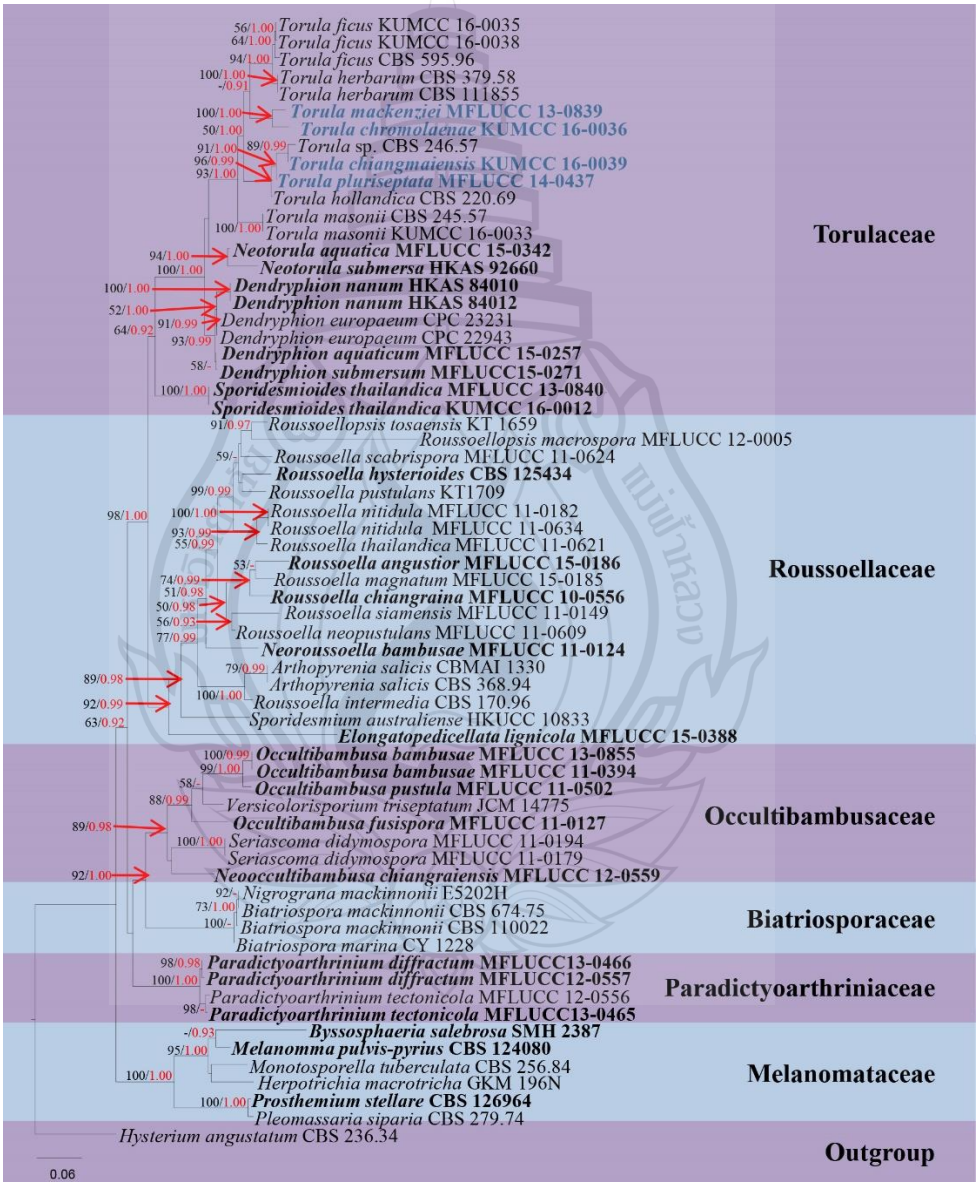


Figure 3.2 Phylogram generated from maximum likelihood analysis of sequences in Torulaceae

Figure 3.2 Phylogenetic construction using RAxML-based analysis of a combined LSU, SSU, TEF1-α and RPB2 dataset. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 50 % and Bayesian posterior probabilities (PP, red) equal to or greater than 0.90 are shown above the nodes. The tree is rooted to *Hysterium angustatum*. The type strains are in black bold and the newly generated sequences are indicated in blue bold.

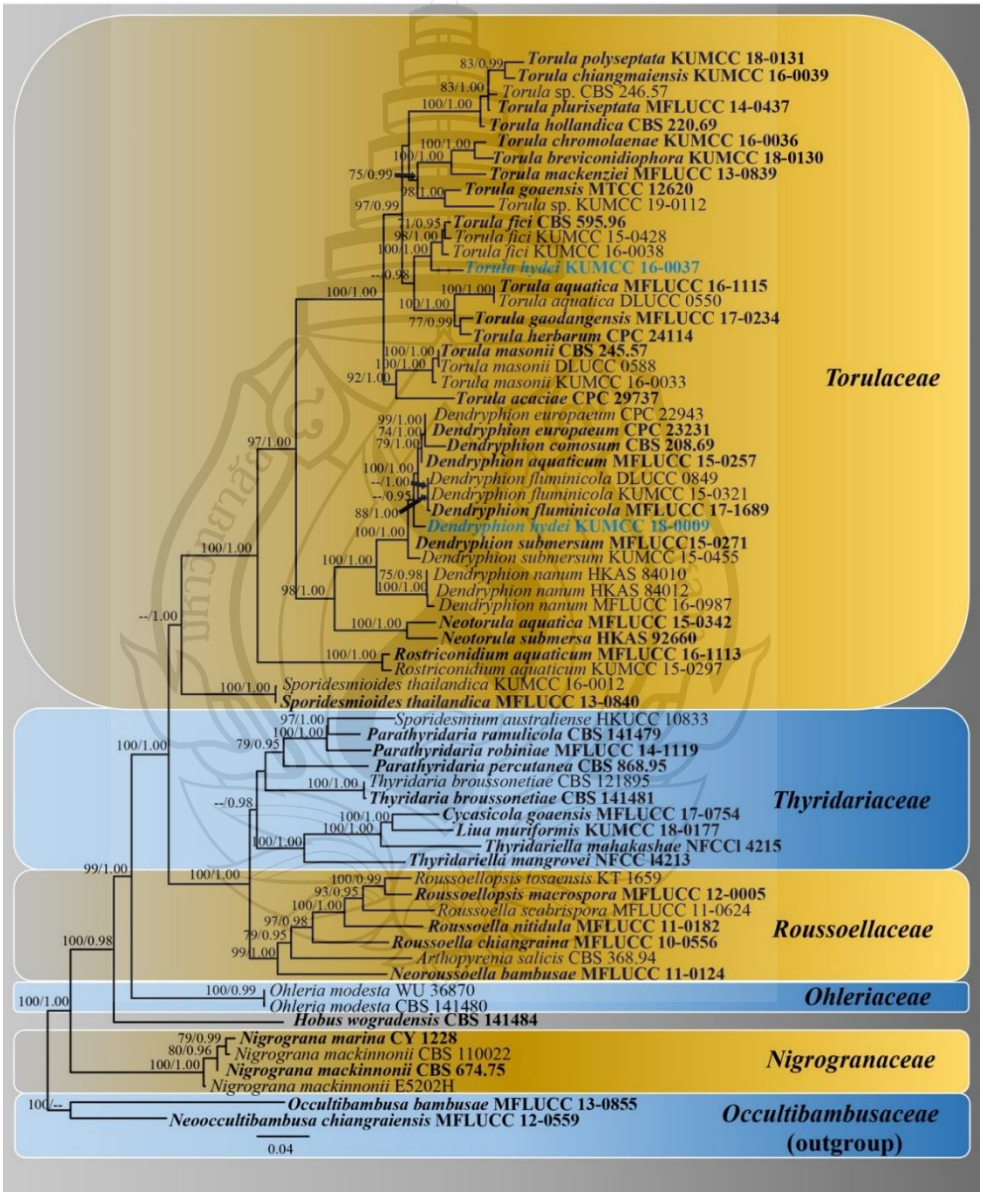


Figure 3.3 Phylogram generated from maximum likelihood analysis of sequences in Torulaceae (Pleosporales)

Figure 3.3 Phylogenetic construction using RAxML-based analysis of a combined LSU, SSU, TEF1-α, RPB2 and ITS DNA sequence dataset. Bootstrap support values for maximum likelihood (ML) equal to or greater than 70% and Bayesian posterior probabilities (PP) equal to or greater than 0.95 are shown as "ML/PP" above the nodes. The tree is rooted to *Occultibambusa bambusae* (MFLUCC 13-0855) and *Neooccultibambusa chiangraiensis*(MFLUCC 12-0559). The type strains are in black bold and the newly generated sequences are indicated in blue bold.

3.4.1 *Sporidesmioides* J.F. Li, R. Phookamsak & K.D. Hyde, gen. nov

MycoBank number: MB 817934, Facesoffungi number: FoF 02515.

Etymology: Named after its conidial similarity to *Sporidesmium*.

Saprobic or *parasitic* on plant litter of wood or herbs. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. *Conidiophores* macronematous, mononematous, straight or curved, septate, smooth, brown or dark brown, unbranched, erect, single or caespitose, smooth, thick-walled, brown to dark brown, septate, cylindrical, arising from a stromatic base akin to pseudoparenchymatous stromata. *Conidiogenous cells* polyblastic, integrated, indeterminate or percurrent, terminal, sometimes becoming intercalary sympodial, often dark and prominent, cylindrical or doliiform. *Conidia* acrogenous, solitary, dry, pyriform, rostrate, straight or curved, fusiform to obclavate, conico-truncate at the base, septate, smooth, brown to dark brown, with paler cells at end, truncate at the base, smooth or verruculose to echinulate, dark brown, subhyaline at the apex, thick-walled. *Conidial secession* schizolytic.

Type species: *Sporidesmioides thailandica* J.F. Li, R. Phookamsak & K.D. Hyde, sp. nov.

Notes: *Sporidesmioides* is characterized by straight or curved, septate, dematiaceous conidiophores arising from a stromatic base, polyblastic conidiogenous cells and fusiform, septate and dark brown conidia with paler cells at ends. *Sporidesmioides thailandica* superficially resembles species in the genus *Sporidesmium* and *Sporidesmium sensu lato* in conidial shape. However, *Sporidesmioides* differs by its large-sized conidia with paler cells and a hyaline, flat flexuous sheath surrounding the tip. Besides, *Sporidesmioides thailandica* forms a separate clade sister to *Roussoellaceae* based on combined LSU, SSU, TEF1-α and RPB2 sequence data. Based on the sequence data and

the distinct morphology, we treat this as a new genus in *Torulaceae*, Pleosporales, Dothideomycetes.

3.4.2 *Sporidesmioides thailandica* J.F. Li, R. Phookamsak & K.D. Hyde, sp. nov.

MycoBank number: MB 817935, Facesoffungi number: FoF00455

Etymology: Named after the country where this fungus was collected, Thailand.

Holotype*:* MFLU14–0827

Saprobic on stem of a dead herb. Sexual morph: Undetermined. Asexual morph: *Mycelium* immersed on the substrate, composed of septate, branched, brown, smooth, grey-coloured hyphae. *Conidiophores* (100–)120–200 µm long × 7–9(–9.5) µm diam ($\bar{x}$ = 178.6 × 8.5 µm, n = 20), macronematous, mononematous, scattered, dark brown to black, slightly paler at the apex, smooth, thick-walled, septate, unbranched, straight to curved, sometimes percurrently proliferating, arising from a stromatic base. *Conidiogenous cells* (13–)14.2–25.5 µm long × 6.6–8 µm diam ($\bar{x}$ = 16.7 × 7.8 µm, n = 20), polyblastic, integrated, terminal, later becoming intercalary, cylindrical, dark brown, smooth, with pustulate, black, conidiogenous scars on conidial secession. *Conidia* 62.5–80(–97) µm long × (16–)20–25 µm diam ($\bar{x}$ = 75.6 × 24.7 µm, n = 20) solitary, simple, dark brown to black, fusiform, minutely verruculose, 6–7-septate, slightly constricted at some septa, with a thick, black, truncate scar at the base, narrower, flattened and paler towards the tip, with a flap-like, hyaline, thick sheath at the tip, with densely granulate cells.

Cultural characteristics: Conidia germinating on PDA within 14 h and germ tubes produced from the apex. Colonies growing on PDA, reaching 5 cm in 10 days at 30ºC, mycelium partly superficial, partly immersed, slightly effuse, radially striated, with irregular edge, light-grey to grey; sexual or asexual spores not formed within 60 days.

Material examined: THAILAND, Chiang Rai Provice, Doi Mae Sailong, on stem of a dead herb, 3 October 2013, Samantha C. Karunarathne, H-3 (MFLU 14-0827, holotype), ex-type living culture MFLUCC 13-0840, KUMCC 16-0012; (isotype in HKAS, under the code of HKAS 85792)

Notes: *Sporidesmioides*, described herein as a monotypic genus and represented by *S. thailandica*, is based on a collection on stem of a dead herb in northern Thailand. This taxon is characterized by its large fusiform, septate and dark brown conidia with paler apical cells and a conspicuous, hyaline, flexuous, sheath at the tip, straight or curved,

septate, dematiaceous, conidiophores with thick wall and dark-coloured, protuberant polyblastic conidiogenous loci. Unlike other species in *Sporidesmium*, the conidiophores in this taxon are fertile all along the upper half. *Sporidesmioides* superficially resembles species in the genus *Sporidesmium* and *Sporidesmium sensu lato* in shape and schizolytic conidial seccession, but differs by larger conidia, polyblastic conidiogenesis and the hyaline flexuous sheath at conidial apex.



Figure 3.4 Morphology of *Sporidesmioides thailandica* (HKAS 85792)

Figure 3.4 (HKAS 85792) a. Colonies on stem of a dead herb. b. Pseudoparenchymatous stromata. c–g. Conidiophores bearing conidia. h–j. Conidia. k. Germinated conidia. Scale bars: a. 500 µm, b. 100 µm, c–j. 20 µm

3.4.3 *Torula pluriseptata* J.F. Li, Phookamsak, Camporesi & K.D. Hyde, sp. nov.

MycoBank number: MB 819534, Facesoffungi number: FOF 02709.

Etymology: Named after its multi-septate conidia.

Holotype*:* MFLU 16-2814

Saprobic on stem of a dead branch of *Clematis vitalba* Linn. (Ranunculaceae). Sexual morph: Undetermined. Asexual morph: *Colonies* effuse on host, black, powdery. *Mycelium* partly immersed, composed of septate, branched, smooth, pale brown hyphae. *Conidiophores* (2.8–)3–4.3 µm long × 2.5–3 µm diam ($\bar{x}$ = 3.8 × 2.8 µm, n = 10), macronematous, mononematous, solitary, erect, pale brown, verruculose, thick-walled, consisting of 1–2 cells or reduced to conidiogenous cells, subcylindrical to subglobose, arising from prostrate hypha. *Conidiogenous cells* (3–)3.2–3.5 µm long × 3.8–4.6 µm diam ($\bar{x}$ = 3.4 × 4.1 µm, n = 10), polyblastic, terminal, dark brown to black, smooth to minutely verruculose, thick-walled, doliiform to ellipsoid. *Conidia* (14–)23.5–36(–55) µm long × (3.3–)3.6–4.4 µm wide ($\bar{x}$ = 30.5 × 4.1 µm, n = 20) solitary to catenate, acrogenous, simple, phragmosporous, dark brown, with apical cell pale brown, minutely verruculose, 3–10-septate, rounded at both ends, composed of subglobose cells, slightly constricted at some septa, chiefly subcylindrical. *Conidial secession* schizolytic.

Cultural characteristics: Conidia germinating on PDA within 14 hrs and germ tubes produced from the tip cell. Colonies growing on PDA, reaching 5 cm in 10 days at 30ºC, mycelium partly immersed to superficial, slightly effuse, hairy, with regular edge, pale to medium brown; sexual or asexual spores not formed within 60 days.

Material examined: ITALY, Forlì-Cesena [FC], Trivella di Predappio, on a dead branch of *Clematis vitalba* Linn. (Ranunculaceae), 19 February 2014, Erio Camporesi, IT 1729 (MFLU 16-2814, holotype), ex-type living culture MFLUCC 14-0437, KUMCC 16-0034; (isotype in HKAS, under the code of HKAS 96294).

Notes: *Torula pluriseptata* is distinct from the other species treated here. *Torula pluriseptata* resembles *T. hollandica* in having subglobose cells and catenate conidia (Crous et al. 2015), but differs in the number of septa (3–10-septate vs. 2–4-septate), slender

conidia (23.5–36 × 3.6–4.4 µm vs. 13–26 × 6–7 µm) and smaller conidiogenous cells (3.2–3.5 × 3.8–4.6 µm vs. 6–7 × 6–7 µm).

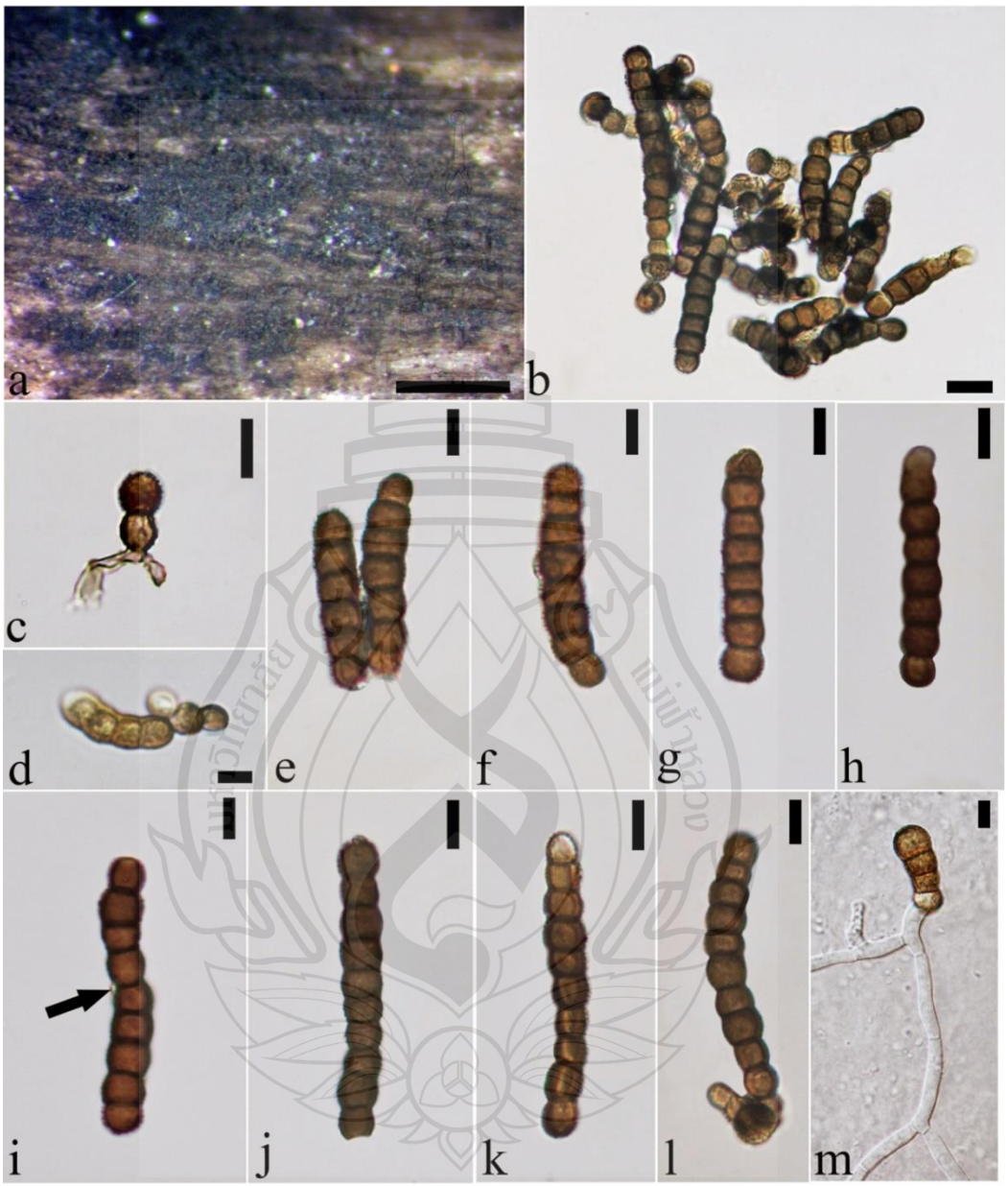


Figure 3.5 Morphology of *Torula pluriseptata* (MFLU 16-2814)

Figure 3.5 a Colonies on dead branch of *Clematis vitalba*. b Mass of conidia. c Conidiophores with conidiogenous cell. d Budding on conidia. e–h, j–l Conidia. i Conidia in catenated chains. m Germinated conidia. Scale bars a 500 µm, b 10 µm, c–m 5 µm

3.4.4 *Torula chiangmaiensis* J.F. Li, Phookamsak & K.D. Hyde, sp. nov.

MycoBank number: MB 819535, Facesoffungi number: FOF 02710

Etymology: Named after the location from where it was collected, Chiang Mai, Thailand.

Holotype: MFLU 16-2815

Saprobic on a branch of dead herbaceous plant. Sexual morph: Undetermined. Asexual morph: *Colonies* effuse on host, black. *Mycelium* partly immersed to superficial on the substrate, composed of septate, branched, smooth, light brown hyphae. *Conidiophores* 8–12.6(–16.1) µm long × 4.5–5.2 µm wide ($\bar{x}$ = 11.8 × 4.9 µm, n = 20), macronematous, mononematous, solitary, erect, subhyaline to paler brown, smooth to minutely verruculose, thick-walled, consisting of 2–3 cells, without apical branches, with ampulliform cells, arising from prostrate hypha. *Conidiogenous cells* 3.4–6.5 µm long × 4.8–7.6 µm diam ($\bar{x}$ = 5.3 × 6.8 µm, n = 20), polyblastic, terminal, dark brown to black, smooth to minutely verruculose, thick-walled, ellipsoid to coronal. *Conidia* (5.4–)25.5–70(–86.5) µm long × 5.6–7.8 µm diam ($\bar{x}$ = 59.6 × 6.6 µm, n = 20) solitary to catenate, acrogenous, phragmosporous, light brown to greyish-brown, smooth to minutely verruculose, 4–12-septate, composed of moniliform cells, slightly constricted at some septa, chiefly subcylindrical. *Conidial secession* schizolytic.

Cultural characteristics: Conidia germinating on PDA within 14 hrs and germ tubes produced from terminal cells. Colonies growing on PDA, reaching 5 cm in 21 days at 30ºC, mycelium partly immersed to superficial, slightly effuse, hairy, with regular edge, maroon to yellowish-brown; sexual morph not formed within 60 days.

Material examined: THAILAND, Chiang Mai, Mae Taeng (Mushroom Research Center), on a branch of dead herbaceous plant, 12 March 2016, Junfu Li, H-MRC45 (MFLU 16-2815, holotype), ex-type living culture KUMCC 16-0039, MFLUCC; (isotype in HKAS, under the code of HKAS 96295)

Notes: *Torula chiangmaiensis* described herein as a new species, resembles *Bahusaganda ambrosiae* in having conidia composed of moniliform cells and coronate conidiogenous cells (Crane and Miller, 2016). However, conidia in *Torula chiangmaiensis* are catenate, with less septa and smaller (4–12-septate, 25.5–70 × 5.6–7.8 µm vs. 6–15-septate, 55–110 × 8–13 µm). Phylogenetic analyses support *T. chiangmaiensis* as a new species in *Torula* as well.

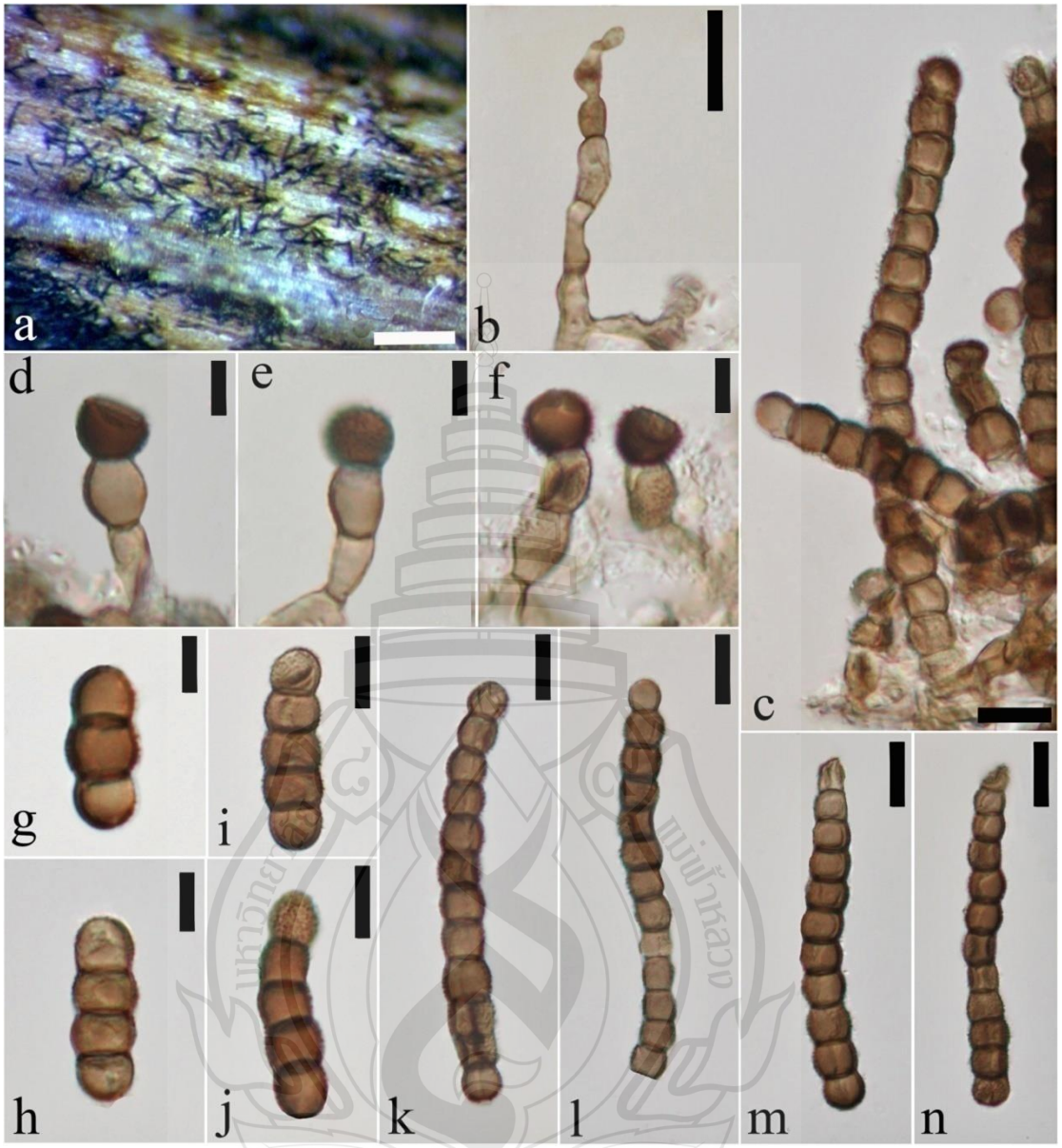


Figure 3.6 Morphology of *Torula chiangmaiensis* (MFLU 16-2815)

Figure. 3.6 a Colonies on branch of dead herbaceous plant. b Hyphae. c Conidia in mass. d–f Conidiophores with conidiogenous cells. g–h Immature conidia. i–n Conidia. Scale bars a 200 µm, b 20 µm, c, k–n 10 µm, i–j 7 µm, d–h 5 µm

3.4.5 *Torula masonii* P.W. Crous, IMA Fungus 6 (1): 192 (2015)

MycoBank number: MB812806, *Facesoffungi number*: FOF 02711

Saprobic on a dead branch of *Iris germanica* Linn. (Iridaceae). Sexual morph: Undetermined. Asexual morph: *Colonies* effuse on host, black, powdery. *Mycelium* immersed on the substrate, composed of septate, branched, smooth, subhyaline hyphae. *Conidiophores* 7.6–20 µm long × 3.5–5.4 µm wide ($\bar{x}$ = 16.8 × 4.5 µm, n = 20), macronematous, mononematous, solitary, straight to flexuous, subhyaline to paler brown, smooth to minutely verruculose, thick-walled, consisting of 2–3 cells, without apical branches, subcylindrical, arising from prostrate hypha. *Conidiogenous cells* 6.4–7.2 µm long × 4.8–6.6 µm diam ($\bar{x}$ = 6.6 × 5.3 µm, n = 20), polyblastic, terminal, dark brown to black, smooth to minutely verruculose, thick-walled, ellipsoid to coronate. *Conidia* 15.6–25.4 µm long × 6.8–11.5 µm wide ($\bar{x}$ = 21.6 × 9.5 µm, n = 20) catenated, acrogenous, phragmosporous, greyish-brown, smooth to minutely verruculose, 2–5-septate, often with a dark coronal conidiogenous cell at apex, chiefly subcylindrical. *Conidial secession* schizolytic.

Cultural characteristics: Conidia germinating on PDA within 14 hrs and germ tubes produced from the apex. Colonies growing on PDA, reaching 5 cm in 15 days at 30ºC, mycelium partly immersed to superficial, slightly effuse, hairy, with regular edge, black; sexual morph not formed within 60 days.

Material examined: ITALY, Forlì-Cesena [FC], Monte Cavallo-Meldola, on a dead branch of *Iris germanica* Linn. (Iridaceae), 16 March 2016, Erio Camporesi, IT2888 (the re-examined specimens deposited in MFLU 16-2816; HKAS 96296), living culture KUMCC 16-0033, MFLUCC.

Notes: *Torula masonii* was introduced by Crous et al. (2015) based on a fungus which sporulated in culture. In this study, we re-examined *T. masonii* on a natural specimen collected in Italy. The study showed that *T. masonii* is also characterized by conidia having a dark terminal coronate cell which is conidiogenous.

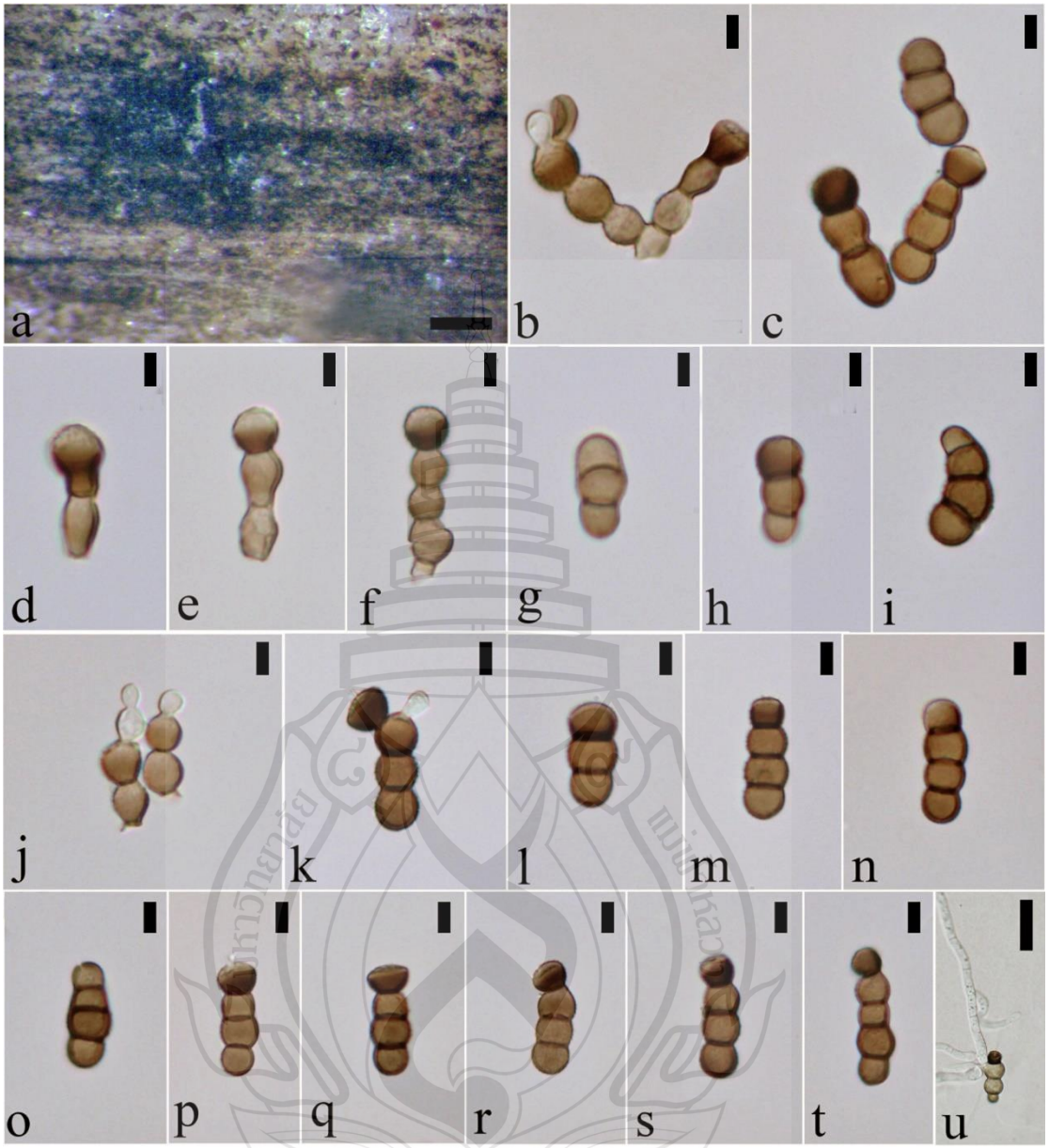


Figure 3.7 Morphology of *Torula masonii* (MFLU 16-2816)

Figure. 3.7 a Colonies on dead branch of *Iris germanica*. b–c, d–f Conidiophores with conidiogenous cell. g–h Immature conidia. j–k Conidial budding. i, l–t Conidia. u Germinated conidia. *Scale bars* a 200 µm, u 20 µm, b–t 5 µm

3.4.6 *Torula ficus* P.W. Crous, IMA Fungus 6 (1): 192 (2015)

MycoBank number: MB812804, Facesoffungi number: FOF 02712.

Saprobic on a dead branch of *Chromolaena odorata* Linn. (Asteraceae). Sexual morph: Undetermined. Asexual morph: *Colonies* effuse on host, black, powdery. *Mycelium* partly immersed to superficial on the substrate, composed of septate, branched, smooth, hyaline hyphae. *Conidiophores* 9.4–12.5 µm long × 3.7–4.5 µm wide ($\bar{x}$ = 10.2 × 3.9 µm, n = 20), macronematous, mononematous, solitary, erect, paler brown to brown, smooth to distinctly verrucose, thick-walled, consisting of 1–2 cells, without apical branches, ampulliform, arising from hypha. *Conidiogenous cells* 5.4–8.5 µm long × 5–7.4 µm wide ($\bar{x}$ = 6.7 × 5.6 µm, n = 20), polyblastic, terminal, dark brown to black, smooth to minutely verruculose, thick-walled, ellipsoid to coronal. *Conidia* 12–20 µm long × 4.6–6.6 µm diam ($\bar{x}$ = 16.3 × 5.4 µm, n = 20) catenate, acrogenous, phragmosporous, light brown to greyish-brown, smooth to distinctly verrucose, 2–4-septate, rounded and paler coloured at apex, slightly constricted at some septa, chiefly subcylindrical. *Conidial secession* schizolytic.

Cultural characteristics: Conidia germinating on PDA within 14 hrs and germ tubes produced from the apex. Colonies growing on PDA, reaching 5 cm in 10 days at 30ºC, mycelium partly immersed to superficial, slightly effuse, cottony, with regular edge, greyish-brown to brown; sexual morph not formed within 60 days.

Material examined: THAILAND, Chiang Rai, Doi Pui, on a dead branch of *Chromolaena odorata* Linn. (Asteraceae), 17 July 2015, Ausana Mapook, DP 59 (the re-examined specimens deposited in MFLU 16-2817, HKAS 96297), living culture KUMCC 16-0035, MFLUCC.

Notes: *Torula ficus*, collected from *Ficus* in Europe was reported by Crous et al. (2015) based on a sporulating culture. *Torula ficus* is morphologically similar to *T. herbarum*, but distinct as it has longer and wider conidia (Crous et al., 2015). In our study, we re-examine *T. ficus* which was collected from *Chromolaena odorata* in Thailand. This is a new record from this host as well as from a tropical region.

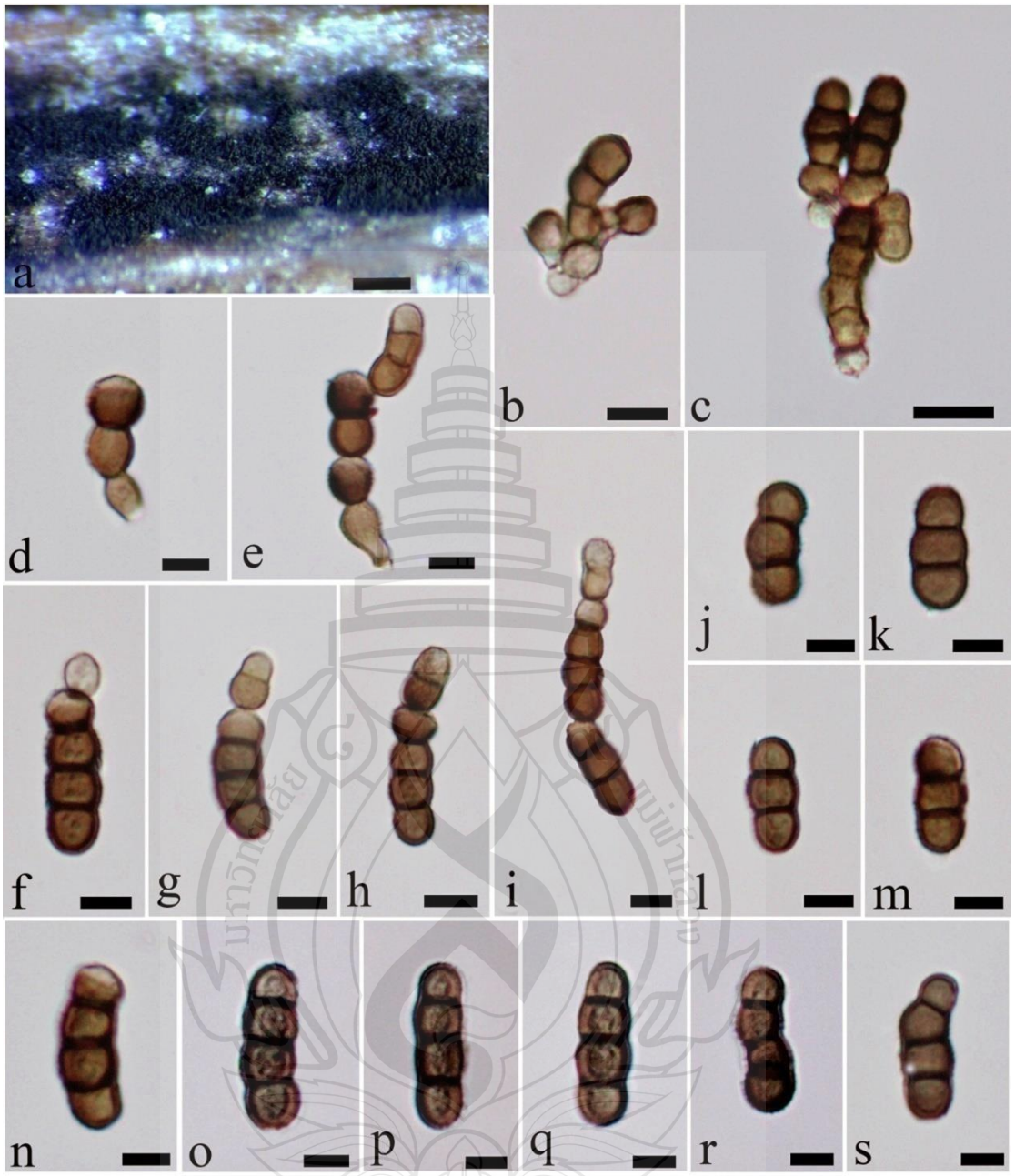


Figure 3.8 Morphology of *Torula ficus* (MFLU 16-2817)

Figure. 3.8 a Colonies on dead branch of *Chromolaena odorata*. b Conidiophores. c, f–i Budding on conida. j–s Conidia. Scale bars a 200 µm, c 10 µm, b, d–s 5 µm

3.4.7 *Torula chromolaenae* J.F. Li, Phookamsak, A. Mapook & K.D. Hyde, sp. nov.

MycoBank number: MB 819536, Facesoffungi number: FOF 02713

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

Holotype: MFLU 16-2819

Saprobic on a dead branch of *Chromolaena odorata* Linn. (Asteraceae). Sexual morph: Undetermined. Asexual morph: *Colonies* discrete on host, black, powdery. *Mycelium* immersed on the substrate, composed of septate, branched, smooth, light brown hyphae. *Conidiophores* 5–6.3 µm long × 3.5–4.6 µm diam ($\bar{x}$ = 5.8 × 4 µm, n = 10), macronematous, mononematous, solitary, erect, light brown, verruculose, thick-walled, consisting of one-celled, without apical branches, ellipsoid to subglobose, arising from hypha. *Conidiogenous cells* (3.5–)4.5–5.1 µm long × 4.8–5.6 µm wide ($\bar{x}$ = 4.7 × 5.4 µm, n = 10), polyblastic, terminal, light brown to brown, paler at apex, smooth to minutely verruculose, thick-walled, doliiform. *Conidia* 12.1–16.5 µm long × (3.6–)4.1–5 µm wide ($\bar{x}$ = 14.5 × 4.3 µm, n = 20) catenated, acrogenous, simple, phragmosporous, light brown to brown, smooth to minutely verruculose, 2–3-septate, rounded at both ends, often paler at apex, slightly constricted at some septa, chiefly subcylindrical. *Conidial secession* schizolytic.

Cultural characteristics: Conidia germinating on PDA within 14 hrs and germ tubes produced from the apex. Colonies growing on PDA, reaching 5 cm in 14 days at 30ºC, mycelium partly immersed to superficial, slightly effuse, cottony, with regular edge, greyish-white to grey; sexual morph not formed within 60 days.

Material examined: THAILAND, Chiang Mai, Mae Taeng (Mushroom Research Center), on a dead branch of *Chromolaena odorata* (Asteraceae), 26 December 2015, Ausana Mapook, MRC1 (MFLU 16-2819, holotype), ex-type living culture KUMCC 16-0036, MFLUCC; (isotype in HKAS, under the code of HKAS 96299)

Notes: *Torula chromolaenae*, described herein as a new species, is distinct from other species in having smaller and doliiform conidogenous cells and 2–3-septate conidia.

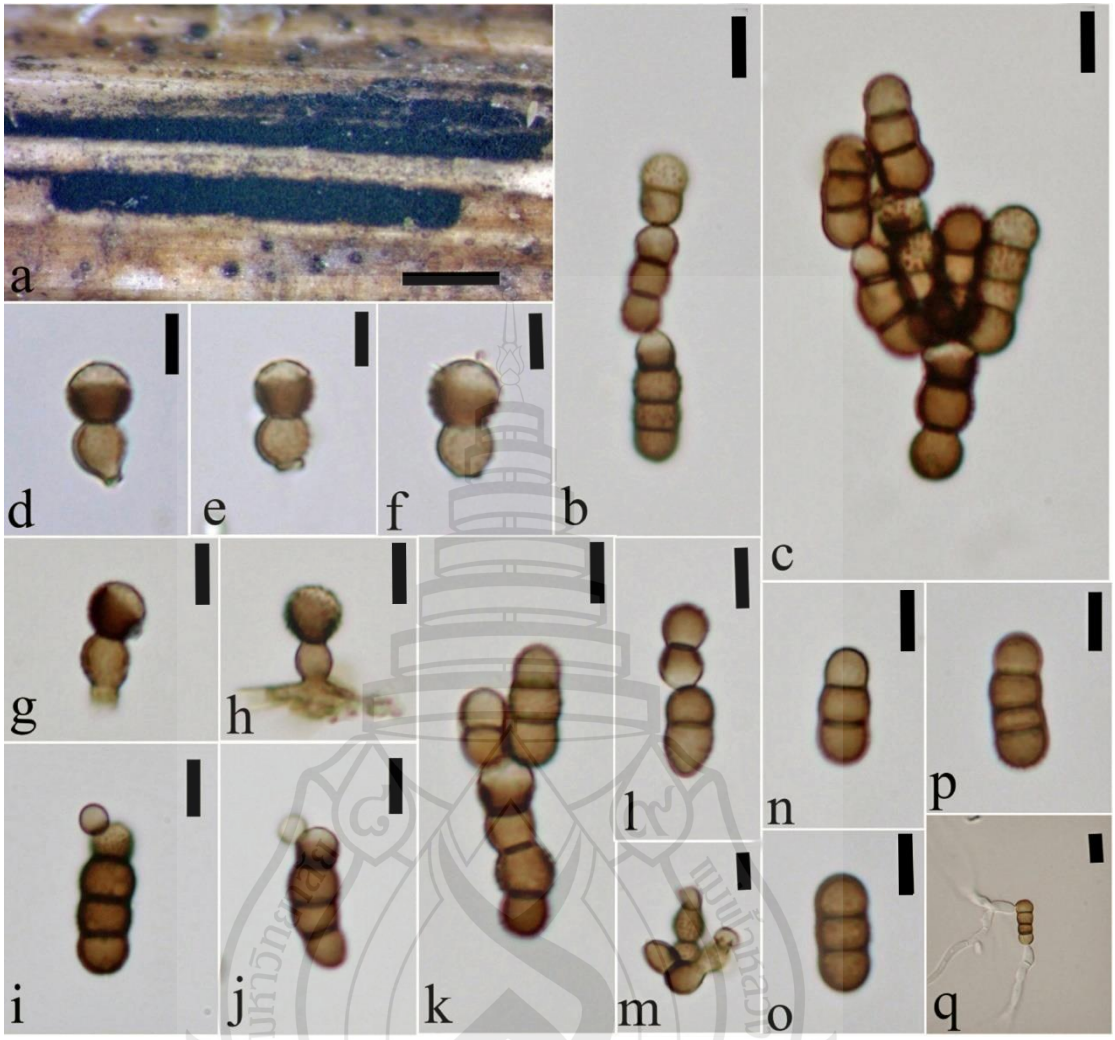


Figure 3.9 Morphology of *Torula chromolaenae* (MFLU 16-2819)

Figure 3.9 a Colonies on dead branch of *Chromolaena odorata*. b, l Conidia in catenated chain. c, k Branched chains of conidia. d–h Conidiophores with conidiogenous cells. i–j Budding on conidia. m Conidiophores. n–p Conidia. q Germinated conidia. Scale bars a 500 µm, q 10 µm, b–p 5 µm

3.4.8 *Torula mackenziei* J.F. Li, Phookamsak & K.D. Hyde, sp. nov.

MycoBank number: MB 819537, Facesoffungi number: FOF 02714

Etymology: Named in honour of the mycologist Dr. E.H.C. McKenzie, in recognition of his contribution to the study of hyphomycetes.

Holotype: MFLU 16-2820

Saprobic on dead branch of *Bidens pilosa* Linn. (Asteraceae). Sexual morph: Undetermined. Asexual morph: *Colonies* sporadic on host, black, powdery. *Mycelium* immersed on the substrate, composed of septate, branched, smooth, light brown hyphae. *Conidiophores* 3–4.3 µm long × 3.4–3.7 µm wide ($\bar{x}$ = 3.8 × 3.5 µm, n = 10), macronematous, mononematous, solitary, erect, light brown, minutely verruculose, thick-walled, consisting of 1–2 cells or reduced to conidiogenous cells, without apical branches, ellipsoid to subglobose, arising from hypha. *Conidiogenous cells* (3.6–)4.3–4.7 µm long × 4–4.8 µm wide ($\bar{x}$ = 4.5 × 4.5 µm, n = 10), polyblastic, terminal, dark brown to black, paler at apex, smooth to minutely verruculose, thick-walled, globose to ellipsoid. *Conidia* 9.4–18.5 µm long × (3.8–)4.4–4.7 µm wide ($\bar{x}$ = 13.8 × 4.5 µm, n = 20) catenated, acrogenous, phragmosporous, light brown to greyish-brown, smooth to minutely verruculose, 2–3-septate, rounded at both ends, often paler at apex, composed of moniliform cells, slightly constricted at some septa, chiefly subcylindrical. *Conidial secession* schizolytic.

Cultural characteristics: Conidia germinating on PDA within 14 hrs and germ tubes produced from the apex. Colonies growing on PDA, reaching 5 cm in 10 days at 30ºC, mycelium partly immersed to superficial, slightly effuse, cottony, with regular edge, white to grey; sexual morph not formed within 60 days.

Material examined: THAILAND, Chiang Rai, Doi Mae Salong, on a dead branch of *Bidens pilosa* Linn. (Asteraceae), 29 September 2013, Junfu Li, H-2 (MFLU 16-2820, holotype), ex-type living culture MFLUCC 13-0839, KUMCC; (isotype in HKAS, under the code of HKAS 96300)

Notes: *Torula mackenziei* is distinct from other *Torula* species in having greyish-brown conidia composed of moniliform cells and 2–3 septa.

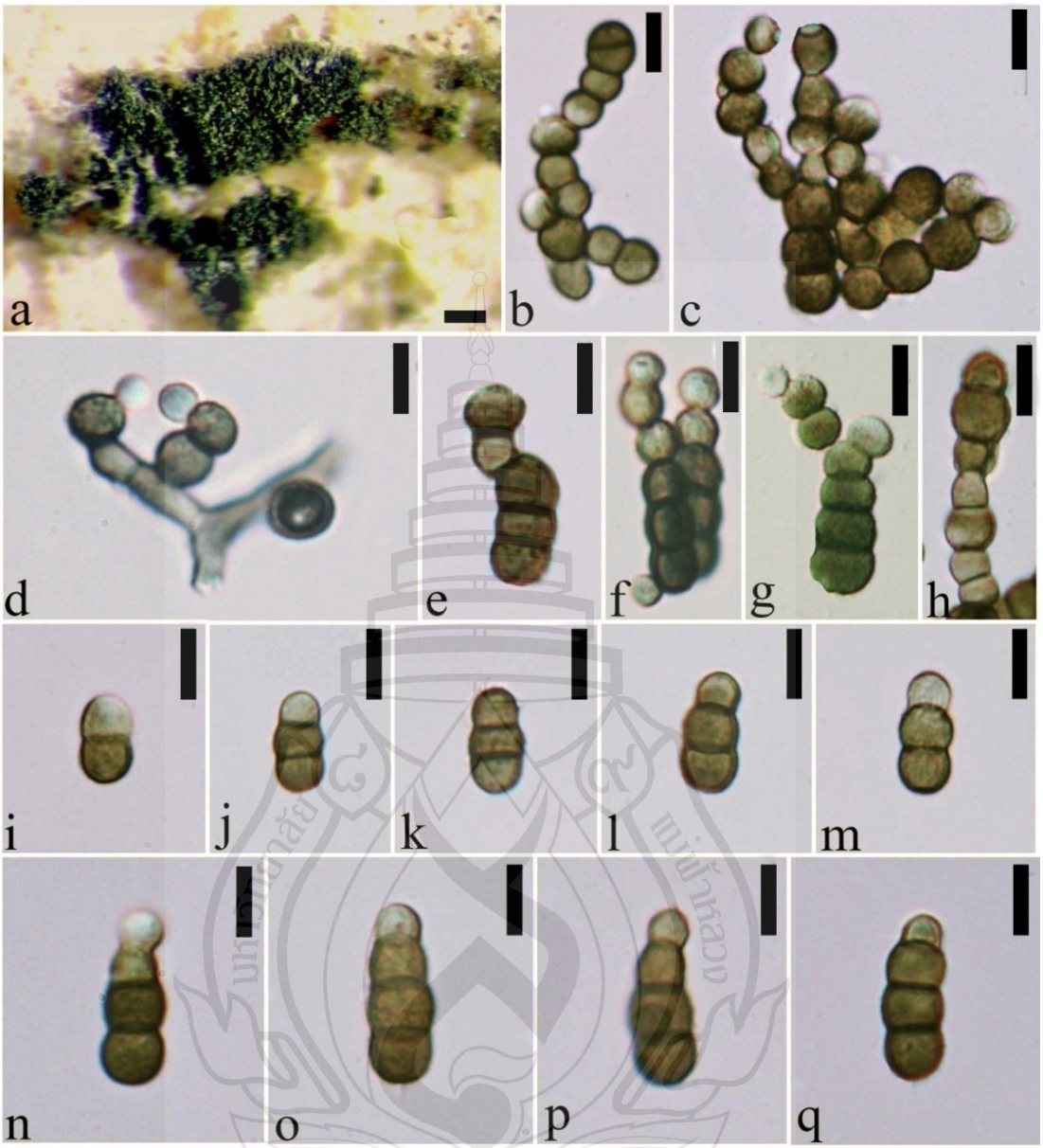


Figure 3.10 Morphology of *Torula mackenziei* (MFLU 16-2820)

Figure 3.10 a Colonies on dead branch of *Bidens pilosa*. b, l Conidia in catenated chain. c, k Branched chains of conidia. d–h Conidiophores with conidiogenous cells. i–j Immature conidia. m Conidiophores. n–q Conidia. Scale bars a 100 µm, b–q 5 µm

3.4.9 *Dendryphion hydei* J.F. Li, Phookamsak & Jeewon, sp. nov.

Indexfungorum: 556746; Facesoffungi number: FOF0457

Etymology: Named in honour of Kevin D. Hyde for his excellent contributionto mycology and on his 65th birthday celebration.

Holotype: KUN-HKAS 97502

Saprobic on a branch litter of *Bidens pilosa* Linn. (Asteraceae). Sexual morph: Undetermined. Asexual morph: *Colonies* on the substratum superficial, effuse, gregarious, hairy, brown to dark brown. *Mycelium* composed of branched, septate, pale brown to brown hyphae. *Conidiophores* 260–395.4 µm long × (4) 7.8–13.8 (16.8 on base) µm diam. ($\overline{x}$ = 349 × 9.3 µm, n = 5) macronematous, mononematous, septate, verrucose, thick-walled, branching simple or penicillate at the tip of primary branches, brown, flexuous. *Conidiogenous cells* 4.5–5 µm long × 5.2–5.5 µm diam. ($\overline{x}$ = 4.8 × 5.1 µm, n = 10) terminal, integrated, pale brown, polytretic. *Conidia* 15–34 µm long × 4.9–7.4 µm diam. ($\overline{x}$ = 23.4 × 5.5 µm, n = 20) single, pale olivaceous-brown, dry, verrucose, monilioid, 2–5-septate, constricted at the septa. Conidial secession schizolytic.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from the apex. Colonies growing on PDA, reaching 5 cm in 21 days at 16–30°C, mycelium partly superficial, partly immersed, slightly effuse, hairy, vertical, with regular edge, white to grayish-brown, not produced pigmentation on media agar.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng District, Mushroom Research Centre, on a branch litter of *Bidens pilosa* Linn., 12 July 2016, J.F. Li, FHP3 (HKAS 97502, holotype), ex-type living culture, MFLUCC 18-0236, KUMCC 18-0009.

Notes: *Dendryphion hydei*is described as a novelspecies here.*Dendryphion hydei* resembles *D. submersum* in morphology but it is unique in having smaller conidiogenous cells and subhyaline conidia. In the phylogenetic tree, *D. hydei* forms a separate lineage from *D. submersum* and nested in between *D. europaeum*, *D. comosum*, *D. aquaticum* and *D. fluminicola*. In this study, we collected *D. hydei* from*Bidens pilosa*, which is a new host record for this species. A morphometric comparison of the new taxon with other similar taxa of *Dendryphion* is provided in Table 3.6.



Figure 3.11 Morphology of *Dendryphion hydei* (HKAS 97479)

Figure 3.11 a Colonies on branch of *Bidens pilosa*. b–c Apex of conidiophores with conidial structures. d–e Conidiophores. f–i Conidiogenous cells. j–q Conidia. Scale bars: a = 100µm, b, d–e = 10µm, c, f–q = 5µm.

3.4.10 *Torula hydei* J.F. Li, Phookamsak & Jeewon, *sp. nov.*

Indexfungorum: 556747; Facesoffungi number: FoF 04573

Etymology: Named in honour of Kevin D. Hyde for his excellent contributionto mycology and on his 65th birthday celebration.

Holotype: HKAS 97478

Saprobic on an aerial dead branch of *Chromolaena odorata* Linn. Sexual morph: Undetermined. Asexual morph: *Colonies* discrete on host, black, powdery. *Mycelium* immersed on the substrate, composed of septate, branched, smooth, light brown hyphae. *Conidiophores* (1.5–)2–3 µm long × 1.5–2 µm diam. ($\bar{x}$ = 2.2 × 1.8 µm, n = 10), macronematous, mononematous, solitary, erect, light brown, verruculose, thick-walled, consist of one cell or reduced to conidiogenous cells, without apical branches, subcylindrical to subglobose, arising from hyphae. *Conidiogenous cells* 3–5.5 µm long × 4.3–5 µm diam. ($\bar{x}$ = 3.8 × 4.5 µm, n = 20), polyblastic, terminal, dark brown to black, smooth to minutely verruculose, thick-walled, doliiform to ellipsoid. *Conidia* (7.5–)8.2–14.3 µm long × 2.6–3.7 µm diam.($\bar{x}$ = 10.4 × 3.4 µm, n = 30) solitary to catenate, acrogenous, simple, phragmosporous, light brown to brown, minutely verruculose, 2–4-septate, rounded at both ends, composed of subglobose cells, slightly constricted at some septa, chiefly sub-cylindrical. *Conidial secession* schizolytic.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from the apex. Colonies growing on PDA, reaching 5 cm in 10 days at 16–30°C, mycelium partly superficial, partly immersed, slightly effuse, hairy, vertical, with regular edge, light brown to brown, not produced pigmentation on media agar; not sporulated on media agar within 2 months.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng District, on an aerial dead branch of *Chromolaena odorata* Linn. (Asteraceae), 26 December 2015, J.F. Li, MRC2 (HKAS 97478, holotype), ex-type living culture, MFLUCC 18-0250, KUMCC 16-0037.

Notes: *Torula hydei* resembles *T. herbarum* and *T. fici* in having 2–4-septate, catenated, brown, verruculose conidia, but differs in having smaller conidia [8]. Phylogenetic analyses showed that *T. hydei* constitutes an independent lineage basal to *T. fici* (100% ML and 1.00 BYPP).

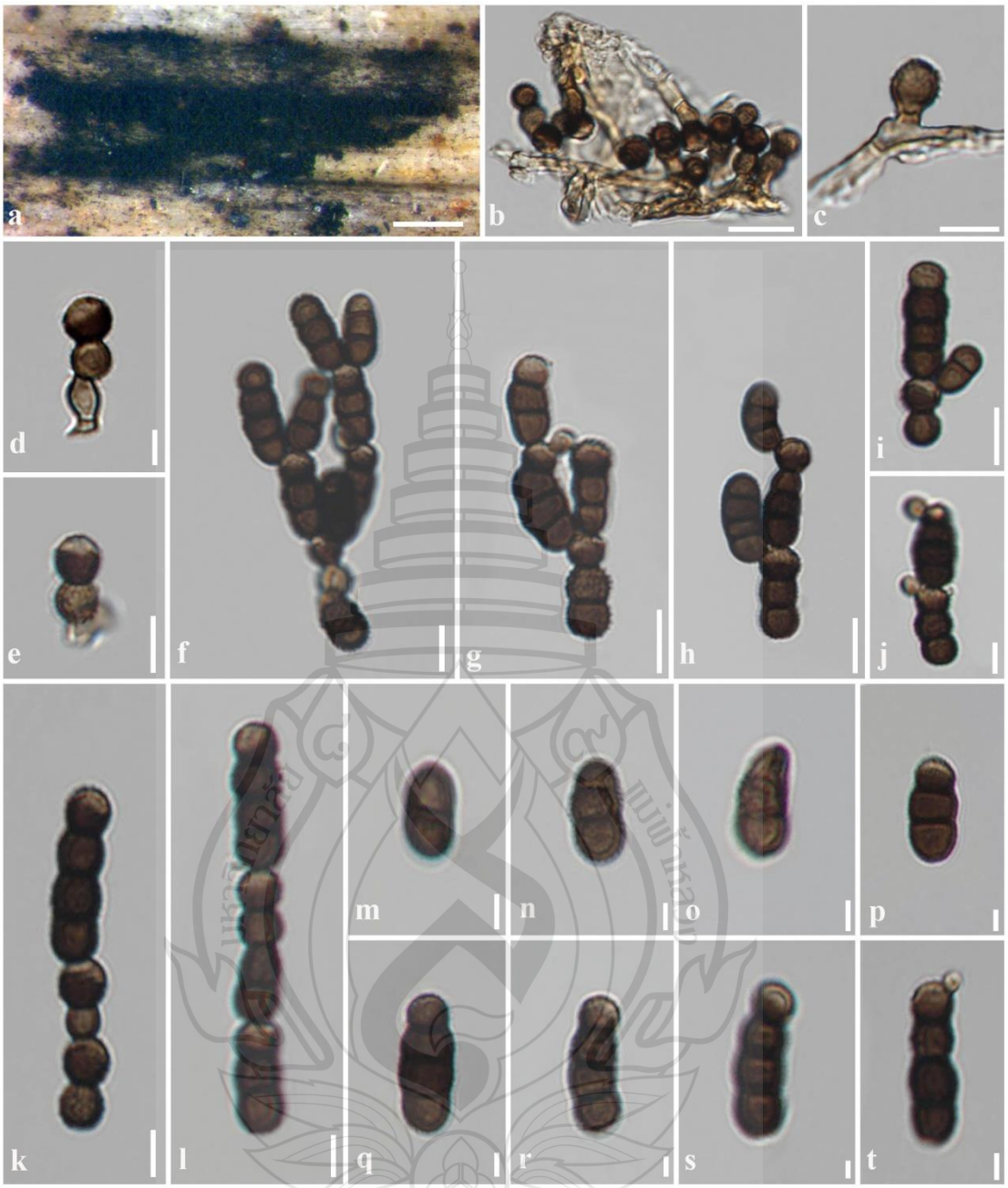


Figure 3.12 Morphology of *Torula hydei* (HKAS 97478)

Figure 3.12 a Colonies on dead branch of *Chromolaena odorata*. b–e, i Conidogenous cell on conidiophres. f–h, j Budding on conidia. i Conidiogenous cell bearing conidia. k–l Conidia in chain. m–t Conidia. Scale bars: a = 100µm, b, f–i = 10 µm, d–e, j–l = 5 µm, m–t = 2 µm.

3.5 Discussion

In this study, the new taxon *Sporidesmioides thailandica*, collected on herbaceous litter from northern Thailand, is recognized as a species different from taxa in the families *Roussoellaceae* and *Torulaceae*, based on morphological distinctiveness. *Sporidesmioides* superficially resembles *Acroconidiellina* (Ellis, 1971), (typified by *A. loudetiae* M.B. Ellis) especially *A. arecae*, by its septate, obclavate, large conidia with a truncate base and macronematous dark brown conidiophores (Bhat, 2010). However, *Sporidesmioides thailandica* differs from the species in *Acroconidiella* by rough, thick-walled, septate conidia, polyblastic conidiogenous cells and conidiophores arising from a stromatic base. The conidiogenesis in *Acroconidiellina* is polytretic (Ellis, 1971). Furthermore, none of the species of *Acroconidiellina* have so far been investigated on a molecular basis. The distinct morphology and molecular segregation from *Sporidesmium sensu lato* and the families *Torulaceae* and *Roussoellaceae*, in the ML tree, together qualify our monotypic new genus *Sporidesmioides* for a new family. However, in view of molecular data of only a single strain, the new genus is retained in *Torulaceae*.

During the fungal diversity studies in Italy and Thailand, I collected a few species with unusual morphologies from herbaceous plant litter. Close microscopic examinations revealed that these species belong to the genus *Torula* and interestingly, four of the taxa could not be ascribed to any known taxa within *Torula* according to recent classification provided by Crous et al. (2015) and Crane and Miller (2016). In addition we also noted morphological differences among extant species and hence five new species, viz *Torula chiangmaiensis* sp. nov., *T. pluriseptata* sp. nov., *T. chromolaenae* sp. nov., *T. mackenziei* sp. nov., *T. hydei* sp. nov., as well as two new host records (*T. masonii* and *T. ficus*) are described and illustrated in this study. The molecular phylogeny based on a concatenated dataset also provides further evidence to support that our newly collected taxa belong to the genus *Torula* with high support. In addition, the new taxa *Dendryphion hydei* is unique in having smaller conidiogenous cells and colourless conidia to compare with other related species in *Dendryphion* (Table 3.2). Our multi-loci phylogeny also positions our new taxon as independent lineage and phylogenetically apart from other species. Phylogenetic

analyses support *D.hydei* as a new species in *Dendryphion*. These aboved tally with recommendations outlined by Jeewon and Hyde (2016) to establish these new species.

Moreover, it is interesting to note that species of Torulaceae have been found to be mostly associated with the host family Asteraceae. In this study our new strains were collected from Asteraceae, and four novel Torulaceae species, *Dendryphion hydei*, *T.orula chromolaenae*, *T. hydei* and *T. mackenziei* from Asteraceae, indicating that Asteraceae harbors a diversity of these fungi. This study recovered *T. ficus* from *Chromolaena odorata*, an herbaceous host (in Thailand), whereas *T. masonii* was collected from dead branches of *Iris germanica* (in Italy). Another peculiar morphological observation noted herein is a characteristic dark corona cell at apex among *T. masonii* but no such details of same have been described in Crous et al. (2015). Indeed, *Dendryphion hydei* collected from an herbaceous host collected in northern Thailand is also the first record on the host (*Bidens pilosa*) and location.

CHAPTER 4

ALTERNARIA: UPDATE ON SPECIES LIMITS, EVOLUTION, MULTI-LOCUS PHYLOGENY, AND CLASSIFICATION [3]

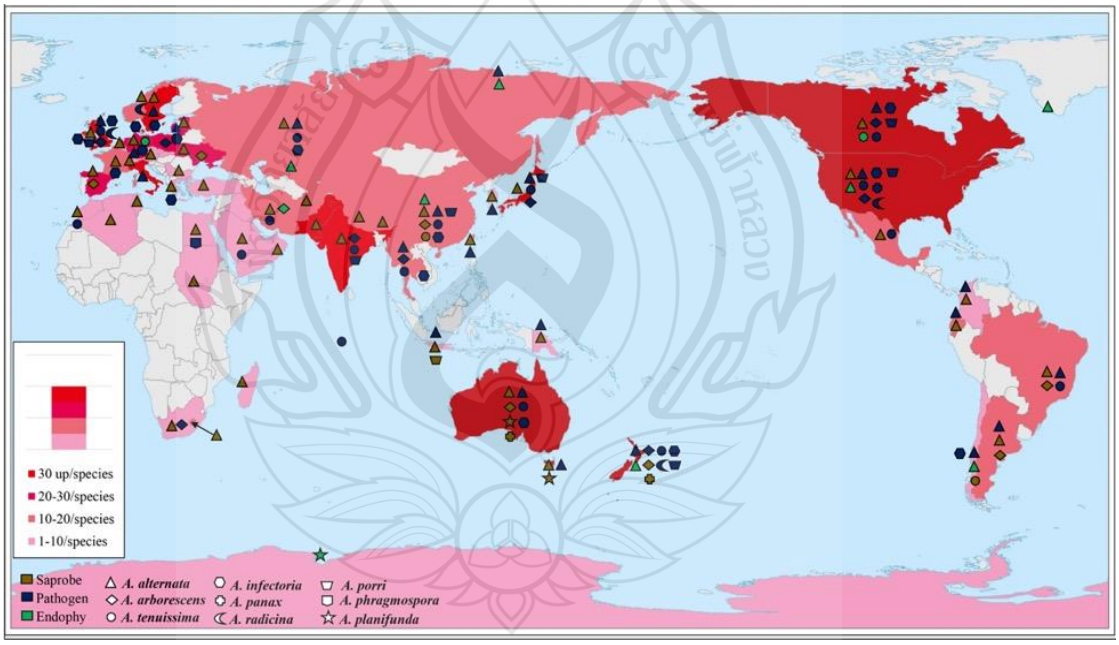
Abstract

Alternaria is a genus of ascomycetous fungi, and the species are known as major plant pathogens as well as common allergens in humans. Recently, there are over 368 accepted species in the genus, which are currently divided into 28 sections. This paper aims to elaborate the taxonomy of *Alternaria* with multi-locus phylogenetic trees derived analyses of a concatenated DNA sequence dataset consisting of ITS, LSU, TEF1-α, RPB2, GAPDH and Alt-a1 loci. Twenty-four new species viz. *Alternaria arctoseptata, A. arundinis, A. baoshanensis, A. breviconidiophora, A. brevirostra, A. ellipsoconidia, A. eupatoriicola, A. falcata, A. lathyri, A. macilenta, A. macroconidia, A. minimispora, A. muriformispora, A. nodulariconidiophora, A. oblongoellipsoidea, A. obpyriconidia, A. orobanches, A. ovoidea, A. phragmiticola, A. phytolaccae, A. pseudoinfectoriae, A. rostroconidia, A. salicicola* and *A torilis* spp. nov. are proposed and classified in sect. *Alternaria*, sect. *Infectoriae*, sect. *Porri* and sect. *Radicina*. *Alternaria alternata* and *A. doliconidium*, with new host and geographical records, in China, Italy, and Thailand are also described. This study further explores the utility of divergent time estimates to gain additional insights into the evolutionary relationships of *Alternaria* in Pleosporales.

[3] This section has been published in Mycosphere, 2021, (In prep).

4.1 Introduction

Alternaria Nees is a ubiquitous genus and well-known as dematiaceous hyphomycete, comprising over 590 legitimate species epithets, and approximately 368 species as accepted within 28 sections (Hongsanan et al., 2020; Wijayawadene et al., 2020; He et al., 2021; Species Fungorum 2021). Species of *Alternaria* occupy diverse ecological niches from endophytes on various asymptomatic plant tissues to saprobes on a wide range of host and substrates (i.e., dead vegetations, paper, and foods), as well as plant, human and animal pathogens worldwide (Thomma, 2003; Ma et al., 2010; Polizzotto et al., 2012; Woudenberg et al., 2013, 2015; Lawrence et al., 2013, 2014, 2016). The genus is cosmopolitan and widely distributed in Asia (e.g., India, Japan), Australia, Europe, and North America (Figure 4.1) (Farr & Rossman, 2021).



Note The nine keys phylogenetically correlate to *Alternaria* species from different sections that occur worldwide are shown with different symbols on the map. The number of *Alternaria* species in each country is shown with different colors, which indicates "richness" from 0 to 30 species and up to 30 species.

Figure 4.1 World distribution of *Alternaria* species

As invasive parasites and pathogens, *Alternaria* species are frequently isolated from different habitats such as atmosphere, dust, indoor environments, soil, and damaged old buildings (Ellis, 1971, 1976; De Hoog & Horre, 2002; Runa et al., 2009; Woudenberg et al., 2013, 2014, 2015). The most prevalent diseases on plants caused by *Alternaria* are leaf spots and defoliation with typical concentric zonatic symptoms featuring brown to black necrotic lesions surrounded by chlorotic areas on leaves (Kinon et al., 1999), but can also present as infected flowers, fruits, roots, seedlings, and stems with different kinds of lesions (Thomma, 2003; Woudenberg et al., 2015; Lawrence et al., 2016). These diseases lead to the reduction of marketing value and economic losses for several important economic crops, such as cabbage, cucumber, fava bean, onion, potato, tomato, and the other ornamental plants (Thomma, 2003; Woudenberg et al., 2013, 2014, 2015; Ariyawansa et al. 2015; Lawrence et al.m 2016; Jayawardena et al.m 2019). Most causal agents are frequently restricted to *Alternaria* sects. *Alternantherae* D.P. Lawr. et al., *Alternaria* D.P. Lawr. et al, *Brassicicola* D.P. Lawr. et al., *Crivellia* (Shoemaker & Inderb.) Woudenb. & Crous, *Gypsophilae* D.P. Lawr. et al., *Nimbya* (E.G. Simmons) Woudenb. & Crous, *Porri* D.P. Lawr. et al., *Radicina* D.P. Lawr. et al., and *Sonchi* D.P. Lawr. et al. occur on over 4000 host and non-host specific plants (Woudenberg et al.m 2013, 2014, 2015; Lawrence et al., 2016; Meena et al., 2016; Tralamazza et al., 2018; Farr & Rossman, 2021). Jayawardena et al. (2019) showed that the majority of pathogenic *Alternaria* species infected a vast array of host species. Effective implementation of control strategies is generally hampered by misidentification.

Alternaria species have the ability to produce a wide spectrum of secondary metabolites. Potential phytotoxins produced by *Alternaria* are beneficial for biotechnological applications as biocontrol agents or mycoherbicides of innumerable plant species under diverse habitable regions (Woudenberg et al., 2013, 2015; Lawrence et al., 2016). Furthermore, *Alternaria* species also produced mycotoxins and are implicated in opportunistic animal and human diseases (e.g., alternariosis) that significantly affect the health of victims and can also contaminate food products. *Alternaria alternata* (Fr.) Keissl. and *A. infectoria* E.G. Simmons have frequently been reported as causative agents of the phaeohyphomycosis in immuno-compromised patients or airborne allergens (Lopes et al., 2013; Mirhendi et al., 2013; Chowdhary et al., 2014; Kustrzeba-Wójcicka et al., 2014; Woudenberg et al., 2013; Lawrence et al., 2016; Lyskova et al., 2017; Cardona et al., 2020).

The genus *Alternaria*, currently belonging to the family Pleosporaceae of Pleosporales, Dothideomycetes (Hongsanan et al., 2020; Wijayawardene et al., 2020), was introduced by Nees (1816), with *A. tenuis* Nees as the type species. Later, Keissler (1912)

considered *A. tenuis* to be conspecific with *Torula alternata* Fr. (Fries, 1832) and synonymized both *A. tenuis* and *T. alternata* with *A. alternata* which is currently designated as the generic type. Extensive morphology-based taxonomy of *Alternaria* was mainly dealt by Emory G. Simmons (1920–2013), who summarized a monograph of *Alternaria* and recognized 275 species in the genus based on the patterns of sporulation and conidial morphology (Simmons, 2007). The latest taxonomic treatment of *Alternaria* carried out by Lawrence et al. (2014, 2016) described the asexual morph of *Alternaria* as alternarioid dematiaceous hyphomycetes with effuse, pigmented colonies, colorless hyphae, mononematous to caespitose, macronematous, simple or branched, pale brown to brown conidiophores, monotretic or polytretic, sympodial, conidiogenous cells, and solitary or in chains, dark pigmented, multi-celled, typically dictyosporous, or rarely phragmosporous, some borne singly and most as catenate conidial structures. The sexual morph of *Alternaria* have only been reported for species in sections *Alternaria*, *Crivellia*, *Embellisioides* Woudenb. & Crous, *Eureka* Woudenb. & Crous, *Infectoriae* Woudenb. & Crous, *Nimbya* and *Panax* D.P. Lawr. et al., and is characterized by small, dark brown, erumpent to superficial, globose to ovoid, glabrous, uni-loculate ascomata, with papillate ostioles, composed of thin-walled of peridium, containing fissitunicate, cylindrical to cylindric-clavate asci, embedded in broad cellular pseudoparaphyses and muriform, ellipsoidal to fusoid, pigmented ascospores (Woudenberg et al., 2013; Ariyawansa et al., 2015; Lawrence et al., 2016).

Over the course of taxonomic discussions of *Alternaria*, many sexual genera were considered to be the sexual morph of *Alternaria*, including *Allewia* E.G. Simmons, *Brachycladium* Corda, *Chalastospora* E.G. Simmons, *Chmelia* Svob.-Pol., *Crivellia* Shoemaker & Inderb., *Embellisia* E.G. Simmons, *Lewia* M.E. Barr & E.G. Simmons, *Nimbya* E.G. Simmons, *Sinomyces* Yong Wang bis & X.G. Zhang, *Teretispora* E.G. Simmons, *Ulocladium* Preuss, *Undifilum* B.M. Pryor et al., and *Ybotromyces* Rulamort (Ariyawansa et al., 2015; Rossman et al., 2015; Lawrence et al., 2016). Moreover, some sexual genera viz. *Clathrospora* Rabenh., *Comoclathris* Clem., *Leptosphaeria* Ces. & De Not., and *Pleospora* Rabenh. ex Ces. & De Not. have also been described with alternarioid asexual morphs, of which *Pleospora* were usually mentioned as the sexual morph of *Alternaria* (Lawrence et al., 2016). However, Simmons (1986) linked *Pleospora* with the asexual genus *Stemphylium* Wallr. Hitherto, *Pleospora* and *Stemphylium* were congeneric,

and *Stemphylium* was recommended to be used instead of *Pleospora* due to its wider use and earlier introduction (Rossman et al., 2015). Woudenberg et al. (2013) demonstrated that *Allewia*, *Brachycladium*, *Chalastospora*, *Chmelia*, *Crivellia*, *Embellisia*, *Lewia*, *Nimbya*, *Sinomyces*, *Teretispora*, *Ulocladium*, *Undifilum* and *Ybotromyces* formed the internal clades within *Alternaria sensu stricto* and thus these genera were synonymized and treated as sections of *Alternaria.* Based on the prior introduction of *Alternaria*, widespread use and number of the species, Rossman et al. (2015) also proposed to use *Alternaria* rather than *Allewia*, *Crivellia* and *Lewia*.

The DNA-based classification of the genus *Alternaria* has so far relied on over ten gene loci, including nuclear ribosomal DNA (LSU, SSU), the intervening ITS regions, mtSSU, protein-coding genes such as ACT, Alt-a1, CAL, GAPDH, RPB2, TEF1-α, THN, Tsr1, and plasma membrane ATPase (Lawrence et al., 2013, 2014, 2016; Woudenberg et al., 2013, 2015; Ghafri et al., 2019; Jayawardena et al., 2019). Multiple molecular methods have been investigated or proposed for distinguishing *Alternaria* species, including random amplified polymorphic DNA (Roberts et al., 2000), amplified fragment length polymorphism (Somma et al., 2011), selective subtractive hybridization (Roberts et al., 2012) and sequence characterized amplified genomic regions (Stewart et al., 2013). However, the standard gene regions and other protein-coding loci (e.g., ACT, CAM, RPB2, TEF1-α, Tsr1, TUB2 and chitin synthase) are not able to delineate species within all the sections of *Alternaria*, such as small spore species-groups like sect. *Alternaria* and sect. *Infectoriae* (Peever et al., 2004; Andrew et al., 2009; Woudenberg et al., 2015; Poursafar et al., 2017, Patriarca et al., 2019). Hong et al. (2005) illustrated that the Alt-a1 locus is reliable for *Alternaria* species identification. Lawrence et al. (2013) used five protein-coding loci (viz. ACT, Alt-a1, CAM, GAPDH, and plasma membrane ATPase) for first clarifying the phylogenetic hypothesis among *Alternaria* lineages and revealed that the plasma membrane ATPase and CAM loci were the most suitable phylogenetic markers for molecular identification of *Alternaria* species. Woudenberg et al. (2013) delineated phylogenetic lineages within *Alternaria,* and allied genera based on the multi-locus phylogeny of SSU, LSU, ITS, GAPDH, RPB2 and TEF1-α gene regions and introduced 16 new *Alternaria* sections. Subsequently, whole-genome sequencing has become an essential tool to delineate ambiguous species in *Alternaria* and other complex species (Woudenberg et al., 2015). Therefore, Woudenberg et al. (2015) used multi-locus

phylogeny based on ITS, GAPDH, RPB2, TEF1-α, Alt-a1, endoPG and OPA10-2 gene loci coupled with whole-genome and transcriptome comparisons to discriminate species in sect. *Alternaria* and accepted only 11 phylogenetic species and one species complex in *Alternaria* sect. *Alternaria*. Woudenberg et al. (2015) also synonymized 35 morphospecies under *A. alternata*. Lawrence et al. (2016) provided a comprehensive taxonomic treatment of the genus *Alternaria* with multi-locus phylogeny and accepted 27 sections in *Alternaria*, but later revised it to 28 accepted sections (Lawrence et al., 2016; Ghafri et al., 2019). As important aspects of plant pathogenicity, further details on phylogeny, diseases and the symptoms as well as morphological characters of the genus *Alternaria* were also discussed by Jayawardena et al. (2019a, 2019b) (also see Table 4.1).

4.1.1 The *Alternaria* Sections

Alternaria sections are recognized based on molecular phylogenies, but these do not always correlate with species-groups that were earlier delineated based on morphological characteristics (Table 4.1) (Woudenberg et al., 2013, 2014; Lawrence et al., 2013, 2014, 2016; Grum-Grzhimaylo et al., 2016). The species-groups *A. alternata*, *A. alternantherae*, *A. brassicicola*, *A. infectoria*, *A. porri*, *A. radicina* and *A. sonchi* were phylogenetically strongly supported by Pryor and Gilbertson (2000), Chou and Wu (2002), de Hoog and Horré (2002), Pryor and Bigelow (2003), Hong et al. (2005), Inderbitzin et al. (2006), Lawrence et al. (2012, 2013), Pryor et al. (2009), Runa et al. (2009), Wang et al. (2011) and Woudenburg et al. (2013, 2015). Lawrence et al. (2013) introduced *A. panax* and *A. gypsophilae* as two species-groups and proposed eight species-groups to sections within *Alternaria*. The *Alternaria* complex comprises the genera *Alternaria*, *Chalastospora* (Simmons, 2007), *Crivellia*, *Embellisia*, *Nimbya*, *Stemphylium*, *Ulocladium*, *Undifilum* and *Sinomyces* together with eight sections of *Alternaria*. Later, based on multigene analyses, Woudenburg et al. (2013) assigned *Alternaria* species complex into 24 sections and Lawrence et al. (2016) generalized the genus with 27 sections. Ghafri et al. (2019) organized *Alternaria* sect. *Omanenses* Al Ghafri et al. by combined seven multi-locus analyses, with the addition of a single species, *A. omanensis* Al Ghafri et al.

Presently, the genus *Alternaria* contains 28 accepted sections viz. sect. *Alternantherae*, sect. *Alternaria*, sect. *Brassicicola*, sect. *Chalastospora*, sect. *Cheiranthus*, sect. *Crivellia*, sect. *Dianthicola*, sect. *Embellisia*, sect. *Embellisioides*, sect. *Euphorbiicola*, sect. *Eureka*, sect.

Gypsophilae, sect. *Infectoriae*, sect. *Japonica*, sect. *Nimbya*, sect. *Omanenses*, sect. *Panax*, sect. *Phragmosporae*, sect. *Porri*, sect. *Pseudoalternaria*, sect. *Pseudoulocladium*, sect. *Radicina*, sect. *Soda*, sect. *Sonchi*, sect. *Teretispora*, sect. *Ulocladioides*, sect. *Ulocladium*, and sect. *Undifilum*. Furthermore, eight species identified in genus *Alternaria* by multigene phylogenetic analyses and not accommodated among the 28 accepted sections of *Alternaria* are *A. argyranthemi* E.G. Simmons & C.F. Hill, *A. brassicae* (Berk.) Sacc., *A. dennisii* M.B. Ellis, *A. helianthiinficiens* E.G. Simmons, Walcz & R.G. Roberts, *A. peucedani* S.H. Yu, *A. soliardae* E.G. Simmons, *A. thalictrigena* K. Schub. & Crous, and *A. thlaspis* (E.G. Simmons & J.C. David) D.P. Lawr., Rotondo & Gannibal (Lawrence et al., 2016).

Section *Alternantherae* was introduced by Lawrence et al. (2013) for species group *Alternaria alternantherae* Holcomb & Antonop., comprising three species previously described as *Nimbya* species viz. *A. celosiicola* Jun. Nishikawa & C. Nakash., *A. gomphrenae* Togashi and *A. perpunctulata* (E.G. Simmons) D.P. Lawr., M.S. Park & B.M. Pryor, and the type species of the section, *A. alternantherae* (Woudenburg et al., 2014a; Lawrence et al., 2016). Subsequently, the other three species were included in the sect. *Alternantherae* viz. *A. crassoides* (Davis) Gannibal, *A. pimpriana* V.G. Rao, and *A. paragomphrenae* Jun. Nishikawa & C. Nakash. that *A. crassoides* and *A. pimpriana* were previously accommodated in the genus *Nimbya* (Gennibal & Lawrence, 2018a; Nishikawa, 2019). Currently seven species are accepted in this section.

Section *Alternaria* was indroduced by Lawrence et al. (2013) to accommodate *Alternaria* species, commonly referred to small-spored *Alternaria* groups. The main morphological feature that can be used to distinguish *Alternaria* sect. *Alternaria* from other sections is the short conidia produced in chains (frequently less than 60 µm *in vitro*) (Lawrence et al., 2013; Woudenberg et al., 2013; Gennibal, 2015). The sexual morph is known from *A. alternata* and described as typically small-sized, erumpent, globose to ovoid, smooth, dark brown, papillate ascomata, cylindrical to cylindric-clavate asci, and ellipsoid to fusoid, brown, eguttulate, smooth-walled ascospores, with 3–7 transverse septa, 1–2 longitudinal septa (Ariyawansa et al., 2015; Lawrence et al., 2016). There were approximately 60 species accommodated in section *Alternaria* based on ITS sequence data (Woudenberg et al., 2013). However, Woudenberg et al. (2015) accepted only 11 phylogenetic species and one species complex in this section and synonymized 35 *Alternaria* morphospecies under *Alternaria alternata*. Gennibal (2015) re-circumscribed

and amended the section based on morphological assessments made by Simmons (2007). Gennibal (2015) included the other 37 morphospecies and accepted 59 species in this section. Subsequently, the other four species were included in this section by Gannibal and Lawrence (2018a) viz. *A. calystegiae* Nelen, *A. diversispora* (Thüm.) E.G. Simmons, *A. guaranitica* (Speg.) E.G. Simmons and *A. macalpinei* E.G. Simmons). Wanasinghe et al. (2018) introduced *A. doliconidium* J.F. Li, Camporesi & K.D. Hyde on *Rosa canina* in Italy. Besides, Jayawardena et al. (2018) also introduced *A. italica* J.F. LI, Camporesi & K.D. Hyde on *Vitis vinifera* in Italy. Nishikawa and Nakashima (2020) also included *A. iridicola* (Ellis & Everh.) J.A. Elliott in this section. Therefore, about 69 species are accommodated in this section.

Section *Brassicicola* was introduced by Lawrence et al. (2013) for *Alternaria brassicicola* (Schwein.) As a wiltshire species-group, it comprises five species viz. *A. brassicicola, A. conoidea* (E.G. Simmons) D.P. Lawr. et al., *A. mimicula* E.G. Simmons, *A. septorioides* (Westend.) E.G. Simmons, and *A. solidaccana* E.G. Simmons (Woudenberg et al., 2013; Lawrence et al., 2016). Multi-locus phylogenetic analyses demonstrated that sect. *Brassicicola* has close phylogenetic relationships with sects. *Sonchi*, *Radicina*, *Gypsophilae*, *Porri*, *Alternaria*, and *Alternanatherae* (Woudenberg et al., 2013). However, the conidial morphology of sect. *Brassicicola* is different from these sections in producing extremely small conidia with heavily melanized transverse septa and usually produced phragmosporous conidia (Lawrence et al., 2013, 2016; Woudenberg et al., 2013).

Section *Chalastospora* was introduced by Woudenberg et al. (2013) for species group which was previously described as *Chalastospora* species. The section is typified by *Alternaria cetera* E.G. Simmons, and the other five species were also initially accommodated in this section, including *A. abundans* (E.G. Simmons) Woudenb. & Crous, *A. armoraciae* E.G. Simmons & C.F. Hill, *A. breviramosa* Woudenb. & Crous, *A. malorum* (Ruehle) U. Braun, Crous & Dugan, and *A. obclavata* (Crous & U. Braun) Woudenb. & Crous (Woudenberg et al., 2013). Interestingly, *A. abundans* and *A. armoraciae* are morphologically distinguished from the other species in sect. *Chalastospora* in producing mostly phragmoconidia that are short and not elongated as in other species of this section (Lawrence et al., 2016). Recently, Marin-Felix et al. (2019) included *A. pobletensis*

Iturrieta-González, Dania García & Gené in this section and thus, seven species are listed in sect. *Chalastospora*.

Section *Cheiranthus* was introduced by Woudenberg et al. (2013) to accommodate *Alternaria cheiranthi* (Lib.) P.C. Bolle, and *A. indefessa* (E.G. Simmons) Woudenberg & Crous (≡ *Embellisia indefessa* E.G. Simmons). Besides, Woudenberg et al. (2013) treated a non-sporulating strain CBS 115.44 which was formally identified as *Alternaria resedae*, in this section. However, *A. resedae* was treated as a synonym of *A. septorioides* E.G. Simmons in sect. *Brassisicola*. Thus, Woudenberg et al. (2013) treated the strain CBS 115.44 as "*Alternaria* sp." Gannibal and Lawrence (2018a) assigned *A. latifunda* E.G. Simmons in this section based on morphological study with conidia producing many longitudinal septa. Hence, three species are accepted in this section (Gannibal & Lawrence, 2018a). Phylogenetic analyses demonstrated this section is sister to sects. *Pseudoulocladium* and *Ulocladioides* (Woudenberg et al., 2013; Lawrence et al., 2013, 2016).

Section *Crivellia* was introduced by Woudenberg et al. (2013) to accommodate the type species of *Crivellia*, *C. papaveracea* (De Not.) Shoemaker & Inderb. (asexual morph known as *Brachycladium penicillatum* Corda), and *B. papaveris* (Sawada) Shoemaker & Inderb. Both species were reported as important pathogens of opium poppy (Lawrence et al., 2016). Phylogenetic analyses based on ITS, GAPDH and TEF1-α sequences revealed that these two species clustered with the Alternaria-complex instead of *Pleospora sensu stricto*. Hence, Woudenberg et al. (2013) transferred these two species to the new section of *Alternaria* as *A. papavericola* Woudenb. & Crous and *A. penicillata* (Corda) Woudenb. & Crous. However, Lawrence et al. (2016) mentioned that the phylogenetic status of this section is uncertain. The sexual morph of *A. penicillata* was interdispersed with dark microsclerotia and macroconidiophores, forming medium-sized (320–400 × 220–300 μm), globose to depressed globose ascomata, with ellipsoidal (20–25 × 6–9 μm) (Lawrence et al., 2016).

Section *Dianthicola* was introduced by Woudenberg et al. (2013) and is typified by *Alternaria dianthicola* Neerg. Three species were accommodated in this section, including *A. dianthicola*, *A. elegans* E.G. Simmons & J.C. David, and *A. simsimi* E.G. Simmons (Woudenberg et al., 2013). Xu et al. (2018) introduced another pathogenic species, *A. kareliniae* B. Xu & Z.D. Jiang, causing leaf spot on *Karelinia caspia* (Pall.) Less. in China.

However, the name is validly introduced by Index Fungorum (2019). Thus, four phylogenetic species are known for this section. Phylogenetic analyses based on protein-coding genes showed that sect. *Dianthicola* has a close relationship with sect. *Ulocladioides* (Woudenberg et al., 2013; Lawrence et al., 2016).

Section *Embellisia* was introduced by Woudenberg et al. (2013) and is typified by *Alternaria embellisia* Woudenb. & Crous (≡ *Helminthosporium allii* Campan.). The section was erected for the species previously described in the genus *Embellisia*, including three species viz. *E. allii* E.G. Simmons, *E. chlamydospora* (Hoes, G.W. Bruehl & C.G. Shaw) E.G. Simmons, and *E. tellustris* E.G. Simmons. The genus *Embellisia* was initially introduced to separate an atypical species of *Helminthosporium* Link (1809) based on conidial and conidiophore morphology which is characterized by successive sympodial proliferations conidiophores and phragmoconidia, with distinctly dark, rigid and thickened transverse septa (Lawrence et al., 2016). Phylogenetic analyses based on GAPDH, ITS and Alt-a1 genes demonstrated the section has close relationships with sects. *Phragmosporae*, *Soda*, *Chalastospora*, *Pseudoalternaria*, and *Infectoriae* (Woudenberg et al., 2013; Lawrence et al., 2016). Therefore, Woudenberg et al. (2013) designated the new name for these three *Embellisia* species and transferred them to *Alternaria* sect. *Embellisia*, namely *Alternaria chlamydosporigena* Woudenb. & Crous, *A. embellisia* Woudenb. & Crous, and *A. tellustris* (E.G. Simmons) Woudenb. & Crous.

Section *Embellisioides* was introduced by Woudenberg et al. (2013) to accommodate six species previously described as *Embellisia* species and named as *Embellisia* group III in Lawrence et al. (2012). The section consists of *Alternaria botryospora* Woudenb. & Crous, *A. hyacinthi* (de Hoog & P.J. Mull. bis) Woudenb. & Crous (type species), *A. lolii* (E.G. Simmons & C.F. Hill) Woudenb. & Crous, *A. planifunda* (E.G. Simmons) Woudenb. & Crous, *A. proteae* (E.G. Simmons) Woudenb. & Crous, and *A. tumida* (E.G. Simmons) Woudenb. & Crous (Woudenberg et al., 2013; Lawrence et al., 2016). These species were obtained from plants or rhizosphere (Lawrence et al., 2016). The sexual morph of species in this section was regarded to the genus *Allewia* and is characterized by ovoid to spherical, dark, thin walled, pseudothecial, papillate ascomata with markedly setose, subellipsoidal to subcylindrical asci and slightly inequilateral subellipsoidal immature ascospores, becoming ellipsoid to subclavate, with multiple transverse septa and discontinuous series of longitudinal septa (Lawrence et al.,

2016). Phylogenetic analyses supported the section as a sister relationship with sect. *Eureka* (Woudenberg et al., 2013; Lawrence et al., 2016).

Section *Euphorbiicola* was introduced by Woudenberg et al. (2014) and is typified by *Alternaria euphorbiicola* E.G. Simmons & Engelhard. Two species are currently accommodated in this section viz. *A. euphorbiicola* and *A. limicola* E.G. Simmons & M.E. Palm (Woudenberg et al., 2014). These two species were obtained from plant host families *Euphorbiaceae* and *Rutaceae* as saprobes and pathogens (Woudenberg et al., 2014; Farr & Rossman, 2021). Woudenberg et al. (2014) established sect. *Euphorbiicola* as a separate section with sect. *Porri* based on the formation of in chains conidia. Multi-locus phylogenetic analyses clearly separated the section from other species in sect. *Porri* (Woudenberg et al., 2014).

Section *Eureka* was introduced by Woudenberg et al. (2013) to accommodate four *Alternaria* species and the other two species previously described as *Embellisia* species which was mentioned as *Embellisia* group IV in Lawrence et al. (2012). Six species are currently known for this section, including *Alternaria anigozanthi* Priest, *A. cumini* E.G. Simmons, *A. eureka* E.G. Simmons (type species), *A. geniostomatis* E.G. Simmons & C.F. Hill, *A. leptinellae* (E.G. Simmons & C.F. Hill) Woudenb. & Crous, and *A. triglochinicola* Alcorn & S.M. Francis. These species were commonly isolated from plants and rhizosphere (Woudenberg et al., 2013; Lawrence et al., 2016). The sexual morph is known for the type species of the section as regarded to *Allewia* and is characterized by spherical to ovoid, thin-walled, dark, papillate ascomata, with conspicuously setose, subcylindrical to subellipsoid asci, somewhat inequilateral, with subellipsoidal and slightly inequilateral juvenile ascospores, becoming subclavate to ellipsoid, with transverse septa, discontinuous series of longitudinal septa when mature (Lawrence et al., 2016). Multi-locus phylogenetic analyses based on the protein-coding genes demonstrated the section has a close relationship with the morphologically similar sect. *Embellisioides* (Woudenberg et al., 2013; Lawrence et al., 2016).

Section *Gypsophilae* was first introduced by Lawrence et al. (2013) to accommodate four *Alternaria* species, comprising *A. gypsophilae* Neerg. (type species), *A. nobilis* (Vize) E.G. Simmons, *A. vaccariae* (Săvul. & Sandu) E.G. Simmons & S.T. Koike and *A. vaccariicola* E.G. Simmons. Woudenberg et al. (2013) recommended the other four species viz. *A. axiaeriisporifera* E.G. Simmons & C.F. Hill, *A. ellipsoidea* E.G.

Simmons, *A. juxtiseptata* E.G. Simmons, and *A. saponariae* (Peck) Neerg. to this section based on multi-gene phylogeny. Based on morphological examination of *Alternaria* species producing conidia with many longitudinal septa, Gannibal and Lawrence (2018a) included *A. longispora* McAlpine in the sect. *Gypsophilae*. Consequently, Gannibal (2019) introduced *A. kamtschatica* Gannibal on leaves of *Dianthus barbatus* from Russia. He et al. (2021a) introduced the other two new species in this section viz. *A. barbata* L. He & J.X. Deng and *A. hispanica* L. He & J.X. Deng from China. Currently, there are 12 species accommodated in this section that are restricted on *Caryophyllaceae* host plant (Woudenberg et al., 2013; Gannibal 2019; He et al., 2021a). The section has a close relationship with sects. *Alternaria, Alternantherae, Euphorbiicola,* and *Porri* (Lawrence et al., 2013, 2016; Woudenberg et al., 2013).

Section *Infectoriae* was introduced by Woudenberg et al. (2013) for *Alternaria infectoria* E.G. Simmons species-group, comprising approximately 50 species (Ariyawansa et al., 2015; Liu et al., 2015; Crous et al., 2016; Gannibal & Lawrence, 2016; Lawrence et al., 2016; Thambugala et al., 2017; Marin-Felix et al., 2019; Iturrieta-González et al., 2020). The human pathogenic genera *Ybotromyces* Rulamort (as *A. caespitosa* (de Hoog & C. Rubio) Woudenb. & Crous) and *Chmelia* (as *A. slovaca* (Svob.-Pol.) Woudenb. & Crous) were also embedded in sect. *Infectoriae* (Woudenberg et al., 2013). The section is typified by *A. infectoria* and taxa in this section are commonly known as saprobes and human pathogens as well as endophytes on apple leaves (Serdani et al.; 2002; Gannibal & Lawrence; 2016; Lawrence et al., 2016). The sexual morph of sect. *Infectoriae* was linked to species in the genus *Lewia* and is characterized by smooth walled ascomata, subcylindrical or subellipsoidal asci and muriform ascospores with 5(−7) transverse septa and 1–2 longitudinal septa in central segments, with or without longitudinal or oblique septum in terminal cells (Lawrence et al., 2016). The main refined morphological features of taxa in sect. *Infectoriae* are small conidia (usually less than 60 µm *in vitro*) and long secondary conidiophores (Lawrence et al., 2013; Woudenberg et al., 2013; Gannibal & Lawrence, 2016). Member of sect. *Infectoriae* are presumed to be homothallic mating-type genes that can produce protoascomata in axenic culture (Andersen et al., 2009; Lawrence et al., 2016). Phylogenetic analyses revealed the section as the sister group to sect. *Pseudoalternaria* and the most suitable genetic markers for distinguishing species in the

sect. *Infectoriae* are ATPase and cmdA genes (Lawrence et al., 2013, 2016; Marin-Felix et al., 2019).

Section *Japonicae* was introduced by Woudenberg et al. (2013) with the type species of the section as *Alternaria japonica* Yoshii. The section was established to accommodate *A. japonica* together with *A. nepalensis* E.G. Simmons based on multi-gene phylogeny. *Alternaria japonica* was previously connected to the *A. brassicicola* species-group (Pryor & Gilbertson, 2000; Pryor & Bigelow, 2003; Lawrence et al., 2013) and this connection was questioned by Hong et al. (2005). Recently, Bessadat et al. (2020) included the additional species *A. telliensis* N. Bessadat, D. Ayad & P. Simoneau in this section; however, this species is invalidly introduced. Species in sect. *Japonicae* were frequently isolated from the *Brassicaceae* hosts (Woudenberg et al., 2013; Lawrence et al., 2016). Phylogenetic status of the sect. *Japonicae* is uncertain within *Alternaria* (Lawrence et al., 2016).

Section *Nimbya* was introduced by Woudenberg et al. (2013) and is typified by *Alternaria scirpicola* (Fuckel) Sivan. The section initially contained four species previously described as *Nimbya* species viz. *A. caricis* (E.G. Simmons) Woudenb. & Crous, *A. scirpicola*, *A. scirpinfestans* (E.G. Simmons & D.A. Johnson) Woudenb. & Crous, and *A. scirpivora* (E.G. Simmons & D.A. Johnson), Woudenb. & Crous. Later, Gannibal (2018) included the other two species, *A. heteroschemos* (Fautrey) Gannibal and *A. juncicola* (E.G. Simmons) Gannibal, to this section. In addition, Ahmadpour (2019) also introduced *A. caricicola* Ahmadp. to this section. Hence, there are currently seven species accommodated in sect. *Nimbya*. The sexual morph of the section was referred to the genus *Macrospora* Fuckel and is characterized by immersed to superficial, subglobose, ostiolate ascomata, with broadly cylindrical or clavate to obovoid asci and broadly ellipsoidal, brown to dark brown, multi-septate ascospores (Ariyawansa et al., 2015; Lawrence et al., 2016). Section *Nimbya* is closely related to sects. *Embellisia*, *Phragmosporae*, *Chalastospora* and *Infectoriae* based on phylogenetic analyses of the combined GAPDH, RPB2 and TEF1-α sequence dataset (Woudenberg et al., 2013).

Section *Omanenses* was introduced by Ghafri et al. (2019), with the single species *Alternaria omanensis* as the type species of the section. *Alternaria omanensis* was isolated from dead wood in Oman as a saprobe and is known for both sexual and asexual morphs. The sexual morph of the section is characterized by superficial, subglobose to globose, or

ovoid to cup-shaped when dry, dark brown to black, carbonaceous ascomata, with a blunt ostiole, cylindrical to subcylindrical asci and pale brown to dark brown, muriform, subclavate to broadly obovoid or ellipsoid ascospores, with 3 transversely septa, 1–2 longitudinal septate at central segments, without septa at the end cells, and constricted at the central septum (asexual morph see Table 4.1). Multiloci phylogenetic analyses of a combined SSU, LSU, ITS, GAPDH, TEF1-α and RPB2 sequence dataset demonstrated that the section has a close relationship with sects. *Embellisioides*, *Eureka* and *Ulocladium* (Ghafri et al., 2019).

Section *Panax* was introduced by Lawrence et al. (2013) and initially consisted of *Alternaria calycipyricola* R.G. Roberts, *A. eryngii* (Pers.) S. Hughes & E.G. Simmons and *A. panax* Whetzel (as *A. panacis* in Deng et al. (2015) and Lawrence et al. (2016); type species). Woudenberg et al. (2013) added *A. avenicola* E.G. Simmons and *A. photistica* E.G. Simmons in this section and the sexual morphs of these two species were known as *Lewia avenicola* Kosiak & Kwaśna (Simmons, 2007) and *L. photistica* E.G. Simmons (Simmons, 1986), respectively. Deng et al. (2015) reported two pathogenic species in this section viz. *A. araliae* H.C. Greene and *A. dendropanacis* S.H. Yu & J.X. Deng that were associated with leaf spot and blight disease on *Araliaceae* in Korea, while Gannibal and Lawrence (2018a) included *A. prasonis* E.G. Simmons based on morphological study. Recently, Hashemlou et al. (2020) described *A. hedjaroudei* Y. Ghosta et al. on stems of *Serratula coriacea* Fisch. & C.A. Mey. from Iran in the sect. *Panax*. Thus, there are currently nine species accommodated in this section. PCR assays of mating-type genes indicated that members in sect. *Panax* are found as both homothallic and heterothallic species that are either capable of sporulating sexual morph *in vitro* or without an identified sexual morph. Phylogenetic analyses based on the GAPDH, RPB2 and TEF1-α sequences suggested that sect. *Panax* has a close relationship with a monotypic lineage *A. thalictrigena* K. Schub. & Crous and sect. *Teretispora* (Woudenberg et al., 2013; Lawrence et al., 2016).

Section *Phragmosporae* was introduced by Woudenberg et al. (2013) and is typified by *Alternaria phragmospora* Emden. The section contains six species viz. *A. chlamydospora* Mouch., *A. didymospora* (Munt.-Cvetk.) Woudenb. & Crous, *A. limaciformis* E.G. Simmons, *A. molesta* E.G. Simmons, *A. mouchaccae* E.G. Simmons, and *A. phragmospora*. These species are known from soil, seawater, seawater plants and

animals, excluding *A. didymospora* which was found from equine nasal mucosa. It has no evidence for species associated with land plants in this section (Lawrence et al., 2016). Phylogenetic results indicated the section is sister to sect. *Embellisia*, with *A. didymospora* and *A. phragmospora* were linked (Woudenberg et al., 2013; Lawrence et al., 2016).

Section *Porri* was introduced by Lawrence et al. (2013) and is typified by *Alternaria porri* (Ellis) Cif. Section *Porri* has been reported as the largest section of *Alternaria* with approximately 63 species revealed in the section based on multi-locus phylogeny (Woudenberg et al., 2013, 2014; Lawrence et al., 2016). Detailed study of this large-spored section was carried out by Woudenberg et al. (2014). The section displays a high level of genetic variation and contains many important plant pathogens such as *A. bataticola* Ikata ex W. Yamam., *A. porri*, *A. solani* Sorauer and *A. tomatophila* E.G. Simmons, causing leaf and stem blight of sweet potato, purple blotch of onion and early blight of potato and tomato, respectively (Woudenberg et al., 2014). Gannibal (2018) included *A. rhapontici* (Nelen) Gannibal to the section. Liu et al. (2019) introduced *A. physalidis* H.F. Liu & J.X. Deng from *Physalis alkekengi* L. (Solanaceae) in China. Cai et al. (2019) also introduced a pathogenic species *A. yunnanensis* Z.Y. Cai et al., which causes leaf spots on rubber tree in China. Hence, about 66 species in known for this section, making this section the second-largest section after sect. *Alternaria*. Multi-locus phylogeny demonstrated that the section is sister to sect. *Euphorbiicola*, and clustered with sects. *Alternaria* and *Alternantherae* (Lawrence et al., 2013, 2016; Woudenberg et al., 2013, 2014).

Section *Pseudoalternaria* was introduced by Lawrence et al. (2016) and is typified by *Alternaria arrhenatheri* D.P. Lawr., Rotondo & Gannibal. The section initially consisted of two species viz. *A. arrhenatheri* and *A. rosae* E.G. Simmons & C.F. Hill based on both phylogeny and morphology. Based on morphological examination, Gannibal and Lawrence (2016) described a new taxon, *A. parvicaespitosa* Gannibal & D.P. Lawr. as a misidentified isolate previously identified as *A. rosae* by Zhu and Xiao (2015). Deng et al. (2018) accommodated a new pathogenic species, *A. brassicifolii* S.H. Yu & J.X. Deng, causing necrotic leaf spots of *Brassica rapa* L. (Brassicaceae) in Korea in the section; however, the species is treated as invalid name (nom. inval.) based on nomenclature article 40.1 (Shenzhen). Subsequently, other four species were included to the section viz. *A. altcampina* Iturrieta-González, Dania García & Gené, *A. ershadii* A. Poursafar, Ghosta

& M. Javan-Nikkhah, *A. inflata* Iturrieta-González, Dania García & Gené., and *A. kordkuyana* A. Poursafar et al. (Poursafar et al., 2018, 2019; Marin-Felix et al., 2019). Currently, eight species are known in sect. *Pseudoalternaria*, all of which were confirmed using multi-locus phylogeny. Section *Pseudoalternaria* showed to be closely related to sects. *Infectoriae* and *Chalastospora* (Lawrence et al., 2016; Marin-Felix et al., 2019).

Section *Pseudoulocladium* was introduced by Woudenberg et al. (2013) to accommodate species previously described as *Ulocladium* species and is typified by *Alternaria chartarum* Preuss. Four species were initially included in the section viz. *A. aspera* Woudenb. & Crous, *A.chartarum*, *A. concatenata* Woudenb. & Crous, and *A. septospora* (Preuss) Woudenb. & Crous (Woudenberg et al., 2013). Based on morphological assessment, Gannibal and Lawrence (2018b) included *A. lanuginosa* (Harz) Sacc. and *A. sylvestris* Gannibal & D.P. Lawr. Section *Pseudoulocladium* morphologically resembles sects. *Ulocladioides* and *Ulocladium* but differs in producing simple or branched chains conidia, whereas sect. *Ulocladioides* usually produces densely geniculate conidiophores with clustered, short chains conidium, and secondary conidiophores are short with several conidiogenous loci. Section *Ulocladium* typically produced small, clustered, single conidia without chains (Gannibal & Lawrence, 2018b). Phylogenetic analyses of protein-coding genes revealed that the section has a sister relationship with sect. *Dianthicola* and clusters with sect. *Ulocladioides* (Woudenberg et al., 2013).

Section *Radicina* was first recognized by Pryor and Gilbertson (2000) and formal established by Lawrence et al. (2013). The section was introduced to accommodate the radicina species-group and is typified by *Alternaria radicina* Meier, Drechsler & E.D. Eddy. Species in this section are pathogens occurring on *Apiaceae* (Wodenberg et al., 2013; Lawrence et al., 2016). Wodenberg et al. (2013) and Lawrence et al. (2016) listed five species in this section, including *A. carotiincultae* E.G. Simmons, *A. petroselini* (Neerg.) E.G. Simmons, *A. radicina*, *A. selini* E.G. Simmons and *A. smyrnii* (P. Crouan & H. Crouan) E.G. Simmons based on multi-locus phylogeny. Subsequently, Marin-Felix et al. (2019) introduced *A. chlamydosporifera* Iturrieta-González, Dania García & Gené, isolated from rabbit dung in Spain, to the section. Recently, He et al. (2021b) introduced two new species in this section viz. *A. divaricatae* L. He & J.X. Deng and *A. vulgaris* L. He & J.X. Deng, both isolated from *Umbelliferae* (*Apiaceae*) in China. Hence, there are currently

eight species accommodated in this section. Phylogenetic analyses demonstrated that the section has a close relationship with sect. *Gypsophilae* (Lawrence et al., 2016).

Section *Soda* was introduced by Grum-Grzhimaylo et al. (2016) to contain three species isolated from soils at the different highly alkaline soda lakes in Russia, comprising *Alternaria kulundae* Bilanenko, Georgieva & Grum-Grzhim. (type species), *A. petuchovskoi* Bilanenko, Georgieva & Grum-Grzhim., and *A. shukurtuzi* Bilanenko, Georgieva & Grum-Grzhim. Species in this section showed a potential alkalitolerant to facultative alkaliphilic type of the adaptation. The sexual morph for the section is not yet been known. Multi-locus phylogeny of SSU, LSU, RPB2, ITS, and GAPDH showed that the section clustered with sects. *Infectoriae*, *Chalastospora*, and *Embellisia* (Grum-Grzhimaylo et al., 2016).

Section *Sonchi* was first described as the species-group by Hong et al. (2005) and validly introduced by Lawrence et al. (2013). Only two species are accommodated in this section viz. *Alternaria cinerariae* Hori & Enjoji and the type species of the section *A. sonchi* Davis. Species in this section occur on a wide range of hosts in family Asteraceae (Woudenberg et al., 2013). The sexual morph of the section is undetermined. Phylogenetic analyses based on the GAPDH, RPB2 and TEF1-α sequences showed that sect. *Sonchi* forms a sister clade with two monotypic lineages, *A. brassicae* (Berk.) Sacc. and *A. helianthiinficiens* E.G. Simmons, Walcz & R.G. Roberts (Woudenberg et al., 2013). Ferreira and Barreto (2019) designated the neotype of *Acroconidiella tropaeoli* (T.E.T. Bond) J.C. Lindq. & Alippi (≡ *Heterosporium tropaeoli* T.E.T. Bond) and proposed the new name for the species as *Alternaria obtusa* B.W. Ferreira & R.W. Barreto. The species is sister to sect. *Sonchi* (Ferreira & Barreto, 2019).

Section *Teretispora* was introduced by Woudenberg et al. (2013) to accommodate a single species, *Alternaria leucanthemi* Nelen, as the type of the section. The species was isolated from *Leucanthemum maximum* (Ramond) DC. (Asteraceae) and is characterized by simple primary conidiophores bearing 1–3 conidiogenous loci and generally solitary, cylindrical conidia, with 7–14(–17) transverse septa, and 3–7 longitudinal septa (Lawrence et al., 2016). The sexual morph has not yet been described for the section. Phylogenetic analyses showed that sect. *Teretispora* is sister to *Alternaria thalictrigena* K. Schub. & Crous, and clustered with sect. *Panax*; thus, Woudenberg et al. (2013) proposed to raise this species as a section, rather than monotypic lineage.

Section *Ulocladioides* was introduced by Woudenberg et al. (2013) and is typified by *Alternaria cucurbitae* Letendre & Roum. The section was introduced to accommodate ten species previously described as *Ulocladium* species based on phylogenetic approach, distinguishing far away from sect. *Ulocladium*. Sect. *Ulocladioides* is morphologically similar to the sect. *Ulocladium* and is characterized by short, geniculate, sympodial proliferations conidiophores with obovoid, single or in chains, non-beaked conidia, with a narrow base (Woudenberg et al., 2013). Gannibal and Lawrence (2018b) included other ten species in this section and thus, 20 species are currently known for this section. Phylogenetic analyses based on the GAPDH, RPB2 and TEF1-α sequences showed that sect. *Ulocladioides* has a close relationship with sects. *Pseudoulocladium* and *Dianthicola* (Woudenberg et al., 2013).

Section *Ulocladium* was introduced by Woudenberg et al. (2013) and is typified by *Alternaria botrytis* (Preuss) Woudenb. & Crous. The section is introduced to accommodate the epitype of the former genus *Ulocladium* as *Alternaria botrytis* (CBS 197.67) and additionally three species viz. *A. alternaria*e (Cooke) Woudenb. & Crous, *A. capsici-annui* Săvul. & Sandu, and *A. oudemansii* (E.G. Simmons) Woudenb. Gannibal and Lawrence (2018b) included *A. manihoticola* (J.M. Yen) Gannibal & D.P. Lawr to the section based on morphological study and thus, five species are known for the section. Phylogenetic analyses based on the GAPDH, RPB2 and TEF1-α sequences showed that sect. *Ulocladium* is sister to the monotypic lineage *A. argyranthemi* E.G. Simmons & C.F. Hill.

Section *Undifilum* was introduced by Woudenberg et al. (2013) and is typified by *Alternaria bornmuelleri* (Magnus) Woudenb. & Crous. The section consists of five species viz. *A. bornmuelleri*, *A. cinerea* (Baucom & Creamer) Woudenb., *A. fulva* (Baucom & Creamer) Woudenb. & Crous, *A. gansuensis* J.Li Liu & Y.Z. Li, and *A. oxytropis* (Q. Wang, Nagao & Kakish.) Woudenb. & Crous (Woudenberg et al., 2013; Liu et al., 2015). Section *Undifilum* morphologically resembles sect. *Embellisia* but can be distinguished by the formation of conidial germination and by the germ tube being a wavy and unbranched (Pryor et al., 2009; Woudenberg et al., 2013). Species in this section were isolated from Fabaceae as endophytes and produced the swaisonine toxic compound, causing a neurological disease of grazing animals (Baucom et al., 2012). Phylogenetic analysis of the GAPDH, RPB2 and TEF1-α sequences showed that the section forms an

independent lineage closely related to a monotypic lineage *Embellisia dennisii* (M.B. Ellis) E.G. Simmons, (CBS 110533, CBS 476.90) which is currently resurrected as *Alternaria dennisii* M.B. Ellis in Woudenberg et al. (2013).

4.1.2 Historical Studies on *Alternaria*

The study of *Alternaria* and their allied genera has been debated for over 200 years. Summarized by Lawrence et al. (2016), there are five stages of *Alternaria* studies. The first stage (1816–1850s) is when the genus *Alternaria* Nees was first described in 1816, with *A. tenuis* Nees 1816 as the type, but it was then confused with genera such as *Macrosporium* and *Stemphylium*. However, the first validly published species name was *Torula alternata* (Fries, 1832). The second stage (1850s–1930s) involved publication of numerous alternarioid species wherein Elliott (1917) first attempted to revise the taxonomy and nomenclature of *Alternaria* and *Macrosporium*, but this resulted in increasing nomenclatural problems within the alternarioid hyphomycetes. The third stage (1930s–1960s) includes various revisions of *Alternaria* made by Wiltshire (1933), Neergaard (1945) and Joly (1964). However, their work did not follow the rules of nomenclature, and despite wide adoption, these are not in practice to date. The fourth stage (1960s–2000s) is when Emory Guy Simmons (1920–2013) presented a complete reappraisal and revision of all names and taxa related to the genus *Alternaria*, representing the most extensive compilations in the taxonomic history of *Alternaria*. The fifth stage (2000s–2015s) involved molecular phylogenetic methods to further investigate taxonomy of *Alternaria*. Taxonomic studies integrating both morphological and molecular data were done by Pryor and Gilbertson (2000), Hong et al. (2005), Lawrence et al. (2012, 2013, 2014), Woudenberg et al. (2013, 2014, 2015) and Grum-Grzhimaylo et al. (2016). In subsequent studies, the utility and reliability of different genes in deciphering phylogenetic relationships was discussed by Woudenberg et al. (2015) and Lawrence et al. (2016).

4.1.3 Evolutionary and Fossil Studies of *Alternaria*

The study of fossil fungi has become an essential tool to understanding fungal evolution and diversification as well as the correlation of fungi with other organisms coupled with historical functions in the ecosystem (Taylor et al., 2015; Samarakoon et al., 2019). The detailed study of fungal fossils was limited in the early stages due to the technical factors used to study fossil fungi and visual matching for identifying similar

extant species as well as poor preservation and unclear morphological characteristics (Tripathi, 2009; Taylor et al., 2015; Samarakoon et al., 2019). Furthermore, the study of fossil fungi received little attention because of lack of interest, expertise and collaboration (Taylor et al., 2015; Samarakoon et al., 2019). Even though the study of fossil fungi is not an essential tool for fungal taxonomy, it is important for understanding the paleoecological conditions and calibrating divergent times of fungal evolution based on molecular clock studies (Samarakoon et al., 2019). Samarakoon et al. (2019) assimilated 16 selected fossil fungi in Ascomycota and provided detailed information based on descriptions, illustrations, minimum age estimations, and phylogenetic affinity, mostly regarding the epiphytic Dothideomycetes and Sordariomycetes, of which the fossil of *Alternaria* has not been yet studied.

The fossil record of *Alternaria* has also not been determined in the Kalgutkar and Jansonius database of fossil fungi (Berbee et al., 2015). However, there is a fossil record referred to *Alternaria* described as *Polycellaesporonites alternariatus* (Kalgutkar & Sigler) Kalgutkar & Janson (≡ *Piriurella alternariata* Kalgutkar & Sigler). *Polycellaesporonites alternariatus* was first described as *Piriurella alternariata* by Kalgutkar and Sigler (1995) for the fungal fossil-produced dictyosporae spore group. The species referred to *Alternaria* in forming muriform, ovoid to obclavate, rostrate, pale brown to brown, conidia, arising singly or in clusters, with transverse septa more prominent and thicker than the longitudinal or oblique septa, broader basal region with distally tapering short or cylindrical beak with or without a dark thickened tip (Kalgutkar & Sigler, 1995). The species was found from Iceberg Bay formation at Kanguk Peninsula, Axel Heiberg Island and Northwest Territories, Canada with estimated age during late Palaeocene or early Eocene (40.4–58.7 MYA) (Kalgutkar & Sigler, 1995). However, the link between *P. alternariatus* and *Alternaria* has not yet been confirmed due to *Alternaria* being variable in shape, size and septation of conidium and showed to be a species complex (Kalgutkar & Sigler, 1995; Lawrence et al., 2012, 2013, 2014; Woudenberg et al., 2013, 2014, 2015).

Evolutionary estimates based on molecular clocks have been increasingly common in fungal taxonomy in recent years (Prieto & Wedin, 2013; Hongsanan et al., 2016, 2017, 2020; Samarakoon et al., 2016; Hyde et al., 2017; Liu et al., 2017, 2019), with some works including the Pleosporales (Phukhamsakda et al., 2016; Liu et al., 2017, 2019). Hyde et al. (2017) compiled the Kingdom Fungi as evolving during the Stenian to Calymmian era

(1000–1600 MYA), the phyla evolved between Devonian to Cambrian (358–541 MYA), the classes evolved during Jurassic to Carboniferous (145–358 MYA) and the orders evolved during Cretaceous to Carboniferous (66–358 MYA). They also determined the higher ranks of fungi based on the divergence time estimations of Sordariomycetes, of which the familial rank would correspond to 50–150 MYA. Liu et al. (2017) recommended that the orders of Dothideomycetes should be evolved between 100 and 220 MYA (crown age) and 130 and 310 MYA (stem age), and the families are ranked between 20–100 MYA (crown age). However, the divergence time estimations of *Alternaria* based on DNA sequence evidence remains unexplored.

In this research survey, we isolated 60 *Alternaria* taxa on different plant hosts collected from Yunnan, China, Italy and Thailand from 2014 to 2019 and introduce 24 novel *Alternatia* species, all of which are represented by the hyphomycetous asexual morphs as saprobes on dead plant tissues. We also provide updated phylogenetic relationships for the sections in *Alternaria* based on phylogenetic analyses of a concatenated dataset from seven gene regions (ITS, LSU, SSU, TEF1-α, RPB2, GAPDH and Alt-a1 loci) and estimated evolutionary divergence time of *Alternaria*.

Table 4.1 Synopsis of *Alternaria* sections based on the asexual morphs

***Alternaria* sections**		**Conidiogenesis structures**	**Ecology and economy**	**References**
Sect. *Alternantherae*	Conidiophores	Short to moderately long, with slightly enlarged conidiogenous tip.	Species in this section are reported as plant pathogens that mainly cause leaf spot.	Lawrence et al. (2012, 2013, 2016), Woudenberg et al. (2013)
	Conidia	Large, ellipsoidal to ovoid, or subcylindrical, rarely narrow ellipsoidal, solitary or rarely paired, disto- and euseptate, transversely septate with no or 1–2 longitudinal or oblique septa, slightly constricted near some septa, with a long apical narrow beak, conidial beak unbranched, septate or aseptate, long filiform, sometimes swollen at the end, internal compartmentation occurs, with cell bright at end, with hexagonal, octagonal or rounded transverse sections lumina.		
Sect. *Alternaria*	Conidiophores	Short to long, straight or curved, simple or branched, with one or several apical conidiogenous loci.	Species in this section are reported as plant pathogens on leaves, stems and fruits, and vegetables. Some species cause opportunistic infections of humans. Species in this section are also reported as resources of potential toxins and secondary metabolites.	Tsuge et al. (2013), Woudenberg et al. (2013, 2015), Lawrence et al. (2016)
	Conidia	Obclavate to long ellipsoid, small or moderate in size, septate, slightly constricted near some septa, with few longitudinal septa, in moderately long to long, simple or branched chains, form tapered beak or secondary conidiophore with one or a few conidiogenous loci.		

Table 4.1 (continued)

Alternaria sections		Conidiogenesis structures	Ecology and economy	References
Sect. *Brassicicola*	Conidiophores	Short to moderately long, simple or branched, with one or several apical conidiogenous loci.	Species in this section mainly cause black spot disease on a wide range of hosts, particular on *Brassica* spp. such as cabbage, Chinese cabbage, cauliflower, oilseeds, broccoli and canola. Species in this section are also reported as sources of antibiotic masses.	Gloer et al. (1988), Lawrence et al. (2013), Pedras and Abdoli (2013, 2017), Woudenberg et al. (2013)
	Conidia	Ellipsoid, ovoid or somewhat obclavate, small or moderate in size, septate, slightly or strongly constricted at most of the transverse septa, with no to many longitudinal septa, in moderately long to long, simple or branched chains, with dark septa and cell walls. Apically or laterally form secondary conidiophores with one or a few conidiogenous loci. Sometimes produced chlamydospores.		
Sect. *Chalastospora*	Conidiophores	Short to long, simple or branched, with one or several conidiogenous loci.	Species in this section are primary reported as saprobes and causal agents of human diseases.	Simmons (2007), Mirhendi et al. (2013), Woudenberg et al. (2013)
	Conidia	Pale to medium brown, narrowly ellipsoidal to ellipsoidal or ovoid, beakless, with no or multiple transverse eusepta and rarely longitudinal septa, solitary or in chains. Apically or laterally form secondary conidiophores with one or a few conidiogenous loci.		
Sect. *Cheiranthus*	Conidiophores	Short to moderately long, simple or branched, with one or several conidiogenous loci.	Species in this section are primarily saprobes and pathogens on various plant hosts.	Simmons (2007), Lawrence et al. (2012), Woudenberg et al. (2013)
	Conidia	Ovoid, broadly ellipsoid with transverse and longitudinal septa, slightly or strongly constricted at the septa, in short to long, simple or branched chains.		

Table 4.1 (continued)

***Alternaria* sections**		**Conidiogenesis structures**	**Ecology and economy**	**References**
Sect. *Crivellia*	Conidiophores	Straight or curved, simple or branched, with geniculate, sympodial proliferations. Apically or laterally form secondary conidiophores. Sometimes produced microsclerotia or chlamydospores.	Species in this section are mainly known as pathogens on opium poppy (*Papaver somniferum* L.), the sexual morph of which links with genus *Crivella*.	Inderbitzin et al. (2006), Woudenberg et al. (2013)
	Conidia	Cylindrical, straight to curved to inequilateral, with transverse septa, rarely constricted at septa, single or in short, simple or branched chains.		
Sect. *Dianthicola*	Conidiophores	Simple or branched, with or without apical geniculate proliferations.	Species in this section mainly cause leaf spot and blight on economic vegetation hosts such as carnation (*Dianthus* sp.) and sesame (*Sesamum indicum* L.).	Woudenberg et al. (2013), Choi et al. (2014), Duan et al. (2015)
	Conidia	Narrowly ovoid or narrowly ellipsoid with transverse and few longitudinal septa, slightly constricted at the septa, with a long (filamentous) beak or apical secondary conidiophore, solitary or in short chains.		
Sect. *Embellisia*	Conidiophores	Simple, septate, straight or with geniculate sympodial proliferation.	Species in this section are reported as pathogens on vegetable crops such as tomato and garlic.	Woudenberg et al. (2013), Sadeghi and Mirzaei (2018), Delgado Ortiz et al. (2019)
	Conidia	Solitary, ovoid to subcylindrical, straight to inequilateral, with transverse septa; septa can be thick, dark and rigid in contrast to the external wall. Sometimes sporulated chlamydospores.		

Table 4.1 (continued)

Alternaria sections		Conidiogenesis structures	Ecology and economy	References
Sect. *Embellisioides*	Conidiophores	Simple, septate conidiophores, straight or with multiple, geniculate, sympodial proliferations.	Species in this section are mainly reported as saprobes in soil and pathogen on plant host.	Simmons (1983, 1990), Woudenberg et al. (2013)
	Conidia	Solitary or in short chains, obovoid to ellipsoid, with transverse and longitudinal septa, transverse septa can be thick, dark and rigid in contrast to the external wall. Apical or lateral, short secondary conidiophores may occur. Sometimes produced sexual morph and chlamydospores.		
Sect. *Eureka*	Conidiophores	Simple, septate conidiophores, straight or with geniculate, sympodial proliferations.	Species in this section are reported as pathogens and endophytes that are active in the biotransformation of some secondary metabolites.	Woudenderg et al. (2013), Özçınar et al. (2018), Karakoyun et al. (2021)
	Conidia	Solitary or in short chains, narrowly ellipsoidal to cylindrical, with transverse and longitudinal septa, slightly constricted at the septa, with a blunt rounded apex. Sometimes form apical or lateral, short secondary conidiophores and sporulated sexual morph and chlamydospores.		
Sect. *Euphorbiicola*	Conidiophores	Short to long, broad, apical and sometimes lateral, secondary conidiophores.	Species in this section served as pathogens on economic plants such as *Euphorbiicola* sp. and *Citrus* sp. and also produced secondary metabolites.	Varejão et al. (2013), Woudenberg et al. (2014)
	Conidia	Medium to large sized, in short to moderately long chains, ovoid, obclavate, disto- and euseptate, with multiple transverse and some longitudinal septa, slightly constricted near some transverse septa, with no or a simple long beak in the terminal conidia.		

Table 4.1 (continued)

Alternaria sections		Conidiogenesis structures	Ecology and economy	References
Sect. *Gypsophilae*	Conidiophores	Simple, or occasionally branched, with one or a few conidiogenous loci.	Species in this section occur on the host family Caryophyllaceae.	Simmons (2007), Lawrence et al. (2013), Woudenberg et al. (2013)
	Conidia	Solitary or in short chains, ellipsoid to long ovoid, with multiple transverse and longitudinal septa, conspicuously constricted near some transverse septa. Apically form secondary conidiophores with one or two conidiogenous loci or laterally with a single conidiogenous locus.		
Sect. *Infectoriae*	Conidiophores	Short to long, simple or branched, with one or several conidiogenous loci.	Species in this section are known as saprobes as well as plant and human pathogens.	Simmons (2007), Lawrence et al. (2013), Woudenberg et al. (2013)
	Conidia	Moderately long to long, branched chains, small or moderate sized, obclavate to long ellipsoidal, septate, slightly constricted near some septa, with few longitudinal septa. Apically or laterally formed long geniculate, multi-locus secondary conidiophores, with meristematic growth.		
Sect. *Japonicae*	Conidiophores	Short to long, simple or occasionally branched, with a single conidiogenous locus.	Species in this section particularly occur on hosts in Brassicaceae.	Lawrence et al. (2013), Woudenberg et al. (2013)
	Conidia	Short to long ovoid with transverse and longitudinal septa, conspicuously constricted at most of the transverse septa, in short chains. Apically formed secondary conidiophores with single conidiogenous locus.		

Table 4.1 (continued)

Alternaria sections		Conidiogenesis structures	Ecology and economy	References
Sect. *Nimbya*	Conidiophores	Simple, short to form moderately long, sometimes one to a few short to long, geniculate, sympodial metastasis.	Species in this section are known as saprobes and plant pathogens. Species in this section produce phytotoxins	Simmons (1989), Tanaka et al. (1994), Lawrence et al. (2012), Woudenberg et al. (2013)
	Conidia	Solitary or in short chains, narrowly elongate-obclavate, gradually tapering apically, with transverse disto- and eusepta, sometimes slightly constricted near eusepta.		
Sect. *Omanenses*	Conidiophores	Long, simple, with multiple geniculate, sympodial metastasis or short conidiogenous loci normally with a terminal cluster of three conidia.	Species in this section consist of a core taxon *A. omanensis* which is saprobic on dead woods.	Ghafri et al. (2019)
	Conidia	Solitary, obovoid and sphaeroid, non-beaked, with transverse and longitudinal septa.		
Sect. *Panax*	Conidiophores	Simple or branched, short to moderately long, with one or a few conidiogenous loci.	Species in this section are known as pathogens causing blight on economic plants such as ginseng and American ginseng (Araliaceae)	Simmons (2007), Deng et al. (2013), Lawrence et al. (2013), Woudenberg (2013)
	Conidia	Solitary, simple or branched, in short chains, obclavate to ovoid, with multiple transverse and longitudinal septa, conspicuously constricted near several transverse septa, apically formed secondary conidiophores with one or several conidiogenous loci, multiple lateral secondary conidiophores with a single conidiogenous locus.		

Table 4.1 (continued)

Alternaria sections		Conidiogenesis structures	Ecology and economy	References
Sect. *Phragmosporae*	Conidiophores	Simple, short to moderately long, with one or multiple geniculate, sympodial metastasis.	Species in this section are mainly known as saprobes from soil and marine environments.	Woudenberg et al. (2013)
	Conidia	Solitary or in simple short chains, broad ovoid to long ovoid, ellipsoidal, curved, or limaciform, with multiple transverse and few to multiple longitudinal septa, some septa darkened, slightly to conspicuously constricted near several transverse septa, apically formed secondary conidiophores with one or several conidiogenous loci.		
Sect. *Porri*	Conidiophores	Short to long, simple, with one or several conidiogenous loci.	Species in this section consist of some important phytopathogens and produce phytotoxins.	Stoessl (1969), Ichihara et al. (1983), Lawrence et al. (2013), Woudenberg et al. (2013, 2014)
	Conidia	Solitary or in short to moderately long chains, with a simple or branched, long to filamentous beak, medium or large size, broadly ovoid, obclavate, ellipsoid, subcylindrical or obovoid, disto- and eusepta, with multiple transverse and longitudinal septa, slightly constricted near some transverse septa, apically or laterally formed secondary conidiophores.		
Sect. *Pseudoalternaria*	Conidiophores	Simple or branched, septate, smooth, medium brown, simple with single apical pore, with short to long, simple to multi-geniculate secondary conidiophores with one to many conidiogenous loci.	Species in this section are known as pathogens on plant hosts.	Lawrence et al. (2016)
	Conidia	Mostly catenulate, ellipsoid to obclavate, medium brown to golden brown, with several transverse and longitudinal septa, smooth, secondary conidiophore may occur as false beak.		

Table 4.1 (continued)

Alternaria sections		Conidiogenesis structures	Ecology and economy	References
Sect. *Pseudoulocladium*	Conidiophores	Simple or branched, with short, geniculate, sympodial metastasis.	Species in this section are reported as phytopathogens for human infection.	Woudenberg et al. (2013)
	Conidia	Obovoid, non-beaked with a narrow base, in simple or mostly branched chains, apically formed secondary conidiophores with multiple conidiogenous loci and laterally secondary conidiophores may occur with a single conidiogenous locus.		
Sect. *Radicina*	Conidiophores	Straight, simple or branched, short or long, with multiple, short geniculate, sympodial proliferations, with one to a few conidiogenous loci at the apex.	Species in this section mainly occur on hosts in family Apiaceae.	Woudenberg et al. (2013)
	Conidia	Solitary or in short chains, moderate in size, broadly ovoid to narrowly ellipsoidal, beakless, with several transverse and longitudinal septa, apically formed solitary, short, secondary conidiophores.		

Table 4.1 (continued)

Alternaria sections		Conidiogenesis structures	Ecology and economy	References
Sect. *Soda*	Conidiophores	Simple or occasionally branched, short to moderately long, with one conidiogenous locus.	Species in this section are isolated from soda lake environments (Western Siberia, Russia).	Grum-Grzhimaylo et al. (2016)
	Conidia	Solitary or in short to long, simple or branched chains, moderate to very large in size, narrowly ellipsoid to elongate-ovoid or somewhat obclavate, septate, with transverse and longitudinal septa, conspicuously constricted at most of the transverse septa, produced microsclerotia or chlamydospores, apical or lateral short secondary conidiophores with a single conidiogenous locus may occur, and conidiogenous tip can be enlarged.		
Sect. *Sonchi*	Conidiophores	Simple or branched, with short, geniculate, with one or several conidiogenous loci.	Species in this section mainly occur on a wide range of hosts within Compositae.	Lawrence et al. (2013)
	Conidia	Single or in short chains, medium to large size, subcylindrical, broadly ovoid, broadly ellipsoid or obclavate, with multiple transverse and few longitudinal septa, slightly constricted at the septa.		

Table 4.1 (continued)

Alternaria sections		Conidiogenesis structures	Ecology and economy	References
Sect. *Teretispora*	Conidiophores	Simple, sometimes extending at the apex with one or two, geniculate, sympodial proliferations.	Species in this section consists of a core species, *Alternaria leucanthemi*, which is a phytopathogen causing plant blight disease.	Simmons (2007), Woudenberg et al. (2013)
	Conidia	Single, long cylindrical, lacking a beak portion, with many transverse and a few longitudinal septa, constricted at most of the transverse septa, secondary conidiophores with single conidium from base of primary conidium and rarely formed apically.		
Sect. *Ulocladioides*	Conidiophores	Short, geniculate, sympodial proliferations.	Species in this section are mainly known as phytopathogens causing leaf spot disease and can be saprobes on a variety of host substrates as well as a causal agent of keratitis.	Woudenberg et al. (2013), Lawrence et al. (2016)
	Conidia	Obovoid, non-beaked with a narrow base, single or in chains, with apical secondary conidiophores.		
Sect. *Ulocladium*	Conidiophores	Simple, with one or two short, geniculate, sympodial proliferations.	Species in this section are mainly isolated from plant litters and rarely from marine environments. Potential bioactivities were reported.	Müller-Stöver and Kroschel (2005), Woudenberg et al. (2013)
	Conidia	Single, obovoid, non-beaked, with a narrow base.		
Sect. *Undifilum*	Conidiophores	Simple, septate, straight or with geniculate sympodial proliferation.	Species in this section mainly occur on hosts in family Fabaceae.	Woudenberg et al. (2013)
	Conidia	Ovate to obclavate to long ellipsoid, straight to inequilateral, single, transverse septa, septa can be thick, dark and rigid, and form unique germ tubes, which are wavy or undulate until branching.		

4.2 Materials and Methods

4.2.1 Sample Collections, Examinations and Isolations

Alternaria species were isolated from various hosts (Figure 4.2) mainly collected in Italy, and partly from China (Yunnan), Russia and Thailand between 2014–2019. Materials were brought to the laboratory in Zip-loc plastic bags and examined under a Motic SMZ 168 stereomicroscope. Morphological studies were conducted following the guidelines by Senanayake et al. (2020). Micromorphological characters of *Alternaria* species were examined under a Nikon ECLIPSE 80i compound microscope and images were captured using a Nikon ECLIPSE 80i compound microscope with a Canon EOS 550D digital camera. Measurements were made with the Tarosoft (R) Image Frame Work and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software. New species were justified based on Jeewon & Hyde (2016) and registered in Faces of Fungi (Jayasiri et al., 2015) and Index Fungorum (2021).

Isolates were derived via single spore isolation following the method of Chomnunti et al. (2014) and Senanayake et al. (2020). Germinating spores were transferred to potato dextrose agar (PDA) or malt extract agar (MEA) media and incubated at 18–25°C. The cultural characteristics such as mycelium color, shape, texture and growth rate were determined after 1–8 weeks. The sporulation *in vitro* was observed after 8 weeks. The living cultures were preserved in PDA, the sterilized 10% glycerol, and double-distilled water (ddH_2O) and deposited at the Mae Fah Luang University Culture Collection (MFLUCC), Culture Collection of Kunming Institute of Botany (KUMCC). The type and other collected specimens were deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and Herbarium of Cryptogams Kunming Institute of Botany, Academia Sinica (KUN-HKAS), China.

4.2.2 DNA Extraction, PCR Amplification and Sequencing

Fungal isolates cultured on PDA or MEA at 25°C–28°C for 25–30 days were used for genomic DNA extraction following the guidelines by Dissanayake et al (2020). Fungal mycelium was scraped off and stored in a sterilized 1.5-ml microcentrifuge for further DNA extraction. Fungal genomic DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit (BSC14S1 (BioFlux®, China), following the manufacturer instructions.

DNA amplifications were conducted by polymerase chain reaction (PCR) with seven gene loci as listed in Table 2. Polymerase chain reaction (PCR) was performed in a ABI Veriti gradient PCR machine (Applied Biosystem, Unite states) with the total 25 µl reaction volume, containing 1 µl of DNA template, 1 µl of each forward and reverse primers, 12.5 µl of 2× Power Taq PCR Master Mix (mixture of EasyTaqTM DNA Polymerase, dNTPs, and optimized buffer, Beijing BioTeke Corporation, P.R. China) and 9.5 µl of sterilized double-distilled water (ddH_2O). PCR thermal cycling conditions of each locus was set up following Woudenberg et al. (2013) but adjusted following conditions presented in Figure 4.3. The PCR fragments were then checked on 1% agarose electrophoresis gels stained with ethidium bromide. The amplified PCR fragments were sent to a commercial sequencing provider (TsingKe Biological Technology (Beijing) Co., Ltd, P.R. China) for purification and sequencing in both forward and reverse directions using PCR primers. Consensus sequences were incorporated with both forward and reverse sequences, computed by Bioedit v.7.1.3.0 (Hall, 1999). All acquired nucleotide sequences were deposited in GenBank (Table S1).



Note **a** *Atriplex* sp. (Amaranthaceae). **b**, **c** *Brassica* sp. (Brassicaceae). **d** *Citrullus lanatus* (Thunb.) Mastum. & Nakai (Cucurbitaceae). **e** *Cucurbita moschata* L. (Cucurbitaceae). **f** *Cucurbita argyrosperma* K. Koch (Cucurbitaceae). **g** *Capsicum annuum* L. (Solannaceae). **h** *Dictylis* sp. (Poaceae). **i** *Digitalis* sp. (Plantaginaceae). **j** *Dimocarpus longan* Lour. (Sapindaceae). **k** *Euphorbia cannabinum* L. (Asteraceae). **l** *Euphorbia esula* L. (Euphorbiaceae). **m** *Lathyrus* sp. (Fabaceae). **n**–**p** Fabaceae spp. (Fabaceae). **q** *Raphanus sativus* L. (Brassicaceae). **r** *Reseda* sp. (Resedaceae). **s** *Scabiosa* sp. (Caprifoliaceae). **t** *Solanum* sp. (Solannaceae). **u** Solannaceae sp. (Solannaceae). **v** *Spartium* sp. (Fabaceae). **w** Poaceae sp. (Poaceae). **x** *Bambusa* sp. (Poaceae). **y** *Zea mays* L. (Poaceae). **z** Plant litters.

Figure 4.2 Diverse host preferences of *Alternaria* species

Table 4.2 Gene loci and primers used in this study

Gene loci	Primers	Sequence 5'–3'	References
Internal transcribed spacer region (ITS, including the 5.8S gene)	ITS5	GGA AGT AAA AGT CGT AAC AAG G	White et al. (1990)
	ITS4	TCC TCC GCT TAT TGA TAT GC	
28S large subunit rDNA (LSU)	LR0R	TCC TGA GGG AAA CTT CG	Vilgalys and Hester (1990)
	LR5	ACC CGC TGA ACT TAA GC	
18S small subunit rDNA (SSU)	NS1	GTA GTC ATA TGC TTG TCT C	White et al. (1990)
	NS4	CTT CCG TCA ATT CCT TTA AG	
Alternaria major allergen (Alt-a1)	ALT-F	CAY CCW GGY TTY ATC AAG AA	Crous et al. (2015)
	ALT-R	CCN GCD ATN TCR TTR TCC ATR TA	
Glyceraldehyde 3-phosphate Dehydrogenase (GAPDH)	GDP-1	GGTAACCAAATCGGTGCTGCTTTC	Crous et al. (2015)
	GDP-2	ACCCTCAGTGTAGTGACCCTTGGC	
Plasma membrane ATPase (ATPase)	ATPDF1	ATC GTC TCC ATG ACC GAG TTC G	Lawrence et al. (2013)
	ATPDR1	TCC GAT GGA GTT CAT GAT AGC C	
The second largest subunit of RNA polymerase II (RPB2)	fRPB2-5f	GAY GAY MGW GAT CAY TTY GG	Liu et al. (1999)
	fRPB2-7cR	CCC ATR GCT TGY TTR CCC AT	
Translation elongation factor 1-α (TEF1-α)	EF1-983F	GCY CCY GGH CAY CGT GAY TTY AT	Rehner (2001)
	EF1-2218R	ATG ACA CCR ACR GCR ACR GTY TG	
	EF1-728F	CATCGAGAAGTTCGAGAAGG	Carbone and Kohn (1999)
	EF1-986R	TACTTGAAGGAACCCTTACC	

4.2.3 Phylogenetic Analyses

The quality of the generated ITS, LSU, SSU, TEF1-α, RPB2, GAPDH, Alt- a1and ATPase sequences was checked using Bioedit v. 7.1.3.0 (Hall, 1999) and subjected to the nucleotide BLAST search engine via the NCBI (https://www.ncbi.nlm.nih.gov/) for checking potential contaminants or erroneous sequences as well as delineating the closely related taxa. All reference sequences were downloaded from GenBank based on recent publications (Woudenberg et al., 2015; Poursafar et al., 2017; Thambugala et al., 2017; Cai et al., 2019; Liu et al. 2019; Marin-Felix et al., 2019; Iturrieta-González et al., 2020; Nishikawa & Nakashima, 2020; He et al., 2021b).

The multiple sequence alignments were automatically generated by MAFFT v. 7.452 (Katoh et al., 2019; https://mafft.cbrc.jp/alignment/software/), and manual improvements were made where necessary using BioEdit v. 7.2 (Hall, 1999). Individual gene alignments were separately analyzed by maximum likelihood (ML) in order to check the congruence of tree topology and thus the combined multi-locus phylogenetic trees were inferred based on Bayesian inference (BI) and maximum likelihood (ML) analyses. Six different datasets were generated to estimate phylogenic relationships of *Alternaria* sections (analysis 1), sect. *Alternaria* (analysis 2), diversity of *A. alternata* (analysis 3), sect. *Infectoriae* (analysis 4), sect. *Porri* (analysis 5), and sect. *Radicina* (analysis 6).

Maximum likelihood (ML) analyses were performed by RAxML (Stamatakis, 2008) implemented in raxmlGUI 1.3 (Silvestro & Michalak, 2012) with 1000 bootstrap replicates and GAMMAI model of nucleotide substitution. MrModeltest v. 2.3 (Nylander et al., 2004) was used to determine the best-fit model of nucleotide substitution for each locus and incorporated into the analyses (Table 4.3). Bayesian inference (BI) analyses were performed by MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). Markov Chain Monte Carlo (MCMC) of six simultaneous Markov chains were run with 1–5 million generations to determine posterior probabilities (PP) (Rannala & Yang, 1996; Zhaxybayeva & Gogarten, 2002) and started from a random tree topology. Trees were frequently sampled at 100^{th} generation and the temperature value of heated chain was set to 0.15. The extra runs were required when the average standard deviation of split frequencies did not lower than 0.01 after one million generation. The first 25% trees represented the burn-in phase of the analyses, were discarded and the remaining trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree. The phylogram were visualized in

FigTree v. 1.4.0 (Rambaut, 2012) and edited in Microsoft Office PowerPoint 2016 (Microsoft Inc., United States). The final alignments and trees were submitted TreeBASE (https://www.treebase.org/).

Table 4.3 The best nucleotide substitution model for each locus based on the Akaike information Criterion (AIC) generated by MrModeltest v. 2.3.

	ITS	LSU	SSU	GAPDH	RPB2	TEF1-α	Alt-a1	ATPase
A1: *Alternaria* sections	GTR+I+G	GTR+G	TrN+I+G	SYM+I+G	GTR+I+G	TIM1+I+G	GTR+I+G	n/a
A2: sect. *Alternaria*	GTR+I+G	GTR+I+G	TIM2+I+G	SYM+I+G	TIM2+I+G	TIM1+I+G	GTR+I+G	n/a
A3: *A. alternata*	GTR+I+G	GTR+I+G	GTR+I+G	GTR+I+G	TIM2 +G	GTR+I+G	GTR+I+G	n/a
A4: sect. *Infectoriae*	GTR+I+G	n/a	n/a	GTR+I+G	n/a	n/a	n/a	SYM+G
A5: sect. *Porri*	SYM+I+G	n/a	n/a	TIM2+I+G	GTR+I+G	GTR+G	GTR+I+G	n/a
A6: sect. *Radicina*	GTR+I+G	n/a	n/a	GTR+I+G	TIM2+G	GTR+I+G	n/a	n/a

4.2.4 Fossil Calibration, Divergence Time and Evolutionary Rate Estimations

Fossil calibrations used in the analyses followed the methodology described in Phukhamsakda et al. (2016). Two fossil and one secondary calibration were applied to estimate all other nodes in the tree. Fossil 1, *Metacapnodium succinum* (Metacapnodiaceae) was used to calibrate the minimum age of Capnodiales (normal distribution, mean = 100, SD = 150, providing 95% credibility interval of 346 MYA) (Rikkinen et al. 2003; Beimforde et al. 2014; Hongsanan et al. 2016, Pérez-Ortega et al. 2016, Phukhamsakda et al. 2016) and fossil 2, *Margaretbarromyces dictyosporus* was used to calibrate the crown age of *Aigialus* (Aigialaceae) (gamma distribution, offset = 35, shape = 1.0, scale = 25, providing 95% credibility interval of 110 MYA) (Mindell et al. 2007, Phukhamsakda et al. 2016). The split between Arthoniomycetes and Dothideomycetes was calibrated using the results from Phukhamsakda et al. (2016) as the secondary calibration (normal distribution, mean = 300, SD = 50, providing 95% credibility interval of 382 MYA).

Evolutionary estimation based on molecular clock analysis was performed by BEAST 1.8.4 (Drummond et al., 2012). Aligned sequence data were partitioned separately for each ITS, LSU, SSU, TEF1-α and RPB2 dataset, and were loaded to prepare an XML file constructed with BEAUti v1.8.4. Clock and substitution models were set to be independently estimated for each gene partition, while the tree prior parameters were set to be linked across partitions. A lognormal relaxed clock (uncorrelated) model was applied with a lognormal distribution of rates for each gene estimated. The best fit of substitution models was selected based on jModeltest2 v.0.1.1 (Darriba & Posada, 2014) for each gene partition, resulting as ITS = GTR+I+G, LSU = GTR+I+G, SSU = TIM2+I+G, TEF1-α = SYM+I+G, RPB2 = TIM2+I+G; Yule processed tree prior with a randomly generated starting tree. The analysis was performed for 100 million generations in BEAST v1.8.4, sampling parameters every 10000 generations. The effective sample sizes (ESS) were checked by Tracer v1.6 (Rambaut et al., 2014) and accepted when ESS values were higher than 200. The first 10% trees were discarded as a burn-in phase. The remaining trees were combined in LogCombiner 1.8.0. A maximum clade creditability (MCC) tree was generated by summarized data and estimated in TreeAnnotator v1.8.0. The tree was visualized with FigTree v1.4.0 (Rambaut, 2012).

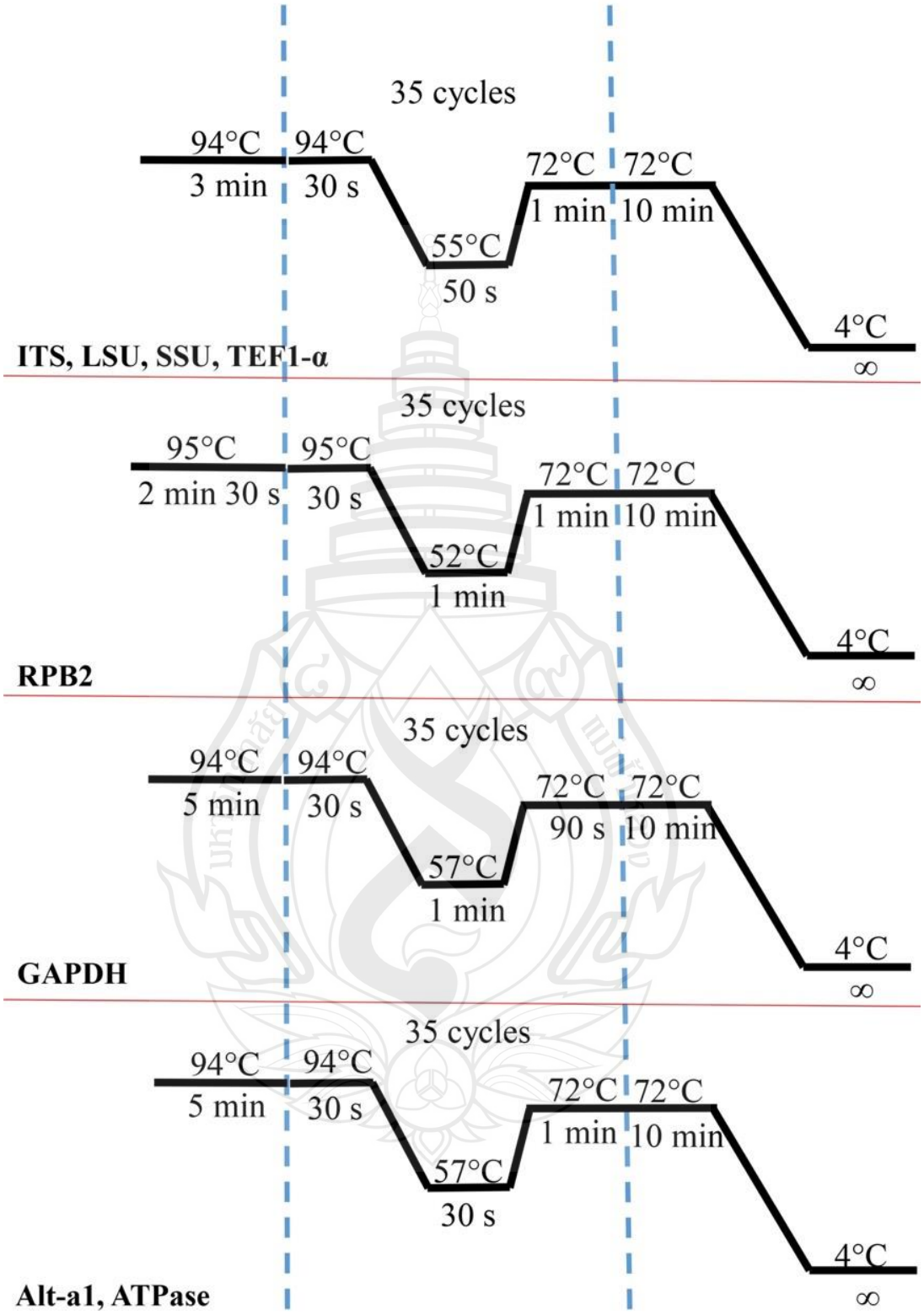


Figure 4.3 PCR thermal cycling condition used to amplify gene loci in this study

4.3 Phylogeny

4.3.1 Phylogeny of Pleosporales and Divergence Time Estimations

Representative strains of taxa in Pleosporales were analyzed based on a combined ITS, LSU, SSU, TEF1-α and RPB2 DNA sequence dataset comprised of 227 strains of ingroup taxa. Four species in Arthoniomycetes (*Arthonia dispersa* UPSC2583, *Dendrographa leucophaea* f-minor, *Roccella fuciformis* Tehler 8171 and *Schismatomma decolorans* Ertz 5003) were selected as the outgroup taxa. The final ML optimization likelihood value of -61934.763756 (ln). RAxML analysis yielded 1831 distinct alignment patterns and 19.69% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.252569, C = 0.228251, G = 0.283442, T = 0.235738, with substitution rates AC = 1.554349, AG = 4.323993, AT = 1.241832, CG = 1.108361, CT = 8.993327, GT = 1.000000. The gamma distribution shape parameter alpha = 0.307857 and the Tree-Length = 19.058776. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with final average standard deviation of split frequencies = 0.009034. The final alignment and tree were submitted in TreeBASE as submission ID: 258527. The phylogenetic results of Pleosporales (Figure 4.4) showed overall similar tree topology with maximum clade credibility (MCC) tree (Figure 4.5). *Alternaria* sections formed well-resolved and stable clades (up to 80% ML, 0.95 PP; Figure 4.4) within Pleosporaceae; while the phylogenetic statuses of sects. *Embellisioides* and *Eureka* (Fig. 4: phylogenetic tree of Pleosporales) are not well-resolved, concurring with phylogenetic results of *Alternaria* sections (Analysis 1; Figure 4.6: phylogenetic tree of genus *Alternaria*).

According to divergence time estimates (Figure 4.5), the stem and crown ages of Dothideomycetes are 358 (266–492) Mya and 310 (230–392) Mya in the Permian Priod, respectively (Figure 4.5). Pleosporales diverged with other orders roughly 253 (184–326) Mya in the Triassic Priod. The crown age of Pleosporales is around 233 (168–301) Mya in the Late Triassic. The crown and stem ages of Dothideomycetes and Pleosporales in the MCC tree (Figure 4.5) are well-supported, falling in the recommended divergence times for class and order status by Liu et al. (2017) and Hongsanan et al. (2020). In Pleosporales, Pleosporinae diverged approximately 120 (84–159) Mya in Cretaceous. The stem age of *Alternaria* is at 62 (42–85) Mya and the crown age of *Alternaria* is at 53 (36–72) Mya in

the age of late Paleocene to early Eocene. The species occurried in the sections that diverged earlier than other sections in *Alternaria* with beakless, rare multi and longitudinal septate conidia, less forming secondary conidiophores such as species in sects. *Crivellia*, *Phragmospora*, *Ulocladium*, and *Undifilum*, while later diverged sections mostly comprise species with beaks or multi-septate conidia forming secondary coniophores with conidiogenous loci (Lawrence et al., 2013, 2016; Woudenberg et al., 2013, 2014, 2015). Divergence times of other sections in the analysis are shown.

4.3.2 Phylogenetic Analyses of *Alternaria* Sections

In this study, six phylogenetic trees were conducted to define the phylogenetic placements of the novel *Alternaria* species and relationships of taxa in *Alternaria* sections. The multi-locus phylogenetic tree (Figure 4.6) demonstrated that 24 novel species were delineated in sects. *Alternaria, Infectoriae, Porri* and *Radicina.* Twenty new species and two new records on host and geography are introduced in sect. *Alternaria* and two novel species are introduced to sect. *Infectoriae*, and the other two new species are introduced in sects. *Porri* and *Radicina*, respectively.

Analyses 1 revealed phylogenetic relationships of the representative *Alternaria* taxa in 28 sections and the novel species introduced in this study. The combined ITS, LSU, SSU, TEF1-α, RPB2, GAPDH and Alt-a1 sequence dataset comprises 181 taxa with *Stemphylium vesicarium* (CBS 191.86) and *Pleospora tarda* (CBS 714.68) as the outgroup taxa. Bayesian Inference (BI) and maximum likelihood (ML) analyses of the combined dataset resulted in phylogenetic reconstructions with largely similar topologies. The best scoring RAxML tree is shown in Figure 4.6, with the final ML optimization likelihood value of – 41879.983934 (ln). The dataset consists of 4689 total characters, including gaps (ITS: 1–679 bp, LSU: 680–1532 bp, SSU: 1533–2459 bp, TEF1-α: 2460–2740 bp, RPB2: 2741–3614 bp, GAPDH: 3415–4053 bp, Alt-a1: 3616–4689 bp). RAxML analysis yielded 1635 distinct alignment patterns and 13.99% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.248122, C = 0.254245, G = 0.257134, T = 0.240499, with substitution rates AC = 1.333971, AG = 3.383335, AT = 1.152678, CG = 0.900077, CT = 5.831606, GT = 1.000000. The gamma distribution shape parameter alpha = 0.215149 and the Tree-Length = 2.947780. The final alignment and tree were submitted in TreeBASE as submission ID: 258521. Bayesian posterior probabilities (BYPP) from

MCMC were evaluated with the final average standard deviation of split frequencies = 0.008882. Present multi-locus analyses (Figure 4.6) demonstrated that most *Alternaria* sections formed well-resolved clades with high support values (up to 80% ML, 0.95 PP), excluding sects. *Cheiranthus, Eureka,* and *Undifilum*. Section *Cheiranthus* clustered with sects. *Dianthicola* and *Pseudoulocladium* with significant support in BI analysis (0.96 PP) but low support in ML analysis. Three representative species in sect. *Cheiranthus* (*A. cheiranthi*, *A. indefessa* and *Alternaria* sp.) grouped together with significant support in ML analyses (72% ML) but low support in BI analysis; while the clades of sect. *Eureka* and sect. *Embellisioides* were not well separated, and grouped together with high support values (93% ML, 0.99 PP). The putative strain of *A. bornmuelleri* (DAOM 231361), represented sect. *Undifilum* and formed an independent lineage (0.95 PP) with *A. dennisii* (CBS 476.90, CBS 110533).

Analyses 2 represented phylogenetic relationships of taxa in sect. *Alternaria* as well as the placement of 20 new species and a new host and geographical records. Phylogenetic construction of sect. *Alternaria* based on a combined ITS, LSU, SSU, TEF1-α, RPB2, GAPDH and Alt-a1 DNA sequence dataset comprises 117 sequences of 39 representative species in sect. *Alternaria* and *A. alternantherae* (CBS 124392) was selected as the outgroup taxon. The best scoring RAxML tree is shown in Figure 4.7 with the final ML optimization likelihood value of -11900.182055 (ln). The dataset consists of 4377 total characters, including gaps (ITS: 1–514 bp, LSU: 515–1368 bp, SSU: 1369–2295 bp, TEF1-α: 2296–2540 bp, RPB2: 2541–3311 bp, GAPDH: 3312–4377 bp). RAxML analysis yielded 544 distinct alignment patterns and 8.35% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.246778, C = 0.253828, G = 0.258720, T = 0.240673, with substitution rates AC = 1.057943, AG = 2.495083, AT = 1.104630, CG = 0.860449, CT = 5.139622, GT = 1.000000. The gamma distribution shape parameter alpha = 0.020000 and the Tree-Length = 0.262541. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with final average standard deviation of split frequencies = 0.008673. The final alignment and tree were submitted in TreeBASE as submission ID: 258522. Twenty new species are introduced in this section, including *A. arctoseptata, A. baoshanensis, A. breviconidiophora, A. ellipsoconidia, A. eupatoriicola, A. falcata, A. lathyri, A. macilenta, A. macroconidia, A. minimispora, A. muriformispora, A. oblongoellipsoidea, A. obpyriconidia, A. orobanches, A. ovoidea, A. phragmiticola, A.*

pseudoinfectoriae, A. rostroconidia, A. salicicola and *A. torilis.* These novel species formed independent well-supported subclades (up to 80% ML and 0.95 PP; Figure 4.7) within sect. *Alternaria*. The new collection, *A. doliconidium* (H-14), clustered with the type strains (HKAS 100840, MFLUCC 17-0263) of *A. doliconidium* with high support values (100% ML, 100 PP; Figure 4.7). However, the species does not form a well-resolved clade and clustered with other strains of *A. alternata* and *A. italica*.

Analyses 3 represented the diversity of *Alternaria alternata* corresponding with their hosts. Phylogenetic construction of *A. alternata* based on a combined ITS, LSU, SSU, TEF1-α, RPB2, GAPDH and Alt-a1 DNA sequence dataset comprised 110 strains with *A. eichhorniae* (CBS 489.92, CBS 119778) as the outgroup taxon. The best scoring RAxML tree is shown in Figure 4.8, with the final ML optimization likelihood value of -6848.962092 (ln). The dataset consists of 4377 total characters including gaps (ITS: 1–514 bp, LSU: 515–1368 bp, SSU: 1369–2295 bp, TEF1-α: 2296–2540 bp, RPB2: 2541–3311 bp, GAPDH: 3312–3897 bp, Alt-a1: 3898–4377 bp). RAxML analysis yielded 74 distinct alignment patterns and 1.54% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.246749, C = 0.253377, G = 0.260395, T = 0.239479, with substitution rates AC = 5.780289, AG = 13.046393, AT = 1.475727, CG = 0.816872, CT = 36.600039, GT = 1.000000. The gamma distribution shape parameter alpha = 0.020000 and the Tree-Length = 0.029480. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with final average standard deviation of split frequencies = 0.008559. The final alignment and tree were submitted in TreeBASE as submission ID: 258523. *Alternaria alternata* strains conducted in this study were obtained from diverse plant hosts and humans. Forty-five new collections of *A. alternata* were included in the present analyses and are reported for different hosts and geography from China, Italy and Thailand (see Table 4.5). Multi-locus phylogenetic analyses (Figure 4.8) showed a high intraspecific genetic variation of *A. alternata.* This phylogenetic result concurs with Woudenberg et al. (2015), who synonymized 35 species under *A. alternata* because the multi-locus phylogeny could not be distinguished between these species. However, the strains of *A. alternata* can be distinguished into five main subclades with significant support (up to 80% ML, 0.95 PP) in this study. Hence, existing strains of *A. alternata* may represent five species rather than a single species, and further work is needed to clarify the phylogeny.

Analyses 4 represented phylogenetic relationships of two novel taxa, *Alternaria arundinis* and *A. nodulariconidiophora*, with other representative species in sect. *Infectoriae* and sect. *Pseudoalternaria*. Phylogenetic construction of sect. *Infectoriae* is based on a combined ITS, GAPDH and ATPase DNA sequence dataset comprising 68 strains of ingroup taxa and two taxa in sect. *Chalastospora* (*A. gossypii* CBS 135.31 and *A. abundans* CBS 534.83) were selected as the outgroup taxa. The best scoring RAxML tree is shown in Figure 4.31 with the final ML optimization likelihood value of -8122.101740 (ln). The dataset consists of 2280 total characters, including gaps (ITS: 1–561 bp, GAPDH: 562–1081 bp, ATPase: 1082–2280 bp). RAxML analysis yielded 428 distinct alignment patterns and 7.89% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.221370, C = 0.311012, G = 0.250900, T = 0.216718, with substitution rates AC = 1.538856, AG = 2.663454, AT = 1.056408, CG = 1.258484, CT = 8.888843, GT = 1.000000. The gamma distribution shape parameter alpha = 0.122301 and the Tree-Length = 0.445658. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with final average standard deviation of split frequencies = 0.008633. The final alignment and tree were submitted in TreeBASE as submission ID: 258524. Three strains of *A. arundinis* (IT2250_A, IT2250_B, IT2240_C) formed a monophyletic subclade (92% ML, 0.99 PP), sister to *A. incomplexa* (CBS 121330) with significant support (89% ML, 1.00 PP), while *A. nodulariconidiophora* (IT2625A_1, IT2625A_2) clustered with *Alternaria* sp. (UTFC-814) and *A. humuli* (CBS 119404) with significant support (88% ML, 0.99 PP). Many *Alternaria* spp. isolated from black head mold-affected wheat and barley in Iran were included in the present analyses and remained phylogenetically unresolved, concurring with Poursafar et al. (2017). Unfortunately, phylogenetic affinities of most species in this section are characterized by internally low support values, also in agreement with Poursafar et al. (2017) and Marin-Felix et al. (2019). More informative phylogenetic markers such as ATPase and cmdA genes, were suggested for ues at species level identification due to species in sect. *Infectoriae* showing high genetic variation (Lawrence et al., 2013, 2016; Marin-Felix et al., 2019).

Analyses 5 represented phylogenetic relationships of the novel species, *Alternaria brevirostra* with other representative species in sect. *Porri*. Phylogenetic construction of *Alternaria* sect. *Porri* based on a combined ITS, GAPDH, TEF1-α, RPB2 and Alt-a1 DNA sequence dataset comprised 114 strains of 65 ingroup species. Five strains of three species

in sect. *Euphorbiicola* (*A. limicola* CBS 483.90, CBS 117360 and *A. euphorbiicola* CBS 119460, CBS 198.86) and sect. *Gypsophilae* (*A. gypsophilae* CBS 107.41) were selected as the outgroup taxa. The best scoring RAxML tree is shown in Fig. 35 with the final ML optimization likelihood value of -11626.467690 (ln). The dataset consists of 2715 total characters, including gaps (ITS: 1–539 bp, GAPDH: 540–1121 bp, TEF1-α: 1122–1463 bp, RPB2: 1464–2239 bp, Alt-a1: 2240–2715). RAxML analysis yielded 597 distinct alignment patterns and 3.43% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.231369, C = 0.294521, G = 0.244128, T = 0.229982, with substitution rates AC = 0.982217, AG = 4.158740, AT = 0.987304, CG = 0.590117, CT = 8.931720, GT = 1.000000. The gamma distribution shape parameter alpha = 0.207829 and the Tree-Length = 0.669039. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with the final average standard deviation of split frequencies = 0.008326. The final alignment and tree were submitted in TreeBASE as submission ID: 258525. Two strains of the novel species, *A. brevirostra* (IT2195_A, IT2028), formed a sister lineage with *Alternaria macrospora* (CBS 117228) and clustered with *A. cucumerina* (CBS 116114), *A. venezuelensis* (CBS 116121) and *A. aragakii* (CBS 594.93), with significant support in ML and BI analyses (74% ML, 0.99 PP; Figure 4.35). According to Woudenberg et al. (2013, 2014), sect. *Porri* displays high genetic variation, and the phylogenetic status of many species was still unable to be resolved in this study.

Analyses 6 represented phylogenetic relationships of the new taxon, *Alternaria phytolaccae*, with other species in sect. *Radicina* and the closely related sect. *Gypsophilae*. Phylogenetic construction of sect. *Radicina* based on a combined ITS, TEF1-α, RPB2, and GAPDH DNA sequence dataset comprised 18 strains of ingroup taxa and *A. helianthiinficiens* (CBS 208.86, CBS 117370) was selected as the outgroup taxon. The best scoring RAxML tree is shown in Fig. 37 with the final ML optimization likelihood value of -5160.537105 (ln). The dataset consists of 2209 total characters, including gaps (ITS: 1–521 bp, TEF1-α: 522–767 bp, RPB2: 768–1636 bp, GAPDH: 1637–2209 bp). RAxML analysis yielded 220 distinct alignment patterns and 8.90% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.246820, C = 0.276063, G = 0.242024, T = 0.235092, with substitution rates AC = 1.221528, AG = 4.038587, AT = 0.589501, CG = 0.595656, CT = 9.444647, GT = 1.000000. The gamma distribution shape parameter alpha 0.191394 and the Tree-Length = 0.206922. Bayesian

posterior probabilities (BYPP) from MCMC were evaluated with final average standard deviation of split frequencies = 0.008477. The final alignment and tree were submitted in TreeBASE as submission ID: 258526. Two strains of the novel species, *A. phytolaccae* (IT2279_A, IT2279_B), formed a strongly support clade (98% ML, 1.00 PP) and clustered with *A. selini* (EGS 25-198), *A. petroselini* (CBS 112.41) and *A. vulgarae* (YZU161234, YZU161235), with significant support in BI analyses (0.97 PP).

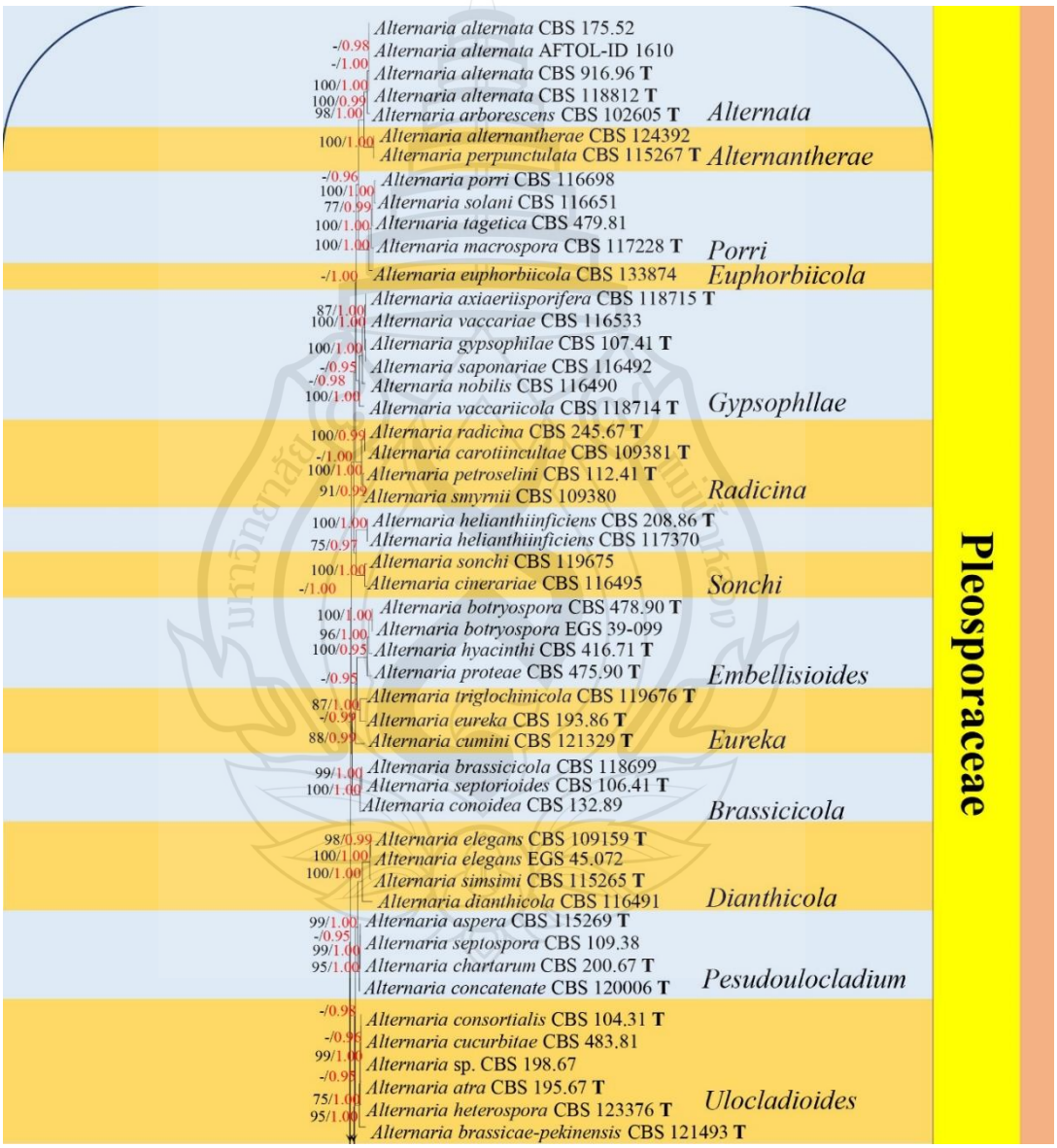


Figure 4.4 Phylogenetic construction of Pleosporales using RAxML-based maximum likelihood analysis of a combined multigens

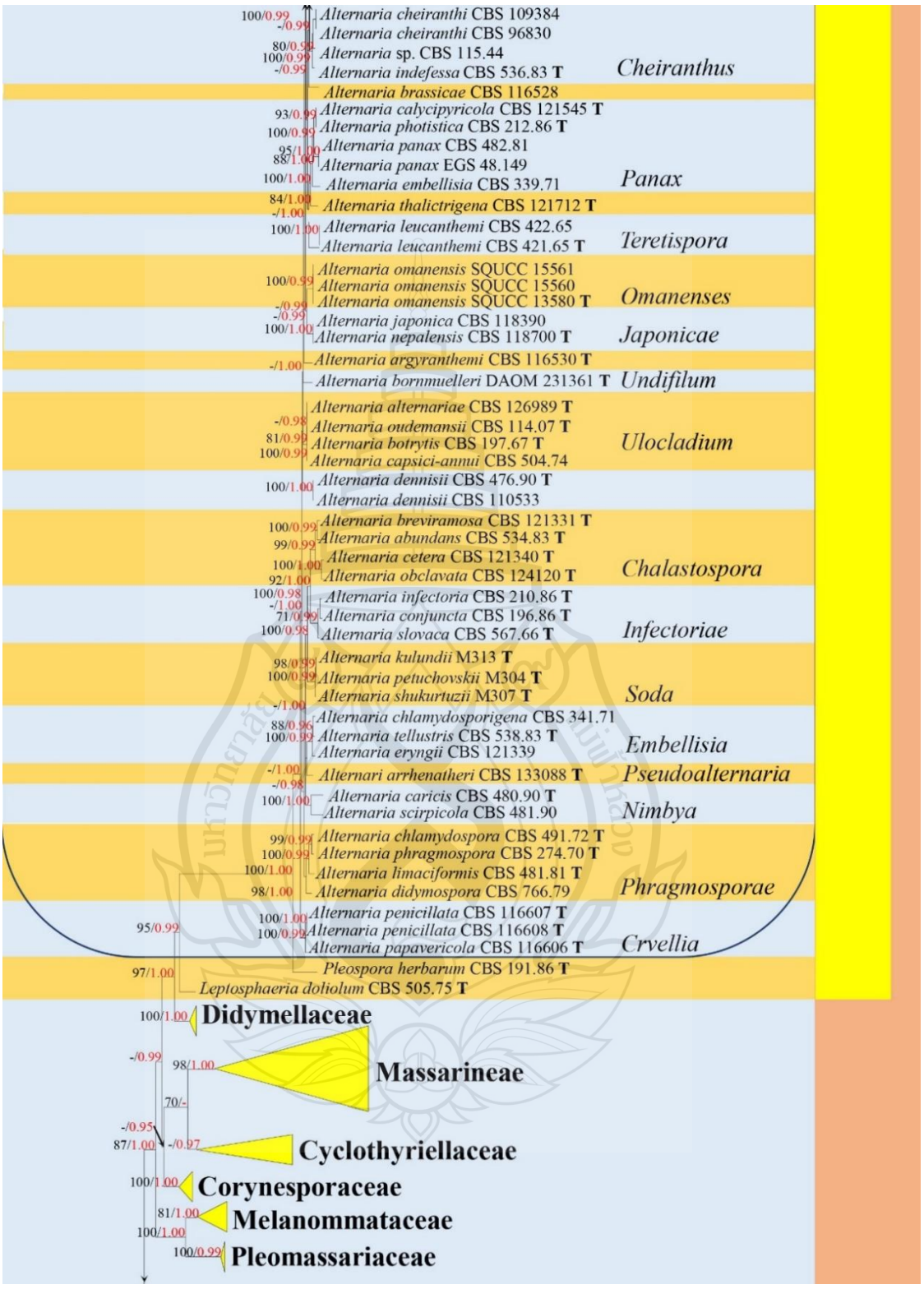


Figure 4.4 (continued)

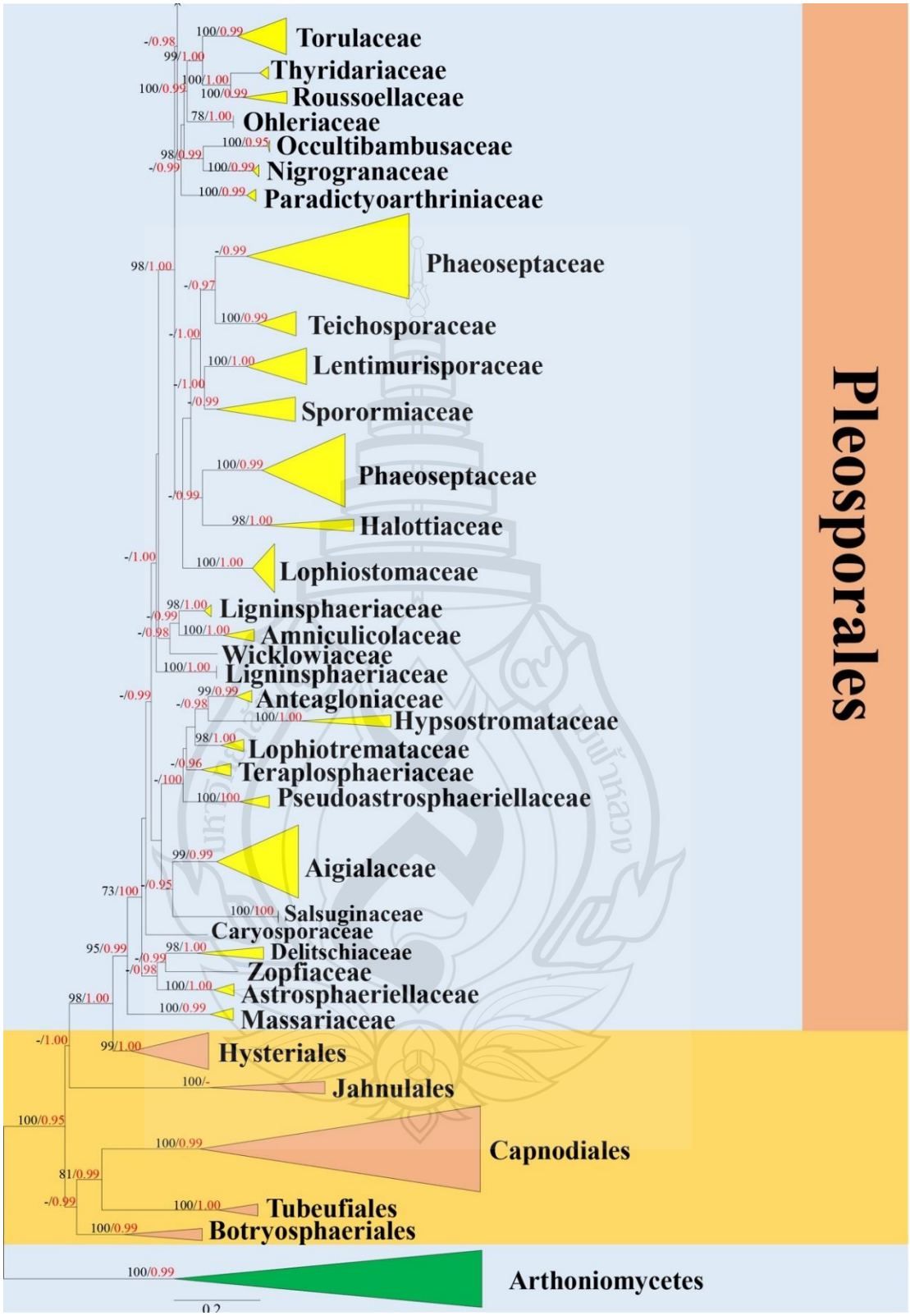


Figure 4.4 (continued)

Figure 4.4 Phylogenetic construction of Pleosporales using RAxML-based maximum likelihood analysis of a combined ITS, LSU, SSU, TEF1-α and RPB2 DNA sequence dataset. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 70% and Bayesian posterior probabilities (PP, red) equal to or greater than 0.95 PP are shown above the nodes. The tree is rooted to Arthoniomycetes (*Arthonia dispersa* UPSC2583, *Dendrographa leucophaea* f-minor, *Roccella fuciformis* Tehler 8171 and *Schismatomma decolorans* Ertz 5003). The type strains are indicated by superscript "T".

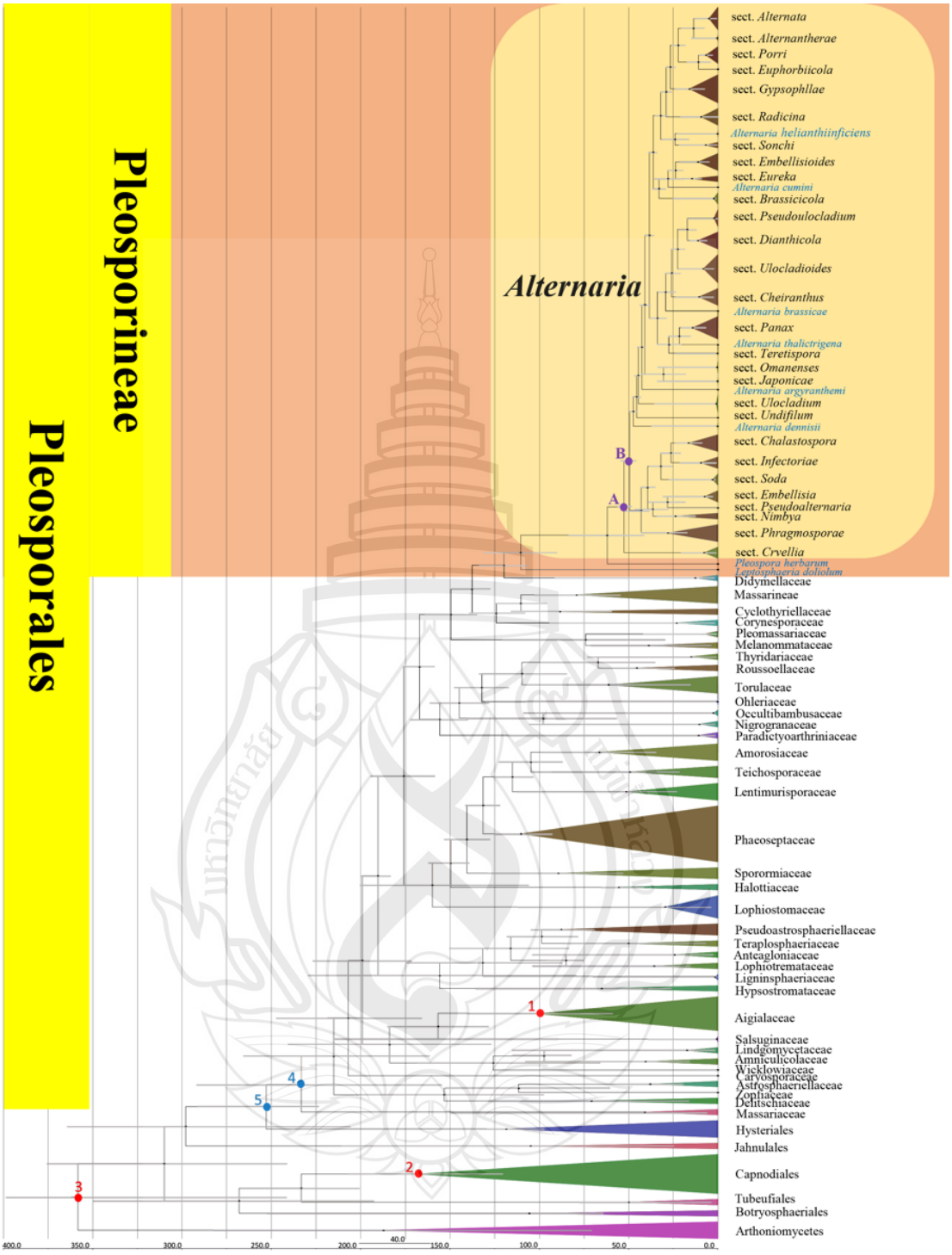


Figure 4.5 Maximum clade credibility (MCC) tree with divergence times estimates obtained from BEAST

Figure 4.5 Maximum clade credibility (MCC) tree with divergence times estimates obtained from BEAST. Numbers in the red color indicate the fossil (1, 2) and secondary (3) points. Numbers in the blue color indicate divergence time estimate of Pleosporales (stem age: 5, crown age: 4). Letters in the purple color indicate divergence time estimate of *Alternaria* (stem age: A, crown age: B). Single lineages of *Alternaria* species are highlighted in blue.

Table 4.4 Divergence times of *Alternaria* sections indicated in MCC tree

Order	Family	Genus	Sections	Divergence times (crown age)	Divergence times (stem age)
Pleosporales				233 (168–301) Mya	252 (184–326) Mya
	Pleosporinaceae			110 (79–148) Mya	120 (84–159) Mya
		Alternaria		53 (36–71) Mya	62 (42–85) Mya
			Alternaria sect. *Alternantherae*	0.4 (0–1.5) Mya	14 (6.7–21) Mya
			Alternaria sect. *Alternaria*	5 (1.7–10) Mya	14 (6.7–21) Mya
			Alternaria sect. *Brassicicola*	2.3 (0.5–5.5) Mya	33 (22–45) Mya
			Alternaria sect. *Chalastospora*	16 (8.8–26) Mya	26 (16–38) Mya
			Alternaria sect. *Cheiranthus*	11 (4.23–20) Mya	26 (16–38) Mya
			Alternaria sect. *Crivellia*	7.6 (1.5–19) Mya	53 (36–71) Mya
			Alternaria sect. *Dianthicola*	11 (5.4–18) Mya	17 (10–27) Mya
			Alternaria sect. *Embellisia*	7.4 (2.5–15) Mya	28 (14–43) Mya
			Alternaria sect. *Embellisioides*	11 (5–19) Mya	24 (14–36) Mya
			Alternaria sect. *Eureka*	14 (5.6–24) Mya	28 (18–44) Mya
			Alternaria sect. *Euphorbiicola*	-	11 (5.6–17) Mya

Table 4.4 (continued)

Order	Family	Genus	Sections	Divergence times (crown age)	Divergence times (stem age)
			Alternaria sect. *Gypsophilae*	16 (7.6–26) Mya	27 (18–37) Mya
			Alternaria sect. *Infectoriae*	9.5 (4–17) Mya	26 (16–38) Mya
			Alternaria sect. *Japonicae*	–	31 (14–47) Mya
			Alternaria sect. *Nimbya*	24 (11–39) Mya	36 (28–51) Mya
			Alternaria sect. *Omanenses*	–	30 (14–47) Mya
			Alternaria sect. *Panax*	14 (6.8–23) Mya	22 (12–33) Mya
			Alternaria sect. *Phragmosporae*	28 (13–44) Mya	42 (28–58) Mya
			Alternaria sect. *Porri*	6.7 (3–11) Mya	11 (5.6–17) Mya
			Alternaria sect. *Pseudoalternata*	–	28 (14–43) Mya*
			Alternaria sect. *Pseudoulocladium*	2.1 (0.4–5.8) Mya	17 (9.5–27) Mya
			Alternaria sect. *Radicina*	9.3 (3.6–18) Mya	29 (19–40) Mya
			Alternaria sect. *Soda*	3 (0.5–8.4) Mya	32 (26–54) Mya
			Alternaria sect. *Sonchi*	6.8 (2–14) Mya	24 (13–36) Mya
			Alternaria sect. *Teretispora*	0.2 (0–1.03) Mya	27 (17–40) Mya
			Alternaria sect. *Ulocladioides*	8.1 (3–17) Mya	22 (13–32) Mya
			Alternaria sect. *Ulocladium*	0.9 (0.1–2.5) Mya	44 (32–60) Mya
			Alternaria sect. *Undifilum*	–	45 (32–62) Mya

Note The age value with "*" means recent result lacking key cording gene strains.

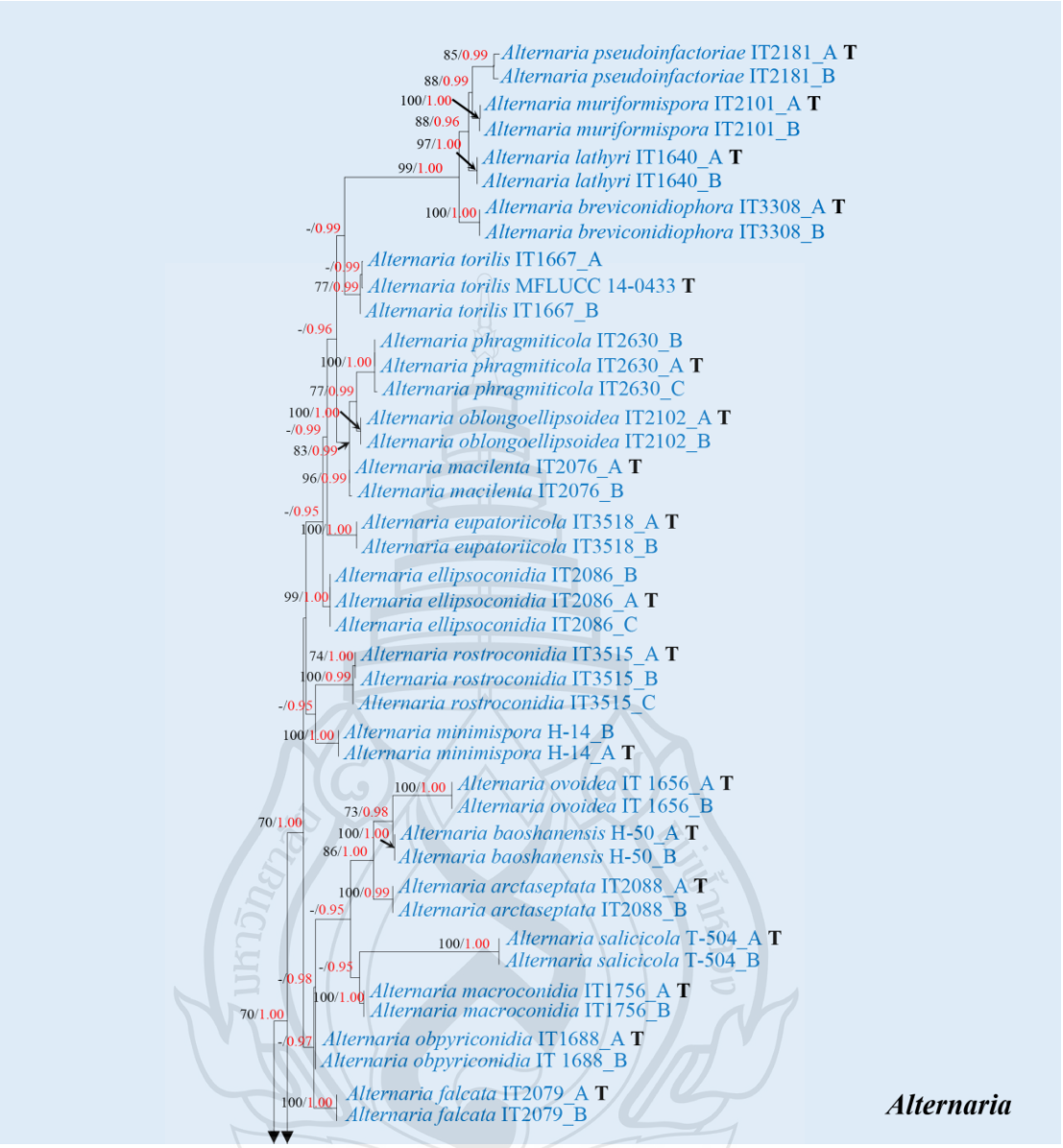


Figure 4.6 Phylogenetic construction of genus *Alternaria* using RAxML-based maximum likelihood analysis of a combined multigenes

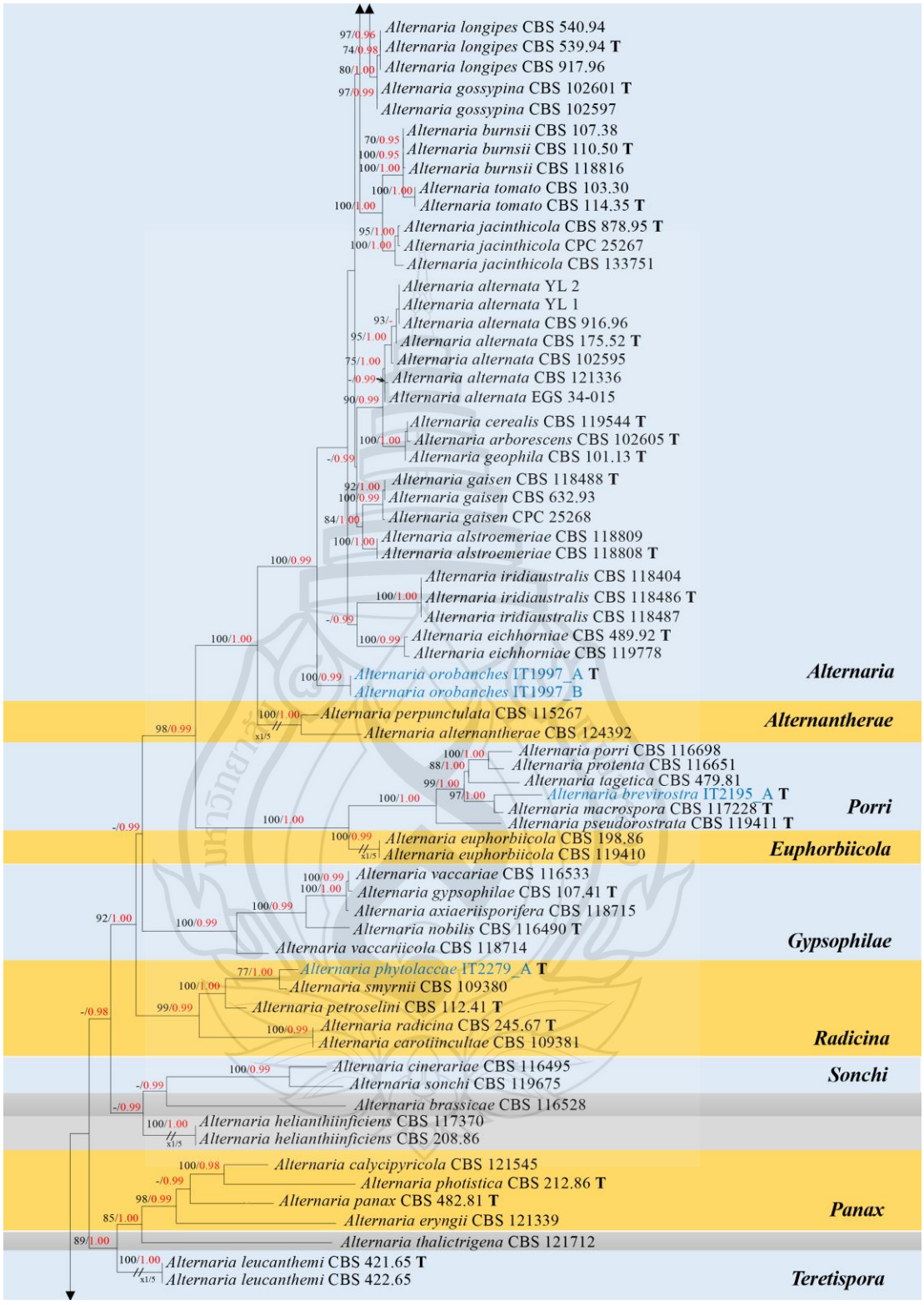


Figure 4.6 (continued)

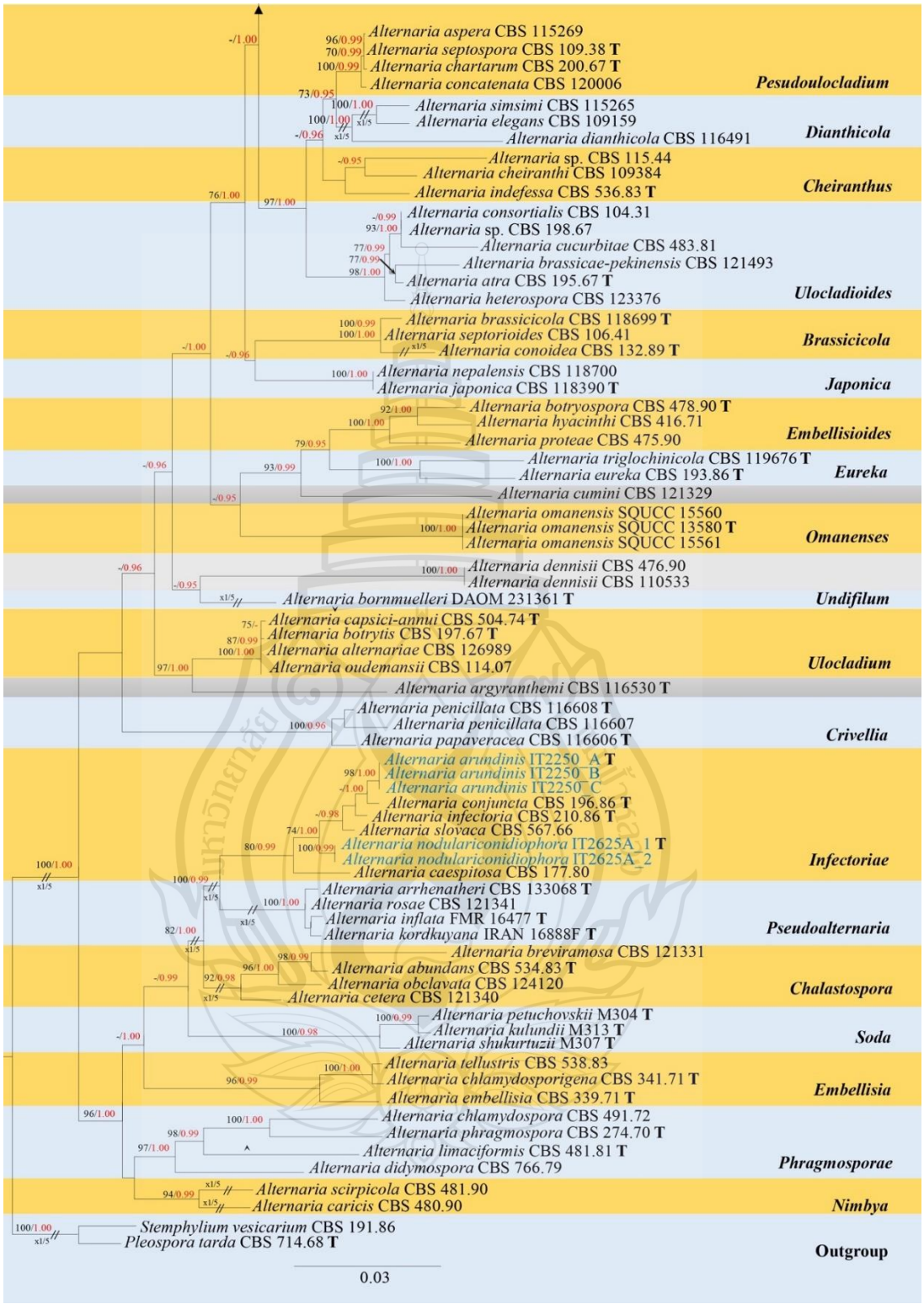


Figure 4.6 (continued)

Figure 4.6 Phylogenetic construction of genus *Alternaria* using RAxML-based maximum likelihood analysis of a combined ITS, LSU, SSU, TEF1-α, RPB2, GAPDH and Alt-a1 DNA sequence dataset. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 70% and Bayesian posterior probabilities (PP, red) equal to or greater than 0.95 PP are shown above the nodes. The tree is rooted to *Stemphylium vesicarium* (CBS 191.86) and *Pleospora tarda* (CBS 714.68). Newly generated strains are in blue, and the type strains are indicated by "T".

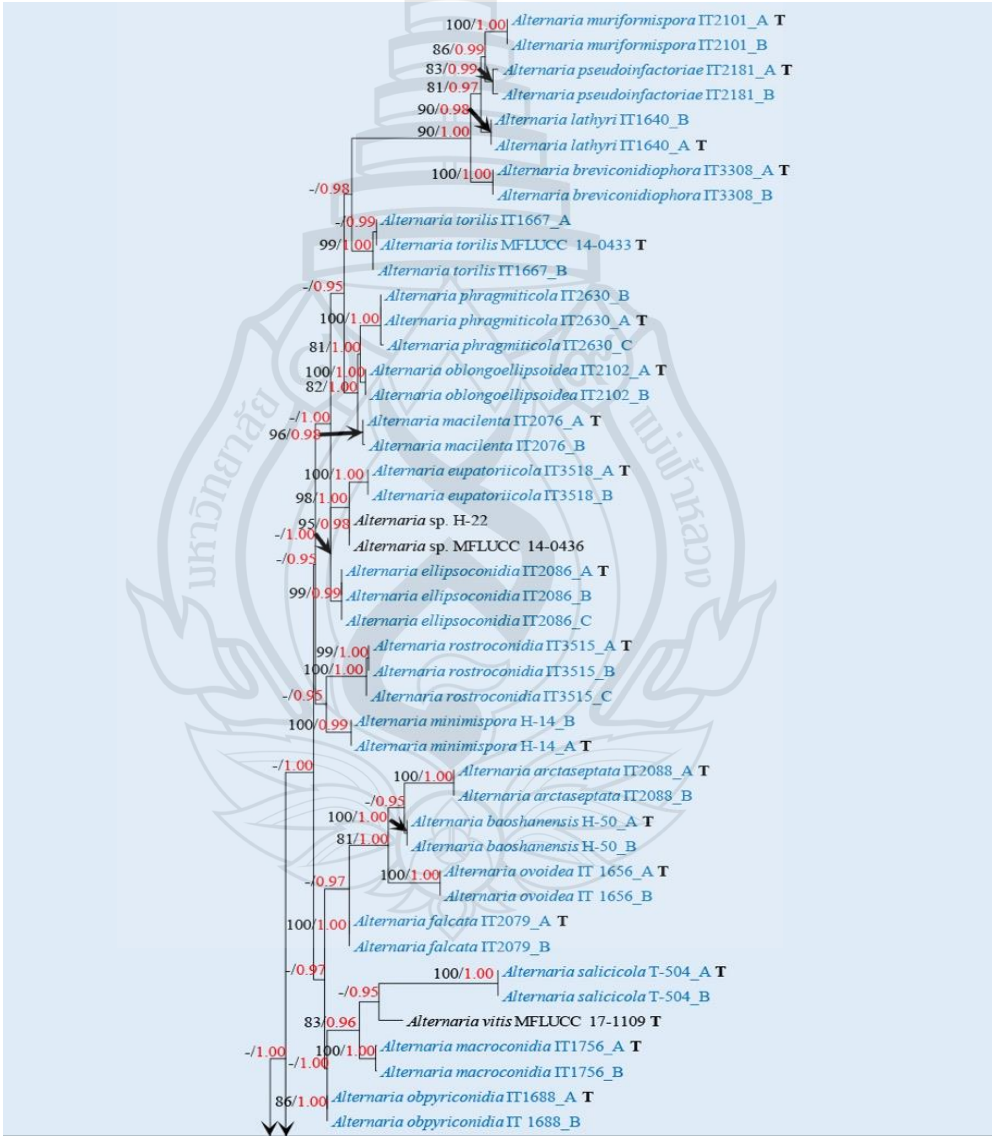


Figure 4.7 Phylogenetic tree of *Alternaria* sect. *Alternaria* using RAxML-based analysis of a combined Multigenes

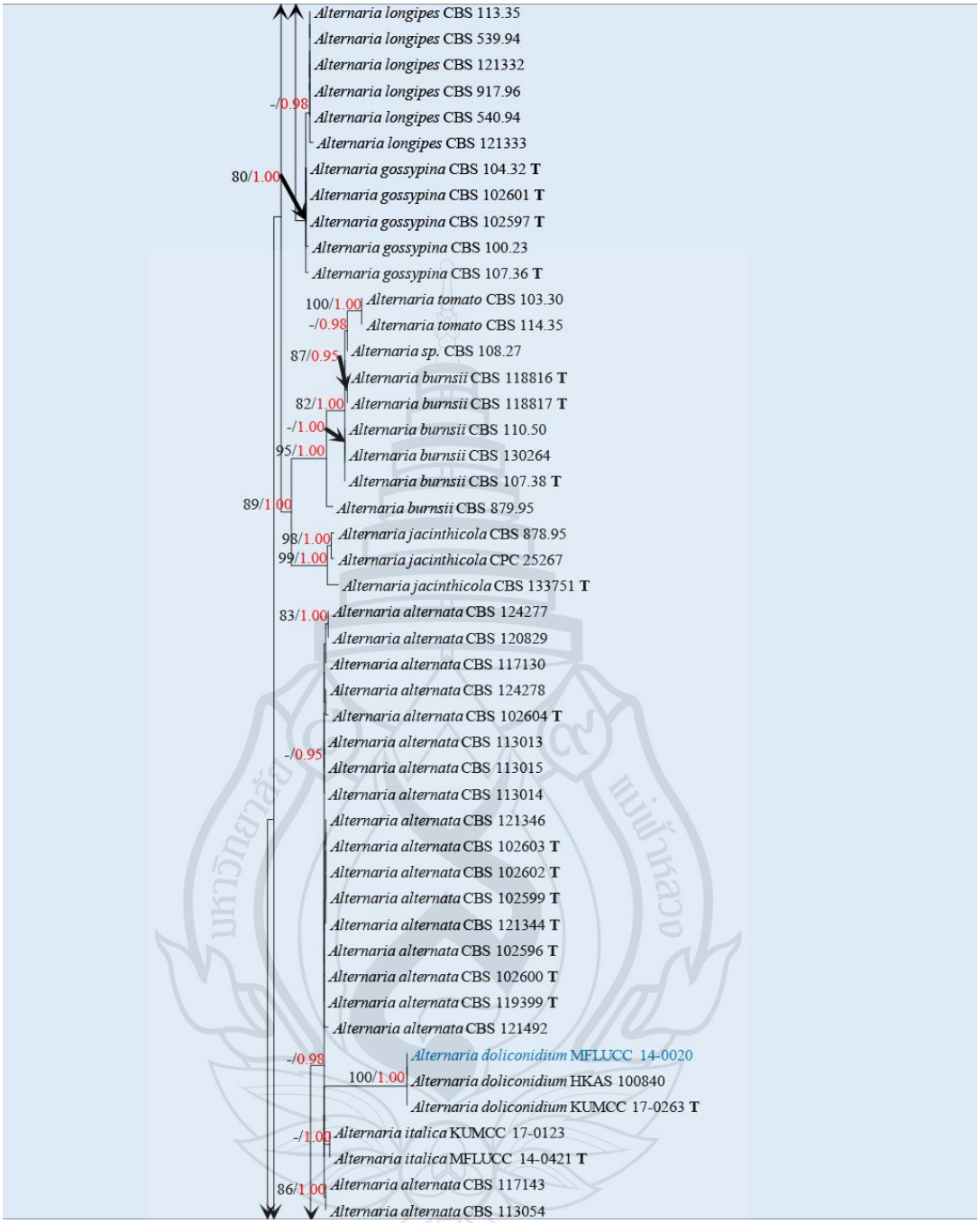
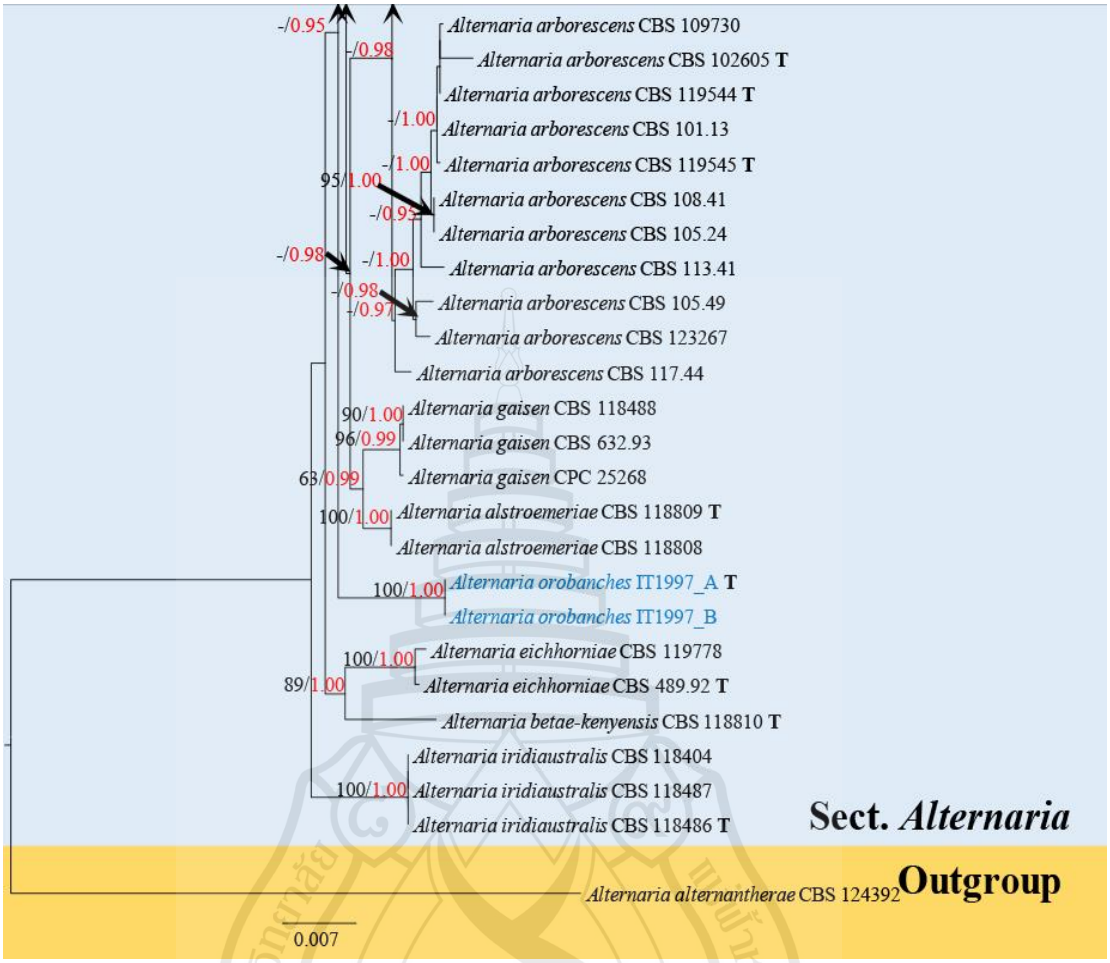


Figure 4.7 (continued)



Note Phylogenetic tree of *Alternaria* sect. *Alternaria* using RAxML-based analysis of a combined ITS, LSU, SSU, TEF1-α, RPB2, GAPDH and Alt-a1 DNA sequence dataset. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 60% and Bayesian posterior probabilities (PP, red) equal to or greater than 0.95 PP are shown above the nodes. The tree is rooted to *Alternaria alternantherae* (CBS 124392). Newly species and generated strains are in blue and the type strains are indicated by "T".

Figure 4.7 (continued)

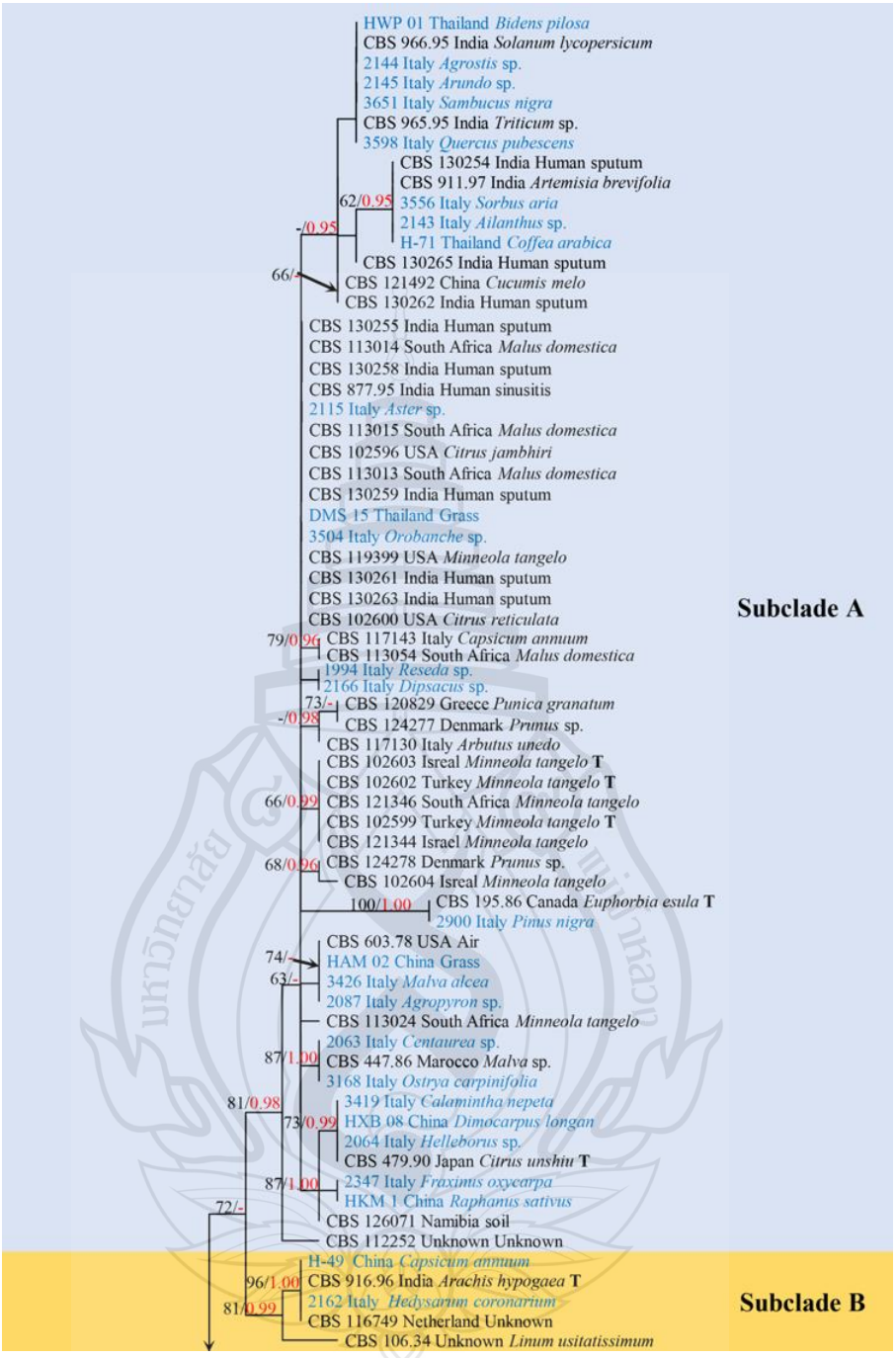
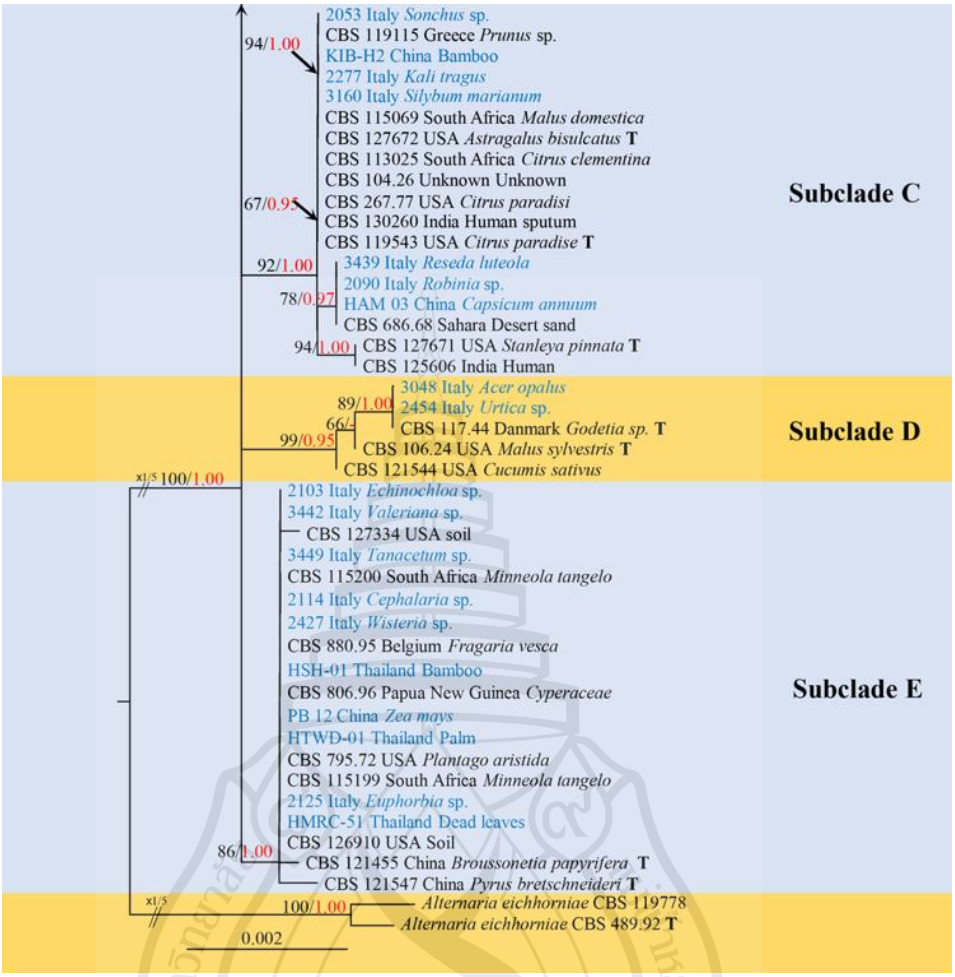


Figure 4.8 Phylogenetic construction of *Alternaria alternata* using RAxML-based analysis of a combined multigenes



Note Phylogenetic construction of *Alternaria alternata* using RAxML-based analysis of a combined ITS, LSU, SSU, TEF1-α, RPB2, GAPDH and Alt-a1 DNA sequence dataset. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 60% and Bayesian posterior probabilities (PP, red) equal to or greater than 0.95 are shown above the nodes. The tree is rooted to *Alternaria eichhorniae* (CBS 489.92) and *Alternaria eichhorniae* (CBS 119778). Newly generated strains are in blue, and the type strains are indicated by "T".

Figure 4.8 (continued)

4.4 Taxonomy

4.4.1 Section *Alternaria* D.P. Lawr., Gannibal, Peever & B.M. Pryor

Type species: *Alternaria alternata* (Fr.) Keissl.

Notes: Simmons (1995) first described the species-groups of *Alternaria alternata*, *A. tenuissima*, *A. cheiranthi* and *A. brassicicola* based on sporulating morphological characteristics. Lawrence et al. (2013) revealed eight distinct asexual lineages of *Alternaria* based on a molecular phylogenetic approach using ten protein-coding loci incorporated extensive taxon sampling (176 species) and proposed eight novel sections for *Alternaria*, in which sect. *Alternaria* was introduced. Woudenberg et al. (2013) assigned an orthographic variant '*Alternata*' for sect. *Alternaria* that is contradictory to ICBN Arts. 22.1 and 22.2. Thus, Lawrence et al. (2015) resurrected the section named *Alternaria*. Most species in this section are small-spored, with concatenated conidia that can be found as saprobes and as pre- or post-harvest diseases in over 100 host plants as well as human pathogens (Lawrence et al., 2016; Woudenberg et al., 2015). Some important plant pathogens in this section such as *A. arborescens* can cause stem canker on tomato, and *A. longipes* caused brown spot disease on tobacco (Woudenberg et al., 2015). Major updated taxonomic treatment of sect. *Alternaria* was circumscribed by Woudenberg et al. (2015). The generic type of *Alternaria*, *A. alternata* is also accommodated in this section. *Alternaria alternata* displays high genetic variation, and thus, Woudenberg et al. (2015) synonymized 35 morpho-species under *A. alternata*, of which three *formae speciales* and two pathotypes of *A. alternata* were recognized according to the detection of host-specific toxins. Woudenberg et al. (2015) mentioned that the genome assembly showed high similarity between the isolates within sect. *Alternaria* (96.7–98.2% genome identity) compared with isolates from other sections (85.1–89.3% genome identity), while the synonymized morpho-species under *A. alternata* showed 1.4–1.5% SNPs in their whole-genome reads. As *Alternaria* isolates were highly polymorphic, the low informative genes of the ITS, LSU and SSU were the least successful in separating the species in sect. *Alternaria*, while GAPDH was commonly used to distinguish all species in the section, except for distinguishing the *A. arborescens* species complex (AASC) from *A. alternata*. The other genes viz. Alt-a1, endoPG, KOG1058, OPA10-2 and RPB2 could separate all

species in the section from *A. alternata* but could not separate different pairs of other species from one another (Woudenberg et al., 2015).

4.4.1.1 *Alternaria alternata* (Fr.) Keissl., Beih. bot. Zbl., Abt. 2 29: 434 (1912)

Index Fungorum number: IF 119834, Facesoffungi number: ****.

Basionym: *Torula alternata* Fr., Syst. Mycol. (Lundae) 3: 500. 1832. (nom. sanct.)

Synonyms:

= *Alternaria amaranthi* (Peck) J. van Hook, Proc. Indiana Acad. Sci. 1920: 214. 1921.

≡ *Macrosporium amaranthi* Peck, Bull. Torrey Bot. Club 22: 493. 1895.

= *Alternaria angustiovoidea* E.G. Simmons, Mycotaxon 25: 198. 1986.

= *Alternaria astragali* Wangeline & E.G. Simmons, Mycotaxon 99: 84. 2007.

= *Alternaria brassicinae* E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 532. 2007.

= *Alternaria broussonetiae* T.Y. Zhang, W.Q. Chen & M.X. Gao, Mycotaxon 72: 439. 1999.

= *Alternaria caudata* (Cooke & Ellis) E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 496. 2007.

≡ *Macrosporium caudatum* Cooke & Ellis, Grevillea 6: 87. 1878.

= *Alternaria citri* Ellis & N. Pierce, Bot. Gaz. (Crawfordville) 33: 234. 1902.

= *Alternaria citriarbusti* E.G. Simmons, Mycotaxon 70: 287. 1999.

= *Alternaria citricancri* E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 542. 2007.

= *Alternaria citrimacularis* E.G. Simmons, Mycotaxon 70: 277. 1999.

= *Alternaria daucifolii* E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 518. 2007.

= *Alternaria destruens* E.G. Simmons, Mycotaxon 68: 419. 1998.

= *Alternaria dumosa* E.G. Simmons, Mycotaxon 70: 310. 1999.

= *Alternaria erumpens* (Cooke) Joly, Le Genre Alternaria: 199. 1964.

≡ *Macrosporium erumpens* Cooke, Grevillea 12: 32. 1883.

= *Alternaria fasciculata* (Cooke & Ellis) l.R. Jones & Grout, Bull. Torrey Bot. Club 24: 257. 1897.

≡ *Macrosporium fasciculatum* Cooke & Ellis, Grevillea 6: 6. 1877.

= *Alternaria godetiae* (Neerg.) Neerg., Aarsberetn. J. E. Ohlens Enkes Plantepatol. Lab. 10: 14. 1945.

≡ *Alternaria tenuissima* var. *godetiae* Neerg., Trans. Brit. Mycol. Soc. 18: 157. 1933.

= *Alternaria herbiphorbicola* E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 608. 2007.

= *Alternaria interrupta* E.G. Simmons, Mycotaxon 70: 306. 1999.

= *Alternaria limoniasperae* E.G. Simmons, Mycotaxon 70: 272. 1999.

= *Alternaria lini* Dey, Indian J. Agric. Sci. 3: 881. 1933.

= *Alternaria mali* Roberts, J. Agric. Res. 2: 58. 1914.

= *Alternaria martindalei* (Ellis & G. Martin) Joly, Le Genre Alternaria: 209. 1964.

≡ *Macrosporium martindalei* Ellis & G. Martin, Amer. Naturalist 18: 189. 1884.

= *Alternaria palandui* Ayyangar, Bull. Agric. Res. Inst., Pusa 179: 14. 1928.

= *Alternaria pellucida* E.G. Simmons, Mycotaxon 37: 102. 1990.

= *Alternaria perangusta* E.G. Simmons, Mycotaxon 70: 303. 1999.

= *Alternaria platycodonis* Z.Y. Zhang & H. Zhang, Flora Fungorum Sin., *Alternaria*: 66. 2003.

= *Alternaria podophylli* (Ellis & Everhart) Joly, Le Genre Alternaria: 212. 1964.

≡ *Macrosporium podophylli* Ellis & Everh., Proc. Acad. Nat. Sci. Philadelphia 43: 92. 1891.

= *Alternaria postmessia* E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 598. 2007.

= *Alternaria pulvinifungicola* E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 514. 2007.

= *Alternaria rhadina* E.G. Simmons, Mycotaxon 48: 101. 1993.

= *Alternaria ribis* Bub´ak & Ranojevi´c, Ann. Mycol. 8: 400. 1910.

= *Alternaria rumicicola* R.L. Mathur, J.P. Agnihotri & Tyagi, Curr. Sci. 31: 297. 1962.

= *Alternaria sanguisorbae* M.X. Gao & T.Y. Zhang, Mycosystema 19: 456. 2000.

= *Alternaria seleniiphila* Wangeline & E.G. Simmons, Mycotaxon 99: 86. 2007.

= *Alternaria soliaegyptiaca* E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 506. 2007.

= *Alternaria tenuis* Nees, Syst. Pilze (Würzburg): 72. 1816 [1816 –1817].

= *Alternaria tenuissima* (Nees & T. Nees: Fr.) Wiltshire, Trans. Brit. Mycol. Soc. 18: 157. 1933.

≡ *Helminthosporium tenuissimum* Kunze ex Nees & T. Nees, Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 9: 242. 1818.

= *Clasterosporium tenuissimum* (Nees & T. Nees: Fr.) Sacc., Sylloge Fungorum (Abellini) 4: 393. 1886.

= *Macrosporium tenuissimum* (Nees & T. Nees) Fr., Syst. Mycol. 3: 374. 1832. (nom. sanct.)

= *Alternaria tenuissima* var. *alliicola* T.Y. Zhang, Mycotaxon 72: 450. 1999.

= *Alternaria tenuissima* var. *verruculosa* S. Chowdhury, Proc. Natl. Acad. Sci. India, Sect. B, Biol. Sci. 36: 301. 1966.

= *Alternaria tomaticola* E.G. Simmons & Chellemi, CBS Biodiversity Ser. (Utrecht) 6: 528. 2007.

= *Alternaria toxicogenica* E.G. Simmons, Mycotaxon 70: 294. 1999.

= *Alternaria turkisafria* E.G. Simmons, Mycotaxon 70: 290. 1999.

= *Alternaria vaccinii* E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 432. 2007.

= *Alternaria viniferae* Yong Wang bis, Y.Y. Than, K.D. Hyde, X.H. Li, Mycol. Progr. 13: 1124. 2014.

= *Alternaria yali-inficiens* R.G. Roberts [as 'yaliinficiens'], Pl. Dis. 89: 142. 2005.

= *Macrosporium inquinans* Cooke & Ellis, Grevillea 7: 39. 1878.

= *Macrosporium maydis* Cooke & Ellis, Grevillea 6: 87. 1878.

= *Macrosporium meliloti* Peck, Rep. (Annual) NewYork State Mus. Nat. Hist. 33: 28. 1880.

= *Macrosporium polytrichi* Peck, Rep. (Annual) NewYork State Mus. Nat. Hist. 34: 31. 1890.

= *Macrosporium pruni-mahalebi* S˘avulescu & Sandu, Hedwigia 75: 228. 1935.

= *Macrosporium seguierii* Allescher, Hedwigia 33: 75. 1894.

Type details: INDIA, on *Arachis hypogaea* (Fabaceae), 1 December 1980, E.G. Simmons, IMI 254138 [epitype; designated by de Hoog & Horré (2002)], ex-epitype living culture, CBS 916.96 = ATCC 66981 = EGS 34.01; GERMANY, on fragments of a pithy stem, C.G.D. Nees von Esenbeck, No. 910, 262-129, a [neotype; designated by Simmons (1967)].

Saprobic on dead branches of *Reseda* sp. Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the substrate, composed of septate, branched, smooth, thin-walled, pale white to grey hyphae. *Conidiophores* (160.5–)179.5–184(–188) × (9–)12–14(–15.5) µm ($\bar{x}$ = 181.4 × 13.2 µm, n = 100), macronematous, mononematous, flexuous, cylindrical, versicolorous, brown to dark brown, septate, unbranched, smooth to rough, with small granules, thick-walled. *Conidiogenous cells* (12–)13–15.5(–16) × (10–)11.5–13(–14) µm ($\bar{x}$ = 14.3 × 12.2 µm, n = 100), mono- to polytretic, integrated, terminal, determinate, percurrent, subhyaline to pale brown, smooth, thin-walled, rounded or doliiform at apex, with 1–3 apical conidiogenous loci. *Conidia* (76–)82.5–91.5(–98) × (22.5–)29–37(–39) µm ($\bar{x}$ = 86.4 × 32.4 µm, n = 100) acrogenous, solitary to catenate, dry, muriform, straight, sometimes curved, ovoid to chiefly obclavate or obpyriform, sometimes formed short, narrow, unbranched beak, grey-brown to brown, 3–4(–8) transverse septate, and 1–2 longitudinal septate, with aseptate or one longitudinal or oblique septum or Y-shaped at the end cell, smooth or verrucose, thin-walled, borne in chain. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all edge cells. Colonies growing on PDA, cottony, pale white to grey, reaching 5 cm in 7 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, subhyaline hyphae. Conidia sporulated on OA media within 15 days, phragmosporous to muriform, obclavate to obpyriform, light brown to brown, with branched or unbranched acicular or doliiform, aseptate, apical beak, formed branched,

apically or laterally secondary conidiophores with 1–2 conidiogenous loci, olivaceous-brown to brown, 1–4 transversely euseptate, 1–3 longitudinal or oblique or Y-shaped septa in transverse divisions, borne in chains, smooth to minutely verruculose.

Material examined: ITALY, Province of Arezzo [AR], near Passo la Calla, on dead aerial stem of *Reseda* sp. (Resedaceae), 13 July 2014, E. Camporesi, IT1994 (MFLU 21-XXXX), living culture = MFLUCC 21-XXXX.

Notes: In this study, we obtained 45 new collections of *Alternaria alternata* from China, Italy and Thailand. Multi-locus phylogeny of ITS, LSU, SSU, TEF1-α, RPB2, GAPDH and Alt-a1 loci confirmed species identification of these 45 strains as *A. alternata*. These 45 strains formed various internal subclades within *A. alternata* and can be separated into five main subclades with significant support (up to 80% ML, 0.95 PP; Figure 4.8). These 45 collections showed a diverse range of host occurrences in families Adoxaceae, Arecaceae, Asteraceae, Betulaceae Brassicaceae, Caprifoliaceae, Fagaceae, Lamiaceae, Malavaceae, Orobanchaceae, Pinaceae, Poaceae, Rubiaceae, Resedaceae, Rosaceae, Sapindaceae, Solanaceae, Urticaceae and some unidentified plant litter.

The major subclade A formed a significant support subclade (81% ML, 0.98 PP) containing 62 strains with several internal lineages. Newly generated strains HWP-01, IT2144, IT2145, IT3598, and IT3651 formed a single lineage with strains CBS 965.95 and CBS 966.95; these isolates occurred on host families Asteraceae, Adoxaceae, Fagaceae, Poaceae and Solanaceae as pathogens and saprobes. The saprobic strains H-71, IT2143, and IT3556 formed a single lineage with strain CBS 130254 (isolated from human sputum in India), and CBS 911.97 (isolated from *Artemisia brevifolia* (Asteraceae) in India) and clustered with CBS 130262, CBS 130265 (isolated from human sputum in India) and CBS 121492 (isolated from *Cucumis melo* (Cucurbitaceae) in China). Six strains isolated from human sputum and sinusitis in India (CBS 877.95, CBS 130255, CBS 130258, CBS 130259, CBS 130261, CBS 130263) clustered with three strains isolated from *Malus domestica* (Rosaceae) in South Africa (CBS 113013, CBS 113014, CBS 113015), strains isolated from *Citrus jambhiri* (CBS 102596), *C. reticulata* (CBS 102600) and *Minneola tangolo* (CBS 119399) in the USA, three new strains isolated from *Aster* sp. (IT2115), *Orobanche* sp. (IT3504) in Italy and unidentified grass (DMS 15) in Thailand. At the same time, the strain CBS 117143 (isolated from *Capsicum annuum* (Solanaceae) in Italy) formed a significant support lineage (79% ML, 0.96 PP) with CBS 113054 (isolated from

M. domestica in South Africa), and clustered with the two new isolates IT 1994 and IT 2166.

The strain CBS 120829 (isolated from *Punica granatum* (Punicaceae) in Greece), formed a single lineage with CBS 124277 (isolated from *Prunus* sp. (Rosaceae) in Denmark) and clustered with CBS 117130 (isolated from *Arbutus unedo* (Ericaceae) in Italy) with significant support in BI analyses (0.98 PP). Five strains isolated from *Minneola tangelo* in Israel, South Africa and Turkey (CBS 102559, CBS 102602, CBS 102603, CBS 121344, and CBS 121346) also formed a significant single lineage (66% ML, 0.99 PP) within subclade A. The strain CBS 124278 (isolated from *Prunus* sp. in Denmark) formed a single lineage with CBS 102604 (isolated from *Minneola tangelo* in Israel) with significant support (68% ML, 0.96 PP), while at the same time, the new isolate IT2900 formed a single lineage with CBS 195.86 (isolated from *Euphorbia esula* (Euphorbiaceae) in Canada) with high support (100% ML, 1.00 PP).

Three new isolates (HAM 02, IT 2087, IT 3426) formed a single lineage with CBS 603.78 (isolated from the air in USA) and clustered with CBS 113024 (isolated from *Minneola tangelo* in South Africa). Two new isolates (IT 2063, IT 3168) formed a significant support lineage (87% ML, 1.00 PP) with CBS 447.86 (isolated from *Malva* sp. (Malvaceae) in Morocco). Three new isolates (HXB 08, IT3419, IT2064) also formed a significant support lineage (73% ML, 0.99 PP) with CBS 479.90 (isolated from *C. unshiu* in Japan) and clustered with IT2347, HKM 1 and CBS 126071 (isolated from soil in Namibia) with significant support (87% ML, 1.00 PP). The strain CBS 112252 formed an independent single lineage basal to subclade A with significant support (81% ML, 1.00 PP).

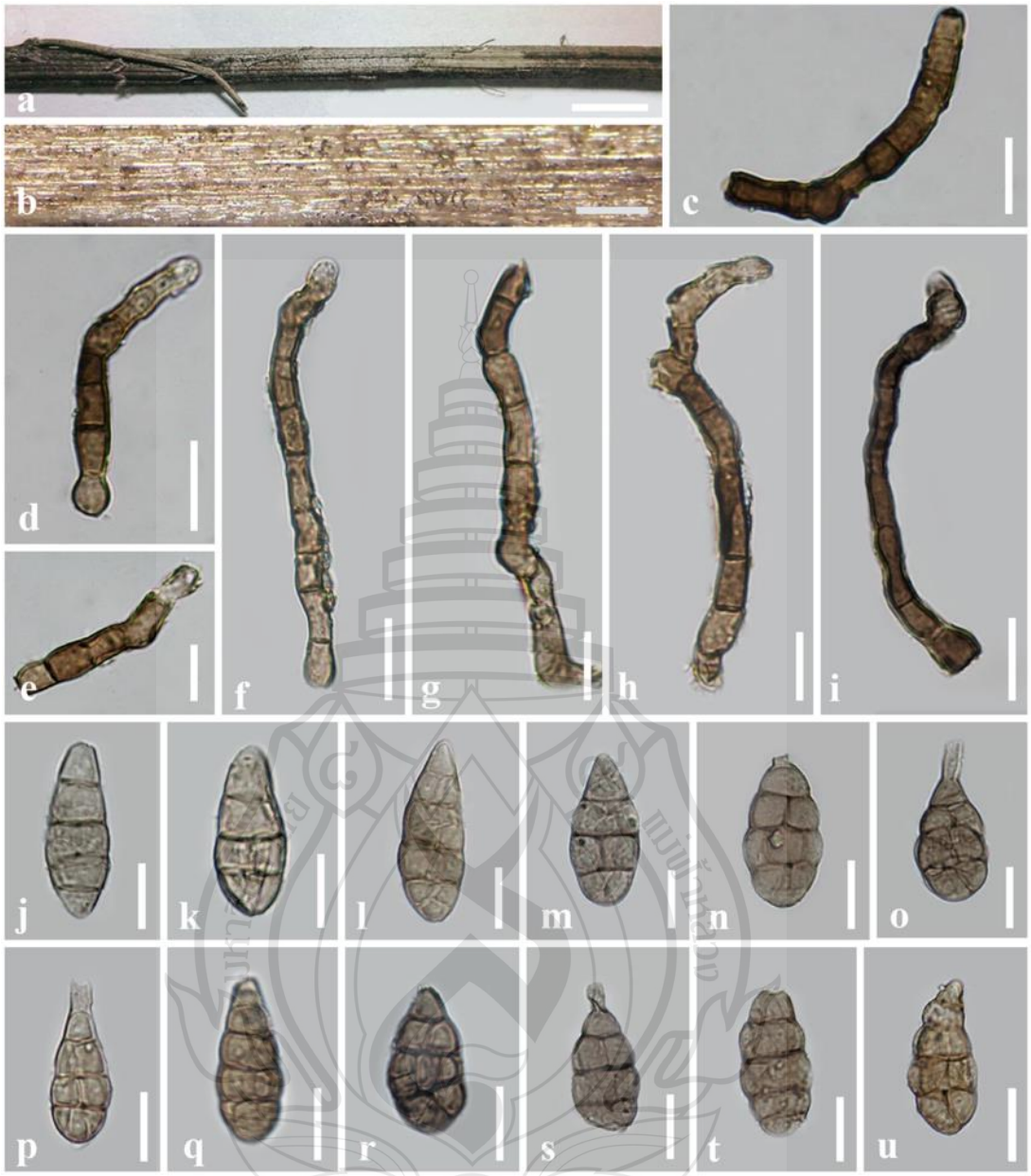
Subclade B was represented by five strains, including the ex-type strain of *Alternaria alternata* (CBS 916.96). Two new isolates (H-49, IT2162) formed a single lineage with the type strain of *A. alternata* (CBS 916.96) and CBS 116749 with high support (96% ML, 1.00 PP), clustering with CBS 106.34 (isolated from *Linum usitatissimum*, Linaceae) with significant support (81% ML, 0.99 PP).

Subclade C contained seven new isolates from China and Italy (IT2053, KIB-H2, IT 2277, IT 3160, IT 3439, IT 2090 and HAM03), clustering with strain CBS 119115 (isolated from *Prunus* sp. in Greece), two strains from South Africa (CBS 115069, CBS113025), four strains from USA (CBS 127672, CBS 267.77, CBS 119543, CBS

127671), two strains from India (CBS 130260, CBS 125606), CBS 686.68 (from Sahara Desert sand) and CBS 104.26, with significant support (92% ML, 1.00 PP).

Subclade D formed a high support subclade (99% ML. 0.95 PP) containing five strains that include two new strains (IT 3048, IT2454) isolated from *Acer opalus* and *Urtica* sp. in Italy. The two new strains (IT 3048, IT2454) formed a single lineage with CBS 117.44 (isolated from *Godetia* sp. in Denmark) with significant support (89% ML, 1.00 PP), clustering with CBS 106.24 (isolated from *Malus sylvestris* in USA) and CBS 121544 (isolated from *Cucumis sativus* in USA).

Subclade E formed a basal lineage with significant support (86% ML, 1.00 PP), including 10 new isolates (IT2103, IT 3442, IT 3449, IT 2114, IT 2427, HSH-01, PB 12, HTWD-01, IT 2125, HMRC-51), two isolates from soil (CBS 127334, CBS 126910) and from *Plantago aristida* (Plantaginaceae) in USA (CBS 795. 72), two strains isolated from *Minneola tangelo* in South Africa (CBS 115200, CBS 115199), an isolate from *Fragaria vesca* in Belgium (CBS 880.95), an isolate from Cyperaceae in Papua New Guinea (CBS 806.96) and two strains isolated from *Broussonetia papyrifera* (CBS 121455) and *Pyrus bretschneideri* (CBS 121547).



Note (MFLU XX-XXXX). a, b Colonies on dead branch. c–i Conidiophores. j–u Conidia. Scale bars: a = 0.1 cm, b = 300 µm, c, d, f, j–u = 30 µm, e, g–i = 20 µm.

Figure 4.9 Morphology of *Alternaria alternata*

Table 4.5 Additional collections of *Alternaria alternata* collected from Yunnan, China, Italy and Thailand in this study

Culture collection	Original code	Herbarium no.	Origin	Host and habitat	Collection date	Collector
MFLUCC XX-XXXX	IT2053	MFLU 14-XXXX	Italy, Province of Forlì-Cesena, Premilcuore	Dead stem of *Sonchus* sp. (Asteraceae)	18 August 2014	E. Camporesi
	IT2063		Italy, Province of Forlì-Cesena, Verghereto, Montecoronaro	Dead hanging stem of *Centaurea* sp. (Asteraceae)	20 August 2014	E. Camporesi
	IT2064		Italy, Province of Forlì-Cesena, Fiumicello di Premilcuore	Dead hanging stem of *Helleborus* sp. (Ranunculaceae).	28 August 2014	E. Camporesi
	IT2087		Italy, Province of Forlì-Cesena, Quattro di Forlì	Dead hanging stem of *Agropyron* sp. (Asteraceae)	1 September 2014	E. Camporesi
	IT2090		Italy, Province of Forlì-Cesena, Predappio, Rocca delle Caminate	Dead leaf petiole of *Robinia* sp. (Fabaceae)	4 September 2014	E. Camporesi
	IT2103		Italy, Province of Forlì-Cesena, Meldola	Dead hanging stem of *Echinochloa* sp. (Poaceae)	8 September 2014	E. Camporesi
	IT2114		Italy, Province of Forlì-Cesena, Tessello	Dead hanging stem of *Cephalaria* sp. (Dipsacaseae)	16 September 2014	E. Camporesi
	IT2115		Italy, Province of Forlì-Cesena, Civitella di Romagna	Dead hanging stem of *Aster* sp. (Asteraceae)	19 September 2014	E. Camporesi
	IT2125		Italy, Province of Arezzo, Stia, Montemezzano	Dead hanging stem of *Euphorbia* sp. (Euphorbiaceae)	22 September 2014	E. Camporesi
	IT2143		Italy, Province of Forlì-Cesena, Galeata, San Zeno	Dead hanging leaf petiole of *Ailanthus* sp. (Simaroubaceae)	30 September 2014	E. Camporesi

Table 4.5 (continued)

Culture collection	Original code	Herbarium no.	Origin	Host and habitat	Collection date	Collector
	IT2144		Italy, Province of Forlì-Cesena, Cabelli di Santa Sofia	Dead hanging stem of *Agrostis* sp. (Poaceae)	2 October 2014	E. Camporesi
	IT2145		Italy, Province of Forlì-Cesena, Monte Mirabello	Dead hanging leaf of *Arundo* sp. (Poaceae)	3 October 2014	E. Camporesi
	IT2162		Italy, Province of Forlì-Cesena, Santa Sofia	Dead hanging stem of *Hedysarum coronarium* L. (Papilionaceae)	7 October 2014	E. Camporesi
	IT2166		Italy, Province of Forlì-Cesena, Meldola, Piandispino	Dead hanging stem of *Dipsacus* sp. (Caprifoliaceae)	7 October 2014	E. Camporesi
	IT2277		Italy, Province of Ravenna, Lido di Dante	Dead hanging stem of *Kali tragus* (L.) Scop. (Amaranthaceae)	2 December 2014	E. Camporesi
	IT2347		Italy, Province of Forlì-Cesena, Collina di Forlì	Several samaras of *Fraxinus oxycarpa* Willd. (Oleaceae)	21 January 2015	E. Camporesi
	IT2427	MFLU 15-1823	Italy, Province of Forlì-Cesena, Forlì, Via Nenni	Dead hanging stem of *Wisteria* sp. (Caprifoliaceae)	30 March 2015	E. Camporesi
MFLUCC XX-XXXX	IT2454	MFLU 14-XXXX	Italy, Province of Forlì-Cesena, Predappio, Rocca delle Caminate	Dead stem of *Urtica* sp. (Urticaceae)	21 April 2015	E. Camporesi
	IT2900	MFLU 16-1116	Italy, Province of Forlì-Cesena, Santa Sofia, Camposonaldo	Dead needles of *Pinus nigra* J.F. Arnold (Pinaceae)	23 March 2016	E. Camporesi
	IT3048	MFLU 16-2277	Italy, Province of Forlì-Cesena, Fiumicello di Premilcuore	Dead hanging stem of *Acer opalus* Mill. (Sapindaceae)	27 July 2016	E. Camporesi

Table 4.5 (continued)

Culture collection	Original code	Herbarium no.	Origin	Host and habitat	Collection date	Collector
	IT3160	MFLU 16-2883	Italy, Province of Forlì-Cesena, Forlì, Ravaldino in Monte	Dead stem of *Silybum marianum* (L.) Gaertn. (Asteraceae)	15 November 2016	E. Camporesi
	IT3168	MFLU 16-2904	Italy, Province of Forlì-Cesena, Forlì, Parco Urbano	Dead hanging fruits of *Ostrya carpinifolia* Scop. (Betulaceae)	19 November 2016	E. Camporesi
	IT3419		Italy, Province of Arezzo, Poppi, Quota	Dead hanging stem of *Calamintha nepeta* (Lamiaceae)	25 July 2017	E. Camporesi
	IT3426		Italy, Province of Arezzo, near Croce di Pratomagno	Dead hanging stem of *Malva alcea* L. (Malavaceae)	1 August 2017	E. Camporesi
	IT3439		Italy, Province of Arezzo, Stia, Montemezzano	Dead hanging stem of *Reseda luteola* L. (Resedaceae)	13 August 2017	E. Camporesi
	IT3442		Italy, Province of Arezzo, Montemignaio	Dead hanging stem of *Valeriana* sp. (Caprifoliaceae)	12 August 2017	E. Camporesi
	IT3449		Italy, Province of Forlì-Cesena, Bagno di Romagna, Riofreddo	Dead hanging stem of *Tanacetum* sp. (Asteraceae)	22 August 2017	E. Camporesi
	IT3504	MFLU 17-1778	Italy, Province of Forlì-Cesena, Bagno di Romagna, Acquapartita	Dead hanging stem of *Orobanche* sp. (Orobanchaceae)	25 September 2017	E. Camporesi
	IT3556		Italy, Province of Forlì-Cesena, Santa Sofia	Dead leaf of *Sorbus aria* Crantz (Rosaceae)	15 November 2017	E. Camporesi
	IT3598		Italy, Province of Ravenna, Faenza, Santa Lucia	Dead leaves of *Quercus pubescens* Willd. (Fagaceae)	13 December 2017	E. Camporesi

Table 4.5 (continued)

Culture collection	Original code	Herbarium no.	Origin	Host and habitat	Collection date	Collector
	IT3651		Italy, Province of Forlì-Cesena, Meldola	Dead hanging branch of *Sambucus nigra* L. (Adoxaceae)	31 December 2017	E. Camporesi
	KIB-H2		China, Yunnan, Kunming Institute of Botany	Dead fallen leave of bamboo (Poaceae)	26 December 2014	J.F. Li
	HKM-1		China, Yunnan, Kunming, Xundian	Dead stem of *Raphanus sativus* L. (Brassicaceae)	13 March 2015	J.F. Li
MFLUCC XX-XXXX	H-49	MFLU 14-XXXX	China, Yunnan, Baoshan	Dead branch of *Capsicum annuum* L. (Solanaceae)	22 October 2015	J.F. Li
	PB-12		China, Yunnan, Pingbian, Dawei Mountain	Dead stem of *Zea mays* L. (Poaceae)	20 September 2017	J.F. Li
	HXB-08		China, Yunnan, Xishuangbanna	Dead fallen leaves of *Dimocarpus longan* Lour. (Sapindaceae)	8 June 2018,	J.F. Li
	HAM-02		China, Yunnan, Honghe, Amu Mountain	Dead fallen leaves of grass (Poaceae)	15 June 2018	J.F. Li
	HAM-03		China, Yunnan, Honghe, Amu Mountain	Dead aerial stem of *Capsicum annuum* (Solanaceae)	15 June 2018	J.F. Li
	HSH-01		Thailand, Chiang Rai, Muang, Singha Park	Dead culms of bamboo (Poaceae)	23 February 2016	J.F. Li
	HMRC-51		Thailand, Chiang Mai, Mae Taeng, Mushroom Research Center (M.R.C)	Dead fallen leaves of unidentified plant	23 March 2016	J.F. Li
	DMS-15		Thailand, Chiang Rai, Doi Mae Salong	Dead leaves of grass (Poaceae)	24 May 2016	J.F. Li
	HTWD-01		Thailand, Chiang Rai, Mae Fah Luang	Dead leaves of palm (Arecaceae)	25 September 2016	J.F. Li

Table 4.5 (continued)

Culture collection	Original code	Herbarium no.	Origin	Host and habitat	Collection date	Collector
	H-71		Thailand, Chiang Rai, Doi Chang	Dead leaves of *Coffea arabica* L. (Rubiaceae)	25 July 2018	J.F. Li
	HWP-01		Thailand, Chiang Rai, Wiang Pa Pao	Dead stems of *Bidens pilosa* L. (Asteraceae)	16 October 2018	J.F. Li

4.4.1.2 *Alternaria arctoseptata* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF ****; Facesoffungi number: FoF****

Etymology: Referring to the constricted septate conidia.

Holotype: MFLU 21-****

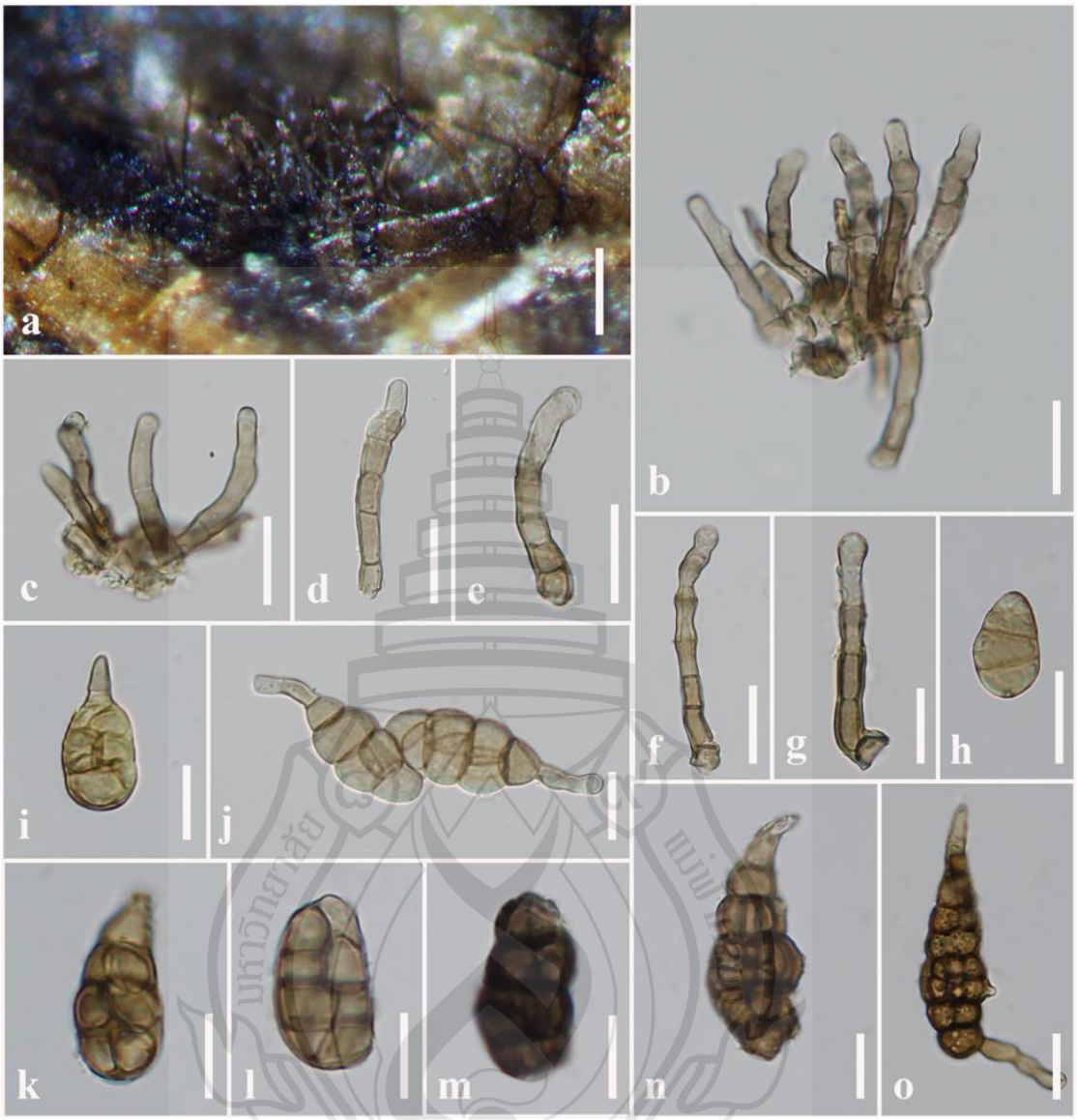
Saprobic on dead handing stem of *Lathyrus* sp. (Fabaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the substrate, composed of dark brown hyphae. *Conidiophores* 50–100 × 8–12 µm ($\bar{x}$ = 82 × 9 µm, n = 30), macronematous, straight or flexuous, cylindrical, pale brown to light brown, septate, smooth, thick-walled, arising from a stomatic base, sometimes with swollen knots. *Conidiogenous cells* 11–12 × 10–14 µm ($\bar{x}$ = 11.5 × 12 µm, n = 20), mono- to polytretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline to pale yellowish, doliiform at apex, with 1–2 conidiogenous loci, smooth, thin-walled. *Conidia* 15–75 × 10–35 µm ($\bar{x}$ = 60 × 25 µm, n = 30) acrogenous, solitary, born in chain, dry, simple, straight or curved, subglobose to ovoid when immature, becoming ellipsoidal to obpyriform or obclavate when mature, with short, narrow, unbranched, aseptate apical beak, occasionally lacking beak, initially yellowish brown or olivaceous brown, laterally brown to dark brown, sectored, 2–3(–6) transverse septa, with 1–2 longitudinal or oblique septa in some transverse divisions, constricted at some transverse septa, smooth or verrucose, thick-walled, sometimes formed apically, or laterally on secondary conidiophores with one conidiogenous loci. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies growing on PDA, hairy, fluffy, brown to dark brown, reaching 5 cm within 7 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, white to brown hyphae; conidia not formed *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Predappio, Fiumana, on dead handing stem of *Lathyrus* sp. (Fabaceae), 2 September 2014, E. Camporesi, IT2088 (MFLU XX-XXXX, holotype), ex-type living culture = MFLUCC XX-XXXX.

Notes: *Alternaria arctoseptata* was isolated from the same host genus *Lathyrus* sp. with *A. lathyri*. Morphologically, *A. arctoseptata* can be distinguished from *A. lathyri* in having paler conidia, with short narrow apical beak or formed secondary conidiophores. Furthermore, the conidiophores of *A. arctoseptata* are shorter, pale brown to light brown

conidiophores with apically swollen knots, arising from stomatic base, which are darker in *A. lathyri*. Phylogenetically two strains of *A. arctoseptata* (IT2088_A, IT2088_B) formed a robust clade (100% ML, 1.00 PP) that clustered with *A. baoshanensis* and *A. ovoidea* with significant support (81% ML, 100 PP; Figure 4.7), and distant from *A. lathyri*. *Alternaria arctoseptata* differs from *A. baoshanensis* in having apical beak, and larger conidia (60 × 25 µm vs. 38 × 18 µm) that are rather constricted at some septa as well as having rather monotretic, larger conidiogenous cells (11.5 µm × 12 µm vs. 6.2 µm × 7.5 µm). *Alternaria arctoseptata* also differs from *A. ovoidea* in having paler brown conidia that are rather constricted at some septa. A comparison of RPB2 nucleotide pairwise shows that *A. arctoseptata* differs from *A. baoshanensis* in 10/559 bp (1.8% difference, no gap) and differs from *A. ovoidea* in 12/565 bp (2.1% difference, no gap). A comparison of Alt-a1 nucleotide pairwise shows that *A. arctoseptata* differs from *A. baoshanensis* in 8/474 bp (1.7% difference, no gap) and differs from *A. ovoidea* in 18/520 bp (3.5% difference, no gap). Based on distinct morphological characteristics and phylogenetic support, *A. arctoseptata* is introduced as a new species in this study.



Note (MFLU XX-XXXX, holotype). a Colonies on dead stem of *Lathyrus* sp. (Fabaceae). b–g Conidiophores bearing conidiogenous cells. h, i Immature conidia. j, o Conidia formed secondary conidiophores. k–n Mature conidia. Scale bars: a = 200 µm, b–f = 30 µm, g, j–o = 20 µm, h, i = 15 µm.

Figure 4.10 Morphology of *Alternaria arctoseptata*

4.4.1.3 *Alternaria baoshanensis* J.F. Li, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF ****; Facesoffungi number: FoF ****

Etymology: Named after the locality, Baoshan (Yunnan, China), where the species is collected.

Holotype: MFLU 21-****

Saprobic on rattan of *Curcubita moschata* (Duch ex Lam.) Duch ex Poiret (Cucurbitaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the substrate, composed of septate, branched, smooth, thin-walled, composed of dark hyphae. *Conidiophores* 80–100 × 12–20 µm ($\bar{x}$ = 48 × 14 µm, n = 20), macronematous, straight or flexuous, unequally cylindrical, versicolorous, light brown to dark brown, smooth, thick-walled, septate, sometimes branched, with several aggregated at the base. *Conidiogenous cells* 5–7 × 6–10 µm ($\bar{x}$ = 6.2 × 7.5 µm, n = 20), mono- to polytretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline to light brown, doliiform apex, with apically or laterally 1–2 conidiogenous loci, smooth, thin-walled, with a distinctive conidiogenous hilum. *Conidia* 25–60 × 12–22 µm ($\bar{x}$ = 38 × 18 µm, n = 30) acrogenous, solitary, straight, curved, varied in shapes, usually subglobose to ellipsoidal, or subcylindrical to obpyriform, occasionally irregular in shape, sometimes formed a short beak, light brown to yellowish brown, 3–6 transverse septa, with 1–2 longitudinal or oblique septa in some transverse divisions, borne in chain, smooth, sometimes verrucose, thin-walled. *Conidial secession* schizolytic.

Cultural characteristics – Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies growing on PDA, hairy fluffy, brown, reaching 5 mm in 10 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, brown hyphae; conidia not formed *in vitro* within 60 days.

Material examined – CHINA, Yunnan, Baoshan, Shuizhai country, on dead rattan of *Curcubita moschata* (Cucurbitaceae), 25 October 2015, J.F. Li, H-50 (MFLU XX-XXX, holotype), ex-type living culture = MFLUCC XX-XXXX.

Notes – *Alternaria baoshanensis* can be distinguished from other related species (*A. acroseptata* and *A. ovoidea*) in having versicolorous conidiophores. Multi-locus phylogeny demonstrated that two strains of *A. baoshanensis* (H-50_A, H-50_B) form a robust clade (100% ML, 1.00 PP), sister to *A. ovoidea* with significant support (73% ML, 0.98 PP) in analyses 1 (Figure 4.6) but are sister to *A. arctoseptata* (0.95 PP) in analyses 2

(Figure 4.7). Detailed morphological comparison between *A. baoshanensis* and *A. ovoidea* referred to the notes of *A. ovoidea*. These three species clustered together in both analysis 1 (86% ML, 1.00 PP; Figure 4.6) and analysis 2 (81% ML, 1.00 PP; Figure 4.7) with significant support.



Note a Colonies on dead rattan of *Curcubita moschata*. b–e Conidiophores with a distinct conidiogenous locus. f–l Variation in shape of conidia. Scale bars: a = 200 µm, b–e = 50 µm, f–l = 20 µm.

Figure 4.11 Morphology of *Alternaria baoshanensis*

4.4.1.4 *Alternaria breviconidiophora* J.F. Li, Camporesi, Phookamsak & K. D. Hyde, *sp. nov*.

Index Fungorum number: IF ****; Facesoffungi number: FoF****

Etymology: Named after its short conidiophores and small conidial structures.

Holotype: MFLU 21-****

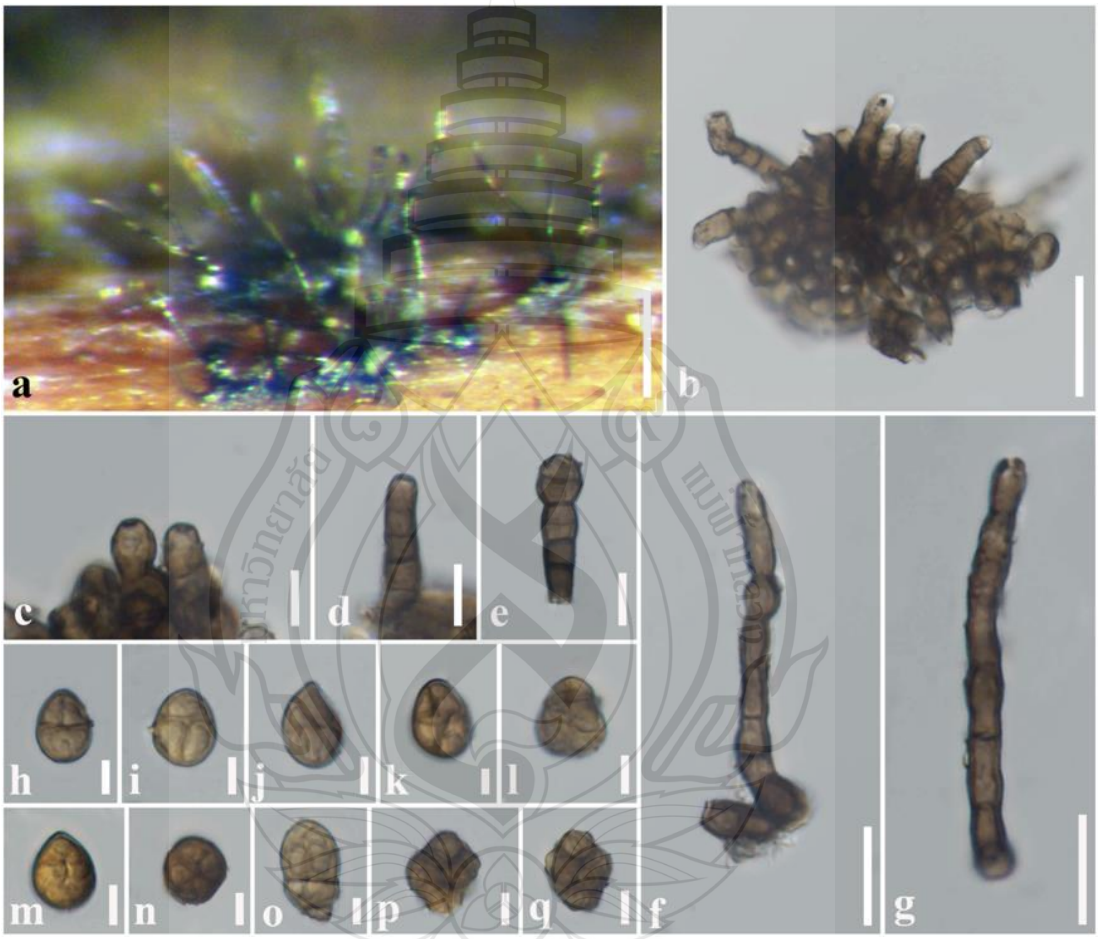
Saprobic on dead hanging branches of *Digitalis* sp. (Scrophulariaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the substrate, composed of brown to dark brown hyphae. *Conidiophores* 25–88 × 6–10 µm ($\bar{x}$ = 45 × 9 µm, n = 40), macronematous, straight or flexuous, cylindrical, dark brown, smooth, thick-walled, septate, unbranched, arising from stromatic base. *Conidiogenous cells* 8–11 × 8–9 µm ($\bar{x}$ = 9.5 × 8 µm, n = 20), mono- to polytretic, integrated, terminal, determinate or percurrent, cylindrical, paler light brown, apically doliiform, with one or a few conidiogenous loci. *Conidia* 8.6–12 × 7–10 µm ($\bar{x}$ = 10 × 8 µm, n = 30) acrogenous, solitary, globose or subglobose, sometimes slightly quadrilateral or ovoid, yellowish brown to brown or dark brown, sectored, 1–2 transversely disto- or eusepta, with 1 longitudinal or oblique or Y-shaped septum, smooth to verruculose, thick-walled. *Conidial secession* schizolytic.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies growing on PDA, cottony, brown to dark brown, reaching 5 mm in 10 days at 25ºC, mycelium superficial, effuse, radially striate, light brown hyphae; conidia not formed *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Rocca San Casciano, on dead hanging branch of *Digitalis* sp. (Scrophulariaceae), 7 April 2017, E. Camporesi, IT3308 (MFLU XX-XXXX, holotype), ex-type living culture = MFLUCCXX-XXXX.

Notes: Multi-locus phylogeny showed that two strains of *Alternaria breviconidiophora* form a robust clade (100% ML, 1.00 PP; Figure 4.6, 4.7) basal to *A. muriformispora, A. lathyri* and *A. pseudoinfectoriae* with significant support in both of analysis 1 (99% ML, 1.00 PP; Figure 4.6) and analysis 2 (90% ML, 1.00 PP; Figure 4.7). However, *A. breviconidiophora* differs from these three species in having small ($\bar{x}$ = 10 × 8 µm), globose or subglobose, non-beaked, 1–2 transversely disto- or euseptate conidia and tiny conidiophores arising from a stomatic base. On the other hand, a comparison of RPB2 nucleotide pairwise showed that *A. breviconidiophora* differs from *A. muriformispora* in 12/570 bp (2.1% difference, no gap), differs from *A. lathyri* in 11/575 bp (1.9% difference,

no gap) and differs from *A. pseudoinfectoriae* in 9/550 bp (1.5% difference, no gap). A comparison of Alt-a1 nucleotides showed that *A. breviconidiophora* differs from *A. muriformispora* in 10/462 bp (2.2% difference, no gap), differs from *A. lathyri* in 9/500 bp (1.8% difference, no gap) and differs from *A. pseudoinfectoriae* in 7/480 bp (1.5% difference, no gap). These tallies along with recommendations outlined by Jeewon and Hyde (2016) establish *A. breviconidiophora* as a new species.



Notes (MFLU XX-XXXX). a Colonies on dead branch. b Conidiophores arising on stomatic base. c–g Conidiophores. h–q Conidia. Scale bars: a = 50 µm, b, f–g = 30 µm, c–e = 10 µm, h–q = 5 µm.

Figure 4.12 Morphology of *Alternaria breviconidiophora*

4.4.1.5 *Alternaria doliconidium* J.F. Li, Camporesi & K.D. Hyde, in Wanasinghe et al., Fungal Diversity: 10.1007/s13225-018-0395-7, [147] (2018)

Index Fungorum number: IF554202; Facesoffungi number: FoF 04041

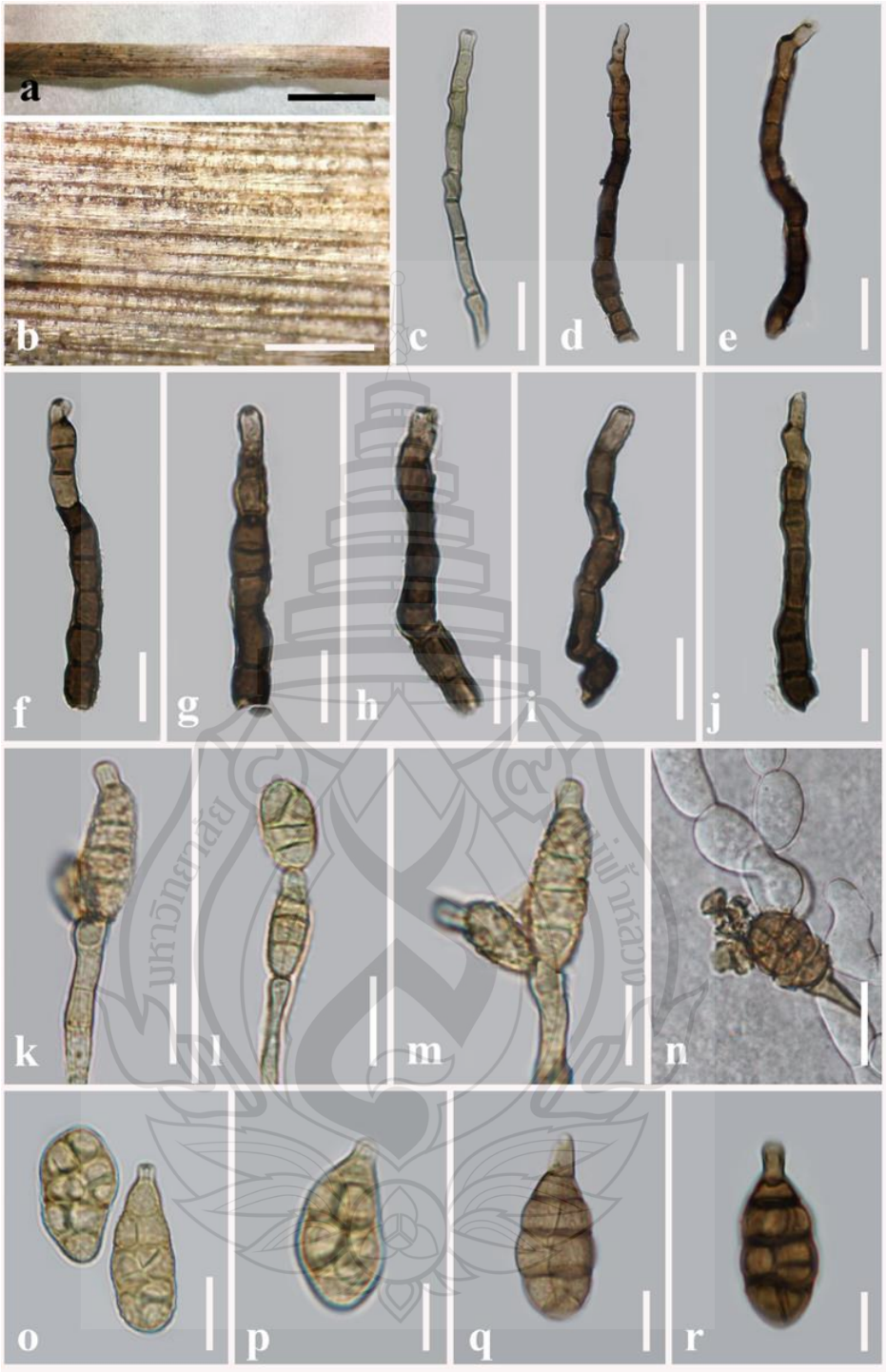
Type details: ITALY, Province of Forli-Cesena [FC], Raggio di Santa Sofia, on dead aerial spines of *Rosa canina* L. (Rosaceae), 16 October 2014, E. Camporesi, IT2165 (KUN-HKAS100840, holotype), ex-type living culture = KUMCC 17-0263.

Saprobic on dead stems of *Reseda* sp. Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the substrate, with dark brown hyphae. *Conidiophores* (140–)167–176(–189.5) × 8–10(–10.5) µm ($\bar{x}$ = 172.4 × 9.5 µm, n = 100), macronematous, mononematous, straight or flexuous, cylindrical, dark brown, paler towards the apex, septate, unbranched, smooth, thick-walled. *Conidiogenous cells* (7.5–)9–10(–12) × (5.5–)8–12.5(–13) µm ($\bar{x}$ = 9.6 × 10.2 µm, n = 100), mono- to polytretic, sympodial, integrated, terminal, determinate or percurrent, cylindrical, subhyaline to paler brown, thick-walled, apically doliiform, with 1–3 conidiogenous cicatrized loci on conidial secession. *Conidia* (56.5–)65.5–71(–79) × (22–)26–30(–34) µm ($\bar{x}$ = 67.3 × 27.8 µm, n = 100) acrogenous, solitary, dry, curved, obclavate to ellipsoidal, or obpyriform, with short, unbranched, aseptate apical beak, or non-beaked, brown to dark brown, 3–5 transversely disto- or eusepta, with 1 longitudinal or oblique or Y-shaped septum in transverse divisions, borne in chains, verruculose to verrucose, thick-walled. *Conidial secession* schizolytic.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from lateral cells. Colonies growing on PDA, hairy fluffy or cottony, grey to brown, reaching 5 cm in 15 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, grey to light brown hyphae. Conidia sporulated on OA within 30 days, phragmosporous to muriform, oblong to subglobose, with short, branched or unbranched, aseptate apical beak, formed apically, or laterally secondary conidiophores with one conidiogenous locus, olivaceous-brown to golden brown, sectored, 2–3 transversely eusepta, with 0–1 longitudinal or oblique or Y-shaped septum in transverse divisions, borne in chains, verruculose to verrucose.

Material examined: CHINA, Yunnan Province, Kunming, Kunyang Town, on dead stem of *Reseda* sp. (Resedaceae), 18 September 2014, J.F. Li, H-11B (MFLU XX-XXXX, new geographical and host record), living culture MFLUCC 14-0020.

Notes: Wanasinghe et al. (2018) described *Alternaria doliconidium* on *Rosa canina* L. (Rosaceae) from Italy. In this study, we collected *A. doliconidium* on *Reseda* sp. as the first record on this host species from China. *Alternaria doliconidium* was isolated from the same host species as *A. alternata* (IT1994) collected in Italy. A comparison nucleotide pairwise showed that our strain MFLUCC 14-0020 differs from *A. alternata* (IT1994) in10/520 bp (1.9% difference, no gap) of GAPDH and 8/500 bp (1.6% difference, no gap) of Alt-a1 gene locus. Multi-locus phylogeny (Fig. 7) showed that our strain (MFLUCC 14-0020) forms a robust clade (100% ML, 1.00 PP; Fig. 7) with the ex-type strains of *A. doliconidium* (HKAS 100840, MFLUCC 17-0263) and clustered with *A. italica* (KUMCC 17-0123, MFLUCC 14-0421) and *A. alternata* (CBS 117143, CBS113054) within *A. alternata* clade. A comparison of nucleotide pairwise showed that *A. doliconidium* is significant distinct from *A. italica*, with *A. alternata A. alternata* (CBS 117143, CBS113054) in ALT-a1, GAPDH, RBP2, and TEF1-α (Table 6). However, the phylogenetic relationship between *A. doliconidium* and *A. italica* with *A. alternata* is unresolved in this study, pending further study.



Note a Dead stem of *Reseda* sp. (Resdaceae). b Colonies on dead stem. c–j Conidiophores on natural substrate. k–m Conidiogenesis sporulated *in vitro*. n Germinated conidium. o–r Conidia (q, r on natural substrate). Scale bars: a = 0.1 cm, b = 500 µm, c–j, n = 30 µm, k = 20 µm, l, m, o–r = 15 µm.

Figure 4.13 Morphology of *Alternaria doliconidium*

Table 4.6 Nucleotide base comparison *Alternaria doliconidium* (H-11B) with closely related taxa

Specie name	Strain no.	Nucleotide difference of gene sequences (no gaps)				
A. doliconidium	H-11B	ITS	TEF1-α	RBP2	GAPDH	ALT-a1
A. alternata	CBS 113054	8/520 bp (1.5%)	14/256 bp (4.7%)	30/875 bp (3.4%)	16/582 bp (2.7%)	12/476 bp (2.5%)
A. alternata	CBS 117143	8/520 bp (1.5%)	14/256 bp (4.7%)	30/875 bp (3.4%)	16/582 bp (2.7%)	12/476 bp (2.5%)
A. doliconidium	KUMCC 17-0263	1/520 bp, (0.2%)	0%	-	-	-
A. italica	MFLUCC 14-0231	6/520 bp, (1.2%)	11/256 bp (4.3%)	25/875 bp, (2.9%)	-	-

4.4.1.6 *Alternaria ellipsoconidia* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF ****, Facesoffungi number: FoF****

Etymology: Named after its ellipsoidal conidia.

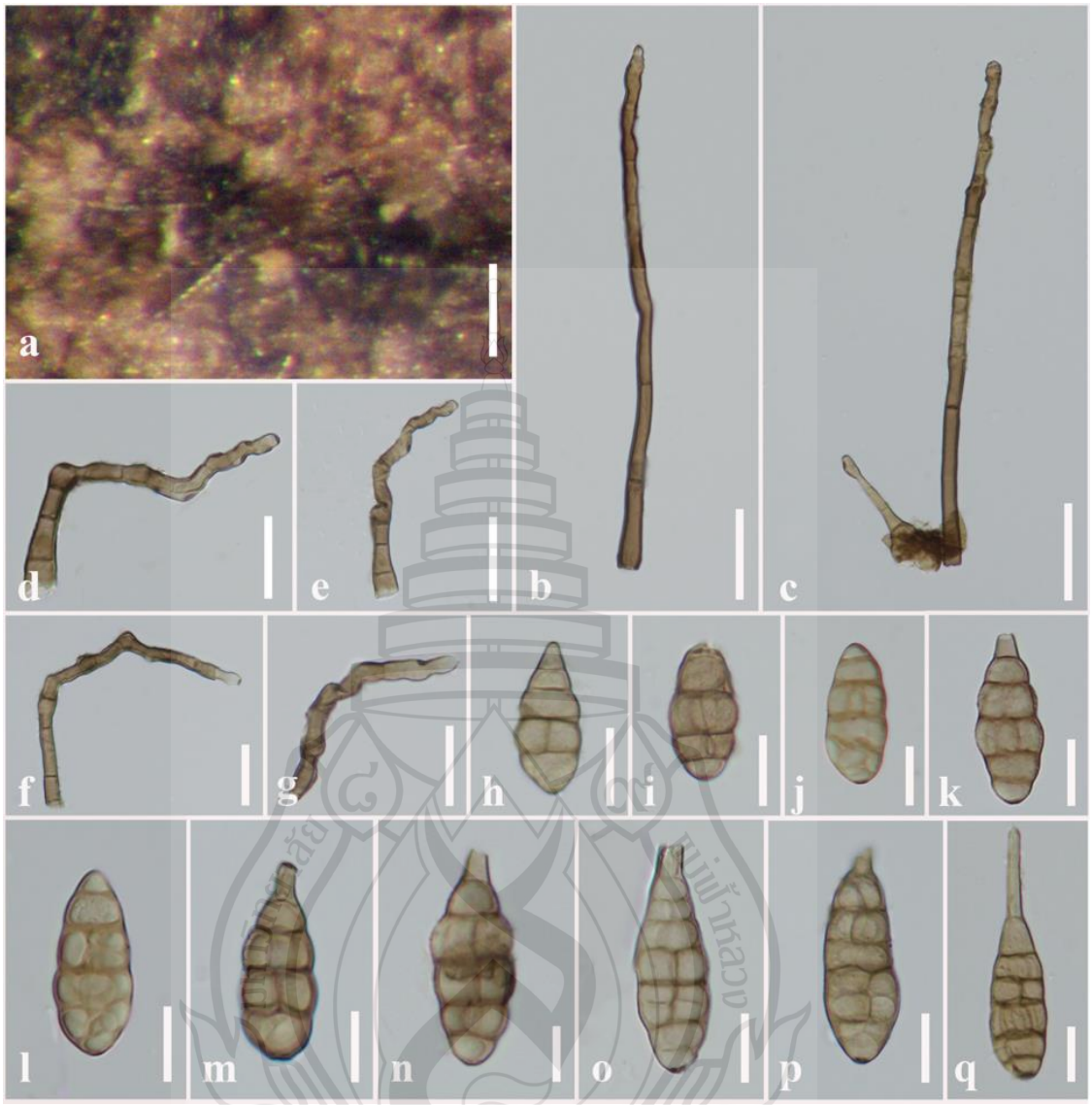
Holotype: MFLU 21-****

Saprobic on dead hanging hulls of *Brassica* sp. (Brassicaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the substrate, with dark brown hyphae. *Conidiophores* 65–188 × 5–8 µm ($\bar{x}$ = 145 × 6.5 µm, n = 30), macronematous, solitary, straight or flexuous, cylindrical, light brown to brown, septate, smooth or verrucose, thick-walled, geniculate near conidiogenous loci. *Conidiogenous cells* 6–9 × 5–7 µm ($\bar{x}$ = 7.5 × 5.5 µm, n = 20), polytretic, sympodial, integrated, terminal, determinate or percurrent, cylindrical, subhyaline to light brown, smooth, thin-walled, apically doliiform, with 1–4 conidiogenous loci. *Conidia* 45–70 × 15–30 µm ($\bar{x}$ = 60 × 28 µm, n = 30) acrogenous, solitary, straight, curved, ellipsoidal to ovoid, or obpyriform, with short to long, aseptate, unbranched apical beak, sometimes lacking beak, pale to yellowish brown, 3–6 transversely disto- or euseptate, with 1 longitudinal or oblique or Y-shaped septum in some transverse divisions, verruculose to verrucose, thick-walled. *Conidial secession* schizolytic.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies growing on PDA, hairy fluffy, brown to dark brown, reaching 5 cm in 10 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, white to grey-white hyphae; conidia not formed *in vitro* within 60 days.

Material examined: ITALY, Province of Arezzo, Stia, Papiano, on dead handing hulls of *Brassica* sp. (Brassicaceae), 1 September 2014, E. Camporesi, IT2086 (MFLU XX-XXXX, holotype**)**, ex-type living culture = MFLUCC XX-XXXX.

Notes: *Alternaria ellipsoconidia* resembles *A. falcata* due to its ellipsoidal conidia with short beak and curved conidiophores. However, *A. ellipsoconidia* differs from *A. falcata* in having solitary conidiophores with several geniculate conidiogenous loci proliferations at apex, which is rather polytretic than *A. falcata*. In multi-locus phylogenetic analyses (Figure 4.6, 4.7), *A. ellipsoconidia* formed an independent clade closely related with *A. eupatoriicola* and distant from *A. falcata*. Morphologically, *A. ellipsoconidia* can be distinguished from *A. eupatoriicola* in having larger (60 × 28 µm vs. 48 × 20 µm), pale to yellowish brown conidia, and the conidiophores are more twisted at the apex in *A. ellipsocondia*. A comparison of RPB2 nucleotide base showed that *A. ellipsoconidia* differs from *A. eupatoriicola* in 9/550 bp (1.6% difference, no gap). In GAPDH, *A. ellipsoconidia* differs from *A. eupatoriicola* in 13/520 bp (2.5% difference, no gap), and in Alt-a1 the species differs from *A. eupatoriicola* in 10/480 bp (2.1% difference, no gap). Therefore, the new species *A. ellipsoconidia* is established.



Note a Colonies on dead hulls of *Brassica* sp. b–g Conidiophores bearing conidiogenous cells with a few apical conidiogenous loci. h–q Conidia. Scale bars: a = 300 µm, b, c, e–g = 30 µm, d, h–q = 20 µm.

Figure 4.14 Morphology of *Alternaria ellipsoconidia*

4.4.1.7 ***Alternaria eupatoriicola*** J.F. Li, Camporesi, Phookamsak & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Named after the host genus "*Eupatorium cannabinum*".

Holotype: MFLU 21-****

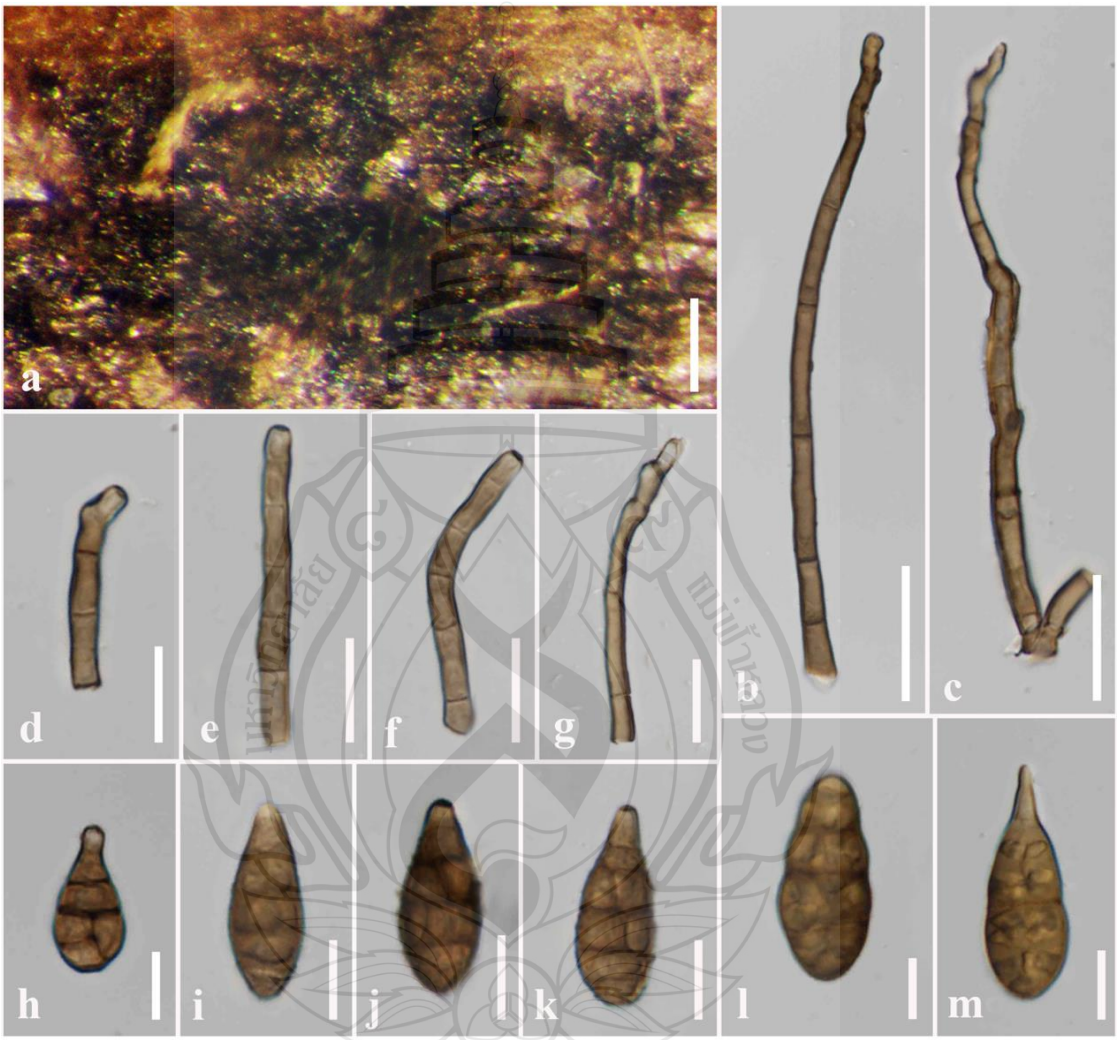
Saprobic on hanging dead stem of *Eupatorium cannabinum* L. (Asteraceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the substrate, thick-walled, with dark hyphae. *Conidiophores* 50–160 × 5–8 µm ($\bar{x}$ = 115 × 7 µm, n = 30), macronematous, mononematous, solitary, straight or flexuous, cylindrical, light brown to brown, septate, unbranched, smooth, thick-walled. *Conidiogenous cells* 6–8 × 4–7 µm ($\bar{x}$ = 7 × 5 µm, n = 20), mono- to polytretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline to light brown, smooth, thin-walled, apically doliiform, with 1–5 conidiogenous loci, sometimes swollen near conidiogenous loci. *Conidia* 40–65 × 15–30 µm ($\bar{x}$ = 48 × 20 µm, n = 30) acrogenous, solitary, straight to curved, ovoid to obpyriform, sometimes with obtuse or coniform, paler brown, short, aseptate, unbranched apical beak, reddish brown to brown, 2–5 transverse septa, with 1 longitudinal or oblique or Y-shaped septum in some transverse divisions, aseptate at the end cells, sometimes constricted at middle transverse septa, borne in chain, verruculose or verrucose, thick-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from both end cells. Colonies growing on PDA, hairy fluffy, brown to dark brown, reaching 5 cm in 20 days at 25ºC, mycelium superficial, effuse, radially striated, with irregular edge, white to light dark brown hyphae; conidia not sporulated *in vitro* within 60 days.

Material examined: ITALY, Province of Arezzo, Badia Prataglia, on dead hanging stem of *Eupatorium cannabinum* (Asteraceae), 2 October 2017, E. Camporesi, IT3518 (MFLU XX-XXXX, holotype), ex-type living culture = MFLUCC XX-XXXX.

Notes: Based on multi-locus phylogenetic analyses, two strains of *Alternaria eupatoriicola* formed a robust clade (100% ML, 1.00PP; Figure 4.7) that clustered with *Alternaria* sp. (H-22) and *Alternaria* sp. (MFLUCC 14-0436) with high support (98% ML, 1.00 PP; Figure 4.7) and had a close relationship with *A. ellipsoconidia*. However, the specimens of *Alternaria* sp. (H-22) and *Alternaria* sp. (MFLUCC 14-0436) were

irrecoverable. Therefore, we could not compare the morphological characteristics of *A. eupatoriicola* with these two species. *Alternaria eupatoriicola* can be easily distinguished from *A. ellipsoconidia* due to its reddish brown to dark brown, beakless conidia, and less conidial septation.



Note a Colonies on dead hanging stem of *Eupatorium cannabinum*. b–f Conidiophores bearing conidiogenous cells. g–q Conidia. Scale bars: a = 300 µm, b = 30 µm, c–m = 20 µm.

Figure 4.15 Morphology of *Alternaria eupatoriicola*

4.4.1.8 *Alternaria falcata* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****, Facesoffungi number: FoF****

Etymology: Referring to the curve conidiophores.

Holotype: MFLU 21-****

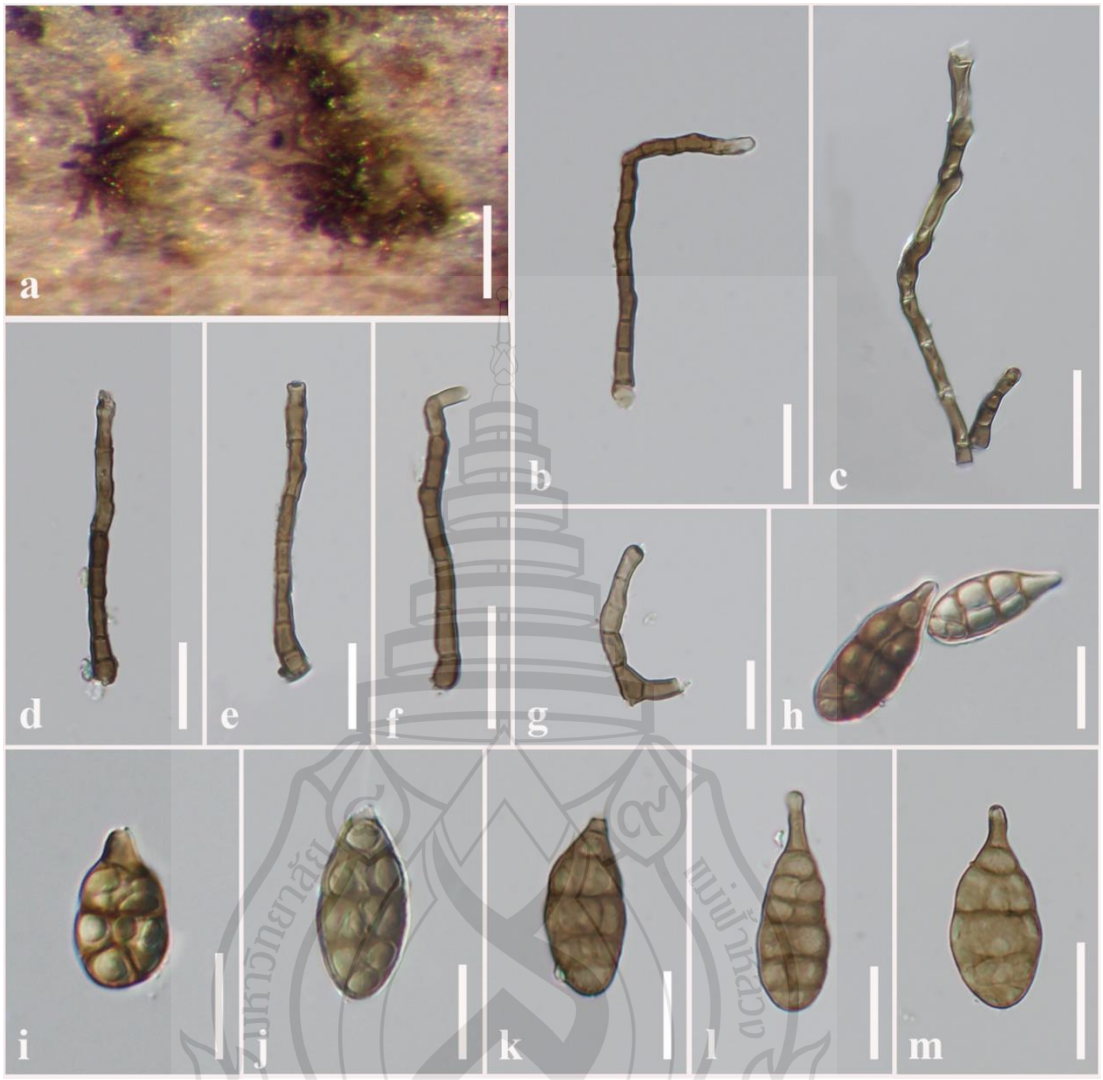
Saprobic on dead hanging stem of *Atriplex* sp. (Chenopodiaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the substrate, composed of dark, septate, branched-hyphae. *Conidiophores* 70–130 × 5–8 µm ($\bar{x}$ = 96 × 7 µm, n = 30), macronematous, mononematous, straight or flexuous, cylindrical, light brown to brown, septate, unbranched, smooth to verruculose, thick-walled. *Conidiogenous cells* 10–11 × 3–5.5 µm ($\bar{x}$ = 10.5 × 4.5 µm, n = 20), mono- to polytretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline, apically doliiform to coronal, with 1–2 conidiogenous loci, smooth, thick-walled. *Conidia* 20–50 × 12–23 µm ($\bar{x}$ = 40 × 15 µm, n = 30) acrogenous, solitary, straight or curved, subglobose to ellipsoidal or obpyriform, with paler brown, short, obtuse, narrow, aseptate, unbranched beak, olivaceous-brown to brown, 2–5 transversely disto- or euseptate, with 1–3 longitudinal or oblique septum in some transverse divisions, sometimes formed Y-shaped septum at lower end cell, smooth to verrucose, thick-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies growing on PDA, hairy or cottony, brown to dark brown, reaching 5 mm in 20 days at 25ºC, mycelium superficial, effuse, radially striated, with irregular edge, white to light grey colored hyphae; conidia not formed *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Fiumicello di Premilcuore, on dead hanging stem of *Atriplex* sp. (Chenopodiaceae), 29 August 2014, E. Camporesi, IT2079 (MFLU XX-XXXX, holotype), ex-type living culture = MFLUCC XX-XXXX.

Notes: In multi-locus phylogenetic analyses, *Alternaria falcata* formed an unstable clade within *Alternaria* sect. *Alternaria*. In analyses 1 (Figure 4.6), two strains of *A. falcata* formed an independent clade (0.97 PP) basal to *A. obpyriconidia* (IT 1688), *A. macroconidia* (IT 1756), *A. salicicola* (T-504), *A. arctoseptata* (IT 2088), *A. baoshanensis* (H-50) and *A. ovoidea* (IT 1656), respectively. In analyses 2 (Figure 4.7), the species has a

close relationship (0.97 PP) with *A. ovoidea* (IT 1656), *A. baoshanensis* (H-50) and *A. arctoseptata* (IT 2088), respectively. Morphologically *A. falcata* differs from *A. arctoseptata* in having smaller (40 × 15 µm vs. 60 × 25 µm), subglobose to ellipsoidal, olivaceous-brown to brown conidia, solitary conidiophores with a curved apex, while its conidia are rather ellipsoidal to obpyriform or obclavate, brown to dark brown, constricted at the septa and conidiophores aggregate on the stomatic base in *A. arctoseptata*. *Alternaria falcata* differs from *A. baoshanensis* in having larger (40 × 15 µm vs. 38 × 18 µm) and longer (96 × 7 µm vs. 48 × 14 µm) solitary concolorous conidiophores, while the species differs from *A. ovoidea* in having smaller (40 µm × 15 µm vs. 55 × 27 µm) and paler brown conidia, with a short beak, and shorter (96 × 7 µm vs. 280 × 8 µm), curved conidiophores. A comparison of RPB2 nucleotide pairwise showed that *A. falcata* differs from *A. arctoseptata* in 10/565 bp (1.8% difference, no gap), differs from *A. baoshanensis* in 8/559 bp (1.4% difference, no gap) and differs from *A. ovoidea* in 9/565 bp (1.6% difference, no gap). A comparison of GAPDH nucleotide pairwise showed that *A. falcata* differs from *A. arctoseptata* in 13/590 bp (2.2% difference, no gap), differs from *A. baoshanensis* in 13/578 bp (2.2% difference, no gap) and differs from *A. oviodea* in 11/570 bp (1.9% difference, no gap). A comparison of Alt-a1 nucleotide pairwise showed that *A. falcata* differs from *A. arctoseptata* in 11/520 bp (2.1% difference, no gap), differs from *A. baoshanensis* in 8/474 bp (1.7% difference, no gap) and differs from *A. ovoidea* in 14/520 bp (2.7% difference, no gap). Thus, the new species *A. falcata* is established base on morphology and phylogeny.



Note a Colonies on dead hanging stem of *Atriplex* sp. b–g Conidiophores bearing conidiogenous cells. h–m Conidia. Scale bars: a = 150 µm, b–f, l, m = 30 µm, g–k = 20 µm.

Figure 4.16 Morphology of *Alternaria falcate*

4.4.1.9 *Alternaria lathyri* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Named after the host genus "*Lathyrus*".

Holotype: MFLU 21-****

Saprobic on dead stem of *Lathyrus* sp. (Fabaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the substrate, with dark hyphae. *Conidiophores* 100–130 × 11–15 µm ($\bar{x}$ = 115 × 14 µm, n = 20), macronematous, mononematous, straight or flexuous, cylindrical, dark brown to black, smooth, septate, unbranched, thick-walled. *Conidiogenous cells* 13–15 × 8–10 µm ($\bar{x}$ = 12.5 µm × 9 µm, n = 20), mono- to polytretic, integrated, terminal, determinate or percurrent, paler or light brown, smooth, thin-walled, apically rounded to doliiform, with 2–4 conidiogenous loci. *Conidia* 40–65 × 18–31 µm ($\bar{x}$ = 55 × 25 µm, n = 20) acrogenous, solitary, straight or curved, ovoid to obpyriform, beakless, occasionally formed a short beak, dark grey-brown, with 2–4 transverse eusepta, with 1–2 longitudinal septa in some transverse divisions, aseptate or with 1–2 oblique or Y-shaped septa at the lower end cell, slightly constricted at the middle transverse septa, verrucose, thick-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 12 hours and germ tubes produced from lateral cells. Colonies growing on PDA, cottony, brown to dark brown, reaching 5 cm in 10 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, brown to dark brown hyphae; conidia not formed *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Galeata, Strada San Zeno, on dead aerial stem of *Lathyrus* sp. (Fabaceae), 11 January 2014, E. Camporesi, IT 1640 (MFLU XX-XXXX, holotype), ex-type living culture = MFLUCC XX-XXXX.

Notes: Multi-locus phylogeny demonstrated *Alternaria lathyri* has a close relationship with *A. muriformispora* (IT 2101) and *A. pseudoinfectoriae* (IT2181) with significant support (88% ML, 0.96 PP; Figure 4.6, 4.7). *Alternaria lathyri* can be distinguished from *A. muriformispora* in having smaller (55 × 25 µm vs. 83 × 29 µm) darker, and beakless conidia, with less conidial septation. The species also differs from *A. pseudoinfectoriae* in having larger (55 × 25 µm vs. 33 × 19 µm), ovoid to obpyriform, dark grey-brown and beakless conidia, while *A. pseudoinfectoriae* has subglobose to obclavate, or obpyriform, light brown conidia that usually formed long secondary

conidiophores. A comparsion of RPB2 nucleotide pairwise showed that *Alternaria lathyri* differs from *A. muriformispora* in 9/570 bp (1.6% differences, no gap), and differs from *A. pseudoinfectoriae* in 12/550 bp (2.2% difference, no gap). In Alt-a1, *A. lathyri* differs from *A. muriformispora* in 8/474 bp (1.7% differences, no gap) and differs from *A. pseudoinfectoriae* in 8/480 bp (1.6% difference, no gap). In this study, *A. lathyri* is described herein as a new species occurring on the same host genus with *A. arctoseptata*.



Note a Colonies on dead stem dead aerial stem of *Lathyrus* sp. b Conidiophores and conidia. c–h Conidiophores bearing conidiogenous cells. i–n Conidia. o Germinated conidium. Scale bars: a = 200 µm, b–h, o = 30 µm, i–n = 20 µm.

Figure 4.17 Morphology of *Alternaria lathyri*

4.4.1.10 *Alternaria macilenta* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Referring to its slender-shaped conidia.

Holotype: MFLU 21-****

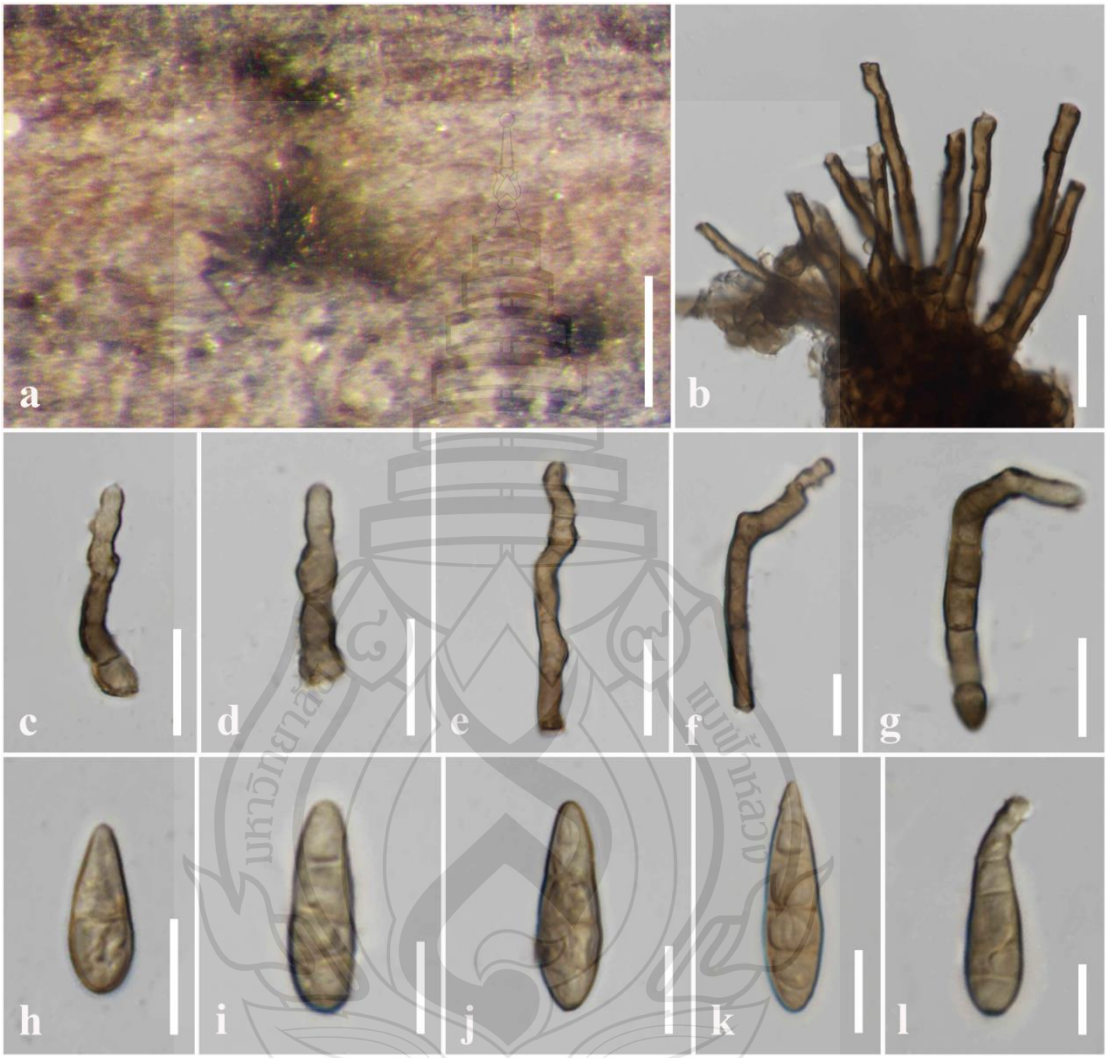
Saprobic on dead hanging stem of *Scabiosa* sp. (Caprifoliaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the substrate, composed of dark hyphae. *Conidiophores* 70–100 × 10–12 µm ($\bar{x}$ = 85 × 11 µm, n = 30), macronematous, brown, straight or flexuous, cylindrical, septate, unbranched, smooth, thick-walled, aggregated, arising from a stomatic base. *Conidiogenous cells* 20–25 × 8–10 µm ($\bar{x}$ = 23.5 µm × 9 µm, n = 20), polytretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline to light brown, smooth, thin-walled, apically rounded or doliiform, with 1–3 conidiogenous loci. *Conidia* 20–50 × 8–12 µm ($\bar{x}$ = 35 × 9.5 µm, n = 30) acrogenous, solitary, straight, sometimes curved at the apex, subcylindrical to obclavate, beakless, pale brown to light greyish brown, 0–4-distoseptate, smooth- to rough-walled, with wrinkle-like, thick-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from both end cells. Colonies growing on PDA, cottony, brown to dark brown, reaching 5 cm in 10 days at 25ºC, mycelium superficial, effuse, radially striated, with irregular edge, pale white to white hyphae; conidia not formed *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Dovadola, on dead hanging stem of *Scabiosa* sp. (Caprifoliaceae), 25 August 2015, E. Camporesi, IT2076 (MFLU XX-XXXX, holotype), ex-type living culture = MFLUCC XX-XXXX.

Notes: Multi-locus phylogenies (Figure 4.6, 4.7) demonstrated that *Alternaria macilenta* clustered with *A. oblongoellipsoidea* and *A. phragmiticola* with significant support (83% ML, 0.99 PP in analysis 1 (Figure 4.6) and 82% ML, 1.00 PP in analysis 2 (Figure 4.7). *Alternaria macilenta* can be easily distinguished from *A. oblongoellipsoidea* and *A. phragmiticola* in having subcylindrical to obclavate, beakless, pale brown to light greyish brown, 0–4-distoseptate conidia, while lacking longitudinal septum and conidiophores aggregated at the base. A comparision of RPB2 nucleotides showed that *A. macilenta* differs from *A. oblongoellipsoidea* 10/560 bp (1.8% difference, no gap) and differs from *A. phragmiticola* in 9/560 bp (1.6% difference, no gap). In Alt-a1, *A. macilenta*

differs from *A. oblongoellipsoidea* in 10/470 bp (2.1% difference, no gap) and differs from *A. phragmiticola* in 8/492 bp (1.6% difference, no gap).



Note a Colonies on dead stem of *Scabiosa* sp. b–g Conidiophores. h–l Conidia. Scale bars: a = 150 µm, b–g = 30 µm, h–l = 15 µm.

Figure 4.18 Morphology of *Alternaria macilenta*

4.4.1.11 *Alternaria macroconidia* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Named after its large conidia (up to 60 µm).

Holotype: MFLU 21-****

Saprobic on branches of *Spartium junceum* L. (Fabaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on host substrate, composed of septate, branched, smooth, thin-walled, white to light brown hyphae. *Conidiophores* 46–80 × 7–12 µm ($\bar{x}$ = 55 × 8.2 µm, n = 100), mononematous, macronematous, straight or flexuous, cylindrical, brown to dark brown, septate, unbranched, smooth, thick-walled, arising from an aggregated base. *Conidiogenous cells* (6–)7–9(–10.5) × (7.5–)8–12 (–17) µm ($\bar{x}$ = 7.9 × 9.6 µm, n = 100), mono- to polytretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline to pale brown, smooth, thin-walled, apically doliiform with one conidiogenous locus. *Conidia* (68.5–)77–89(–95.5) × (20–) 26–28(–30.5) µm ($\bar{x}$ = 75.6 × 27.7 µm, n = 100) acrogenous, solitary, dry, curved, obclavate to ovoid, or obpyriform, with short to long, narrow, pale to greenish brown, aseptate, unbranched beak, olivaceous brown to golden brown or brown, 3–5 transversely disto- or euseptate, with 1 longitudinal or oblique or Y-shaped septum in some transverse divisions, borne in chain, usually rostrate apex when mature, minutely verruculose, thin-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 12 hours and germ tubes produced from lateral cells. Colonies growing on PDA, cottony, white to light brown, reaching 5 cm in 7 days at 25ºC, mycelium superficial, effuse, radially striate, with regular edge, white to light brown hyphae. Conidia sporulated on OA media within 15 days, muriform, ovoid to obclavate, with aseptate, short, paler brown, acicular apical rostrum, light brown to brown, 2–4 transversely eusepta, 0–1 longitudinal septum in transverse division, born in chains, smooth, laterally formed branched or unbranched conidiophores with 1–2 conidiogenous loci.

Material examined: ITALY, Province of Forlì-Cesena, Santa Sofia, Collina di Pondo, on dead aerial branches of *Spartium junceum* (Fabaceae), 6 March 2014, E. Camporesi, IT1756 (MFLU XX-XXXX, holotype), ex-type living culture = MFLUCC XX-XXXX.

Notes: *Alternaria macroconidia* resembles *A. phragmiticola* in having obclavate conidia, with a short to long beak or rostrate apex when mature. However, *A. macroconidia* differs from *A. phragmiticola* in having slightly larger (75.6 × 27.7 µm vs. 70 × 25 µm), olivaceous brown to golden brown or brown conidia, rather monotretic conidiogenous cells and shorter (55 × 8.2 µm vs. 90 × 6.5 µm), aggregated conidiophores at the base. In multi-locus phylogenetic analyses, *A. macroconidia* formed a separate lineage basal to *A. vitis* (MFLUCC 17-1109) and *A. salicicola* (T-504) with significant support (83% ML, 0.96 PP; Figure 4.7), also clustering with *A. obpyriconidia*. *Alternaria macroconidia* differs from *A. vitis* (MFLUCC 17-1109) and *A. salicicola* (T-504) in having larger (75.6 × 27.7 µm vs. 39 × 15 µm vs. 45 × 32 µm), olivaceous brown to golden brown or brown conidia, with 3–5 transversely disto- or eusepta, and 1 longitudinal or oblique or Y-shaped septum in some transverse divisions. *Alternaria vitis* (MFLUCC 17-1109) has brown to dark greyish-brown conidia, with 1–3 transversely eusepta, and indistinct 0–1 longitudinal or oblique or Y-shaped septum in some transverse divisions (Jayawardena et al. 2018), while *A. salicicola* (T-504) has subglobose to obclavate or obpyriform, light yellowish-brown to light brown conidia, with a longer beak, sectored, with several transverse and longitudinal distosepta. A comparision of ITS nucleotide pairwise showed that *A. macroconidia* differs from *A. vitis* in 10/490 bp (2% difference, no gap). In GAPDH, *A. macroconidia* differs from *A. salicicola* in 23/569 bp (4% difference, no gap) and differs from *A. obpyriconidia* in 12/569 bp (2.1% difference, no gap). In Alt-a1, *A. macroconidia* differs from *A. salicicola* in 12/520 (2.3% difference, no gap) and differs from *A. obpyriconidia* in 13/500 bp (2.1% difference, no gap). Based on distinct morphological characteristics and phylogenetic support, *A. macroconidia* is introduced as a new species in this study.



Note a Specimen examined of *Spartium junceum* (Fabaceae). b Colonies on dead aerial branch of *Spartium junceum*. c–j Conidiophores bearing conidiogenous cells. k–n Immature conidia. o–s Mature conidia. t Germinated conidium. Scale bars: a = 0.5 cm, b = 1000 µm, d, e = 30 µm, c, f–t = 20 µm.

Figure 4.19 Morphology of *Alternaria macroconidia*

4.4.1.12 *Alternaria minimispora* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Named after its tiny conidia.

Holotype: MFLU 21-****

Saprobic on rotten peel of *Citrullus lanatus* (Thumb.) Matsum et Nakai (Cucurbitaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the substrate, with dark hyphae. *Conidiophores* 65–175 × 8–13 µm ($\bar{x}$ = 129 × 10 µm, n = 30), macronematous, mononematous, straight or flexuous, cylindrical, dark yellowish-brown, septate, unbranched, smooth, thick-walled. *Conidiogenous cells* 5–10 × 4–6 µm ($\bar{x}$ = 8.5 × 5 µm, n = 20), mono- to polytretic, integrated, terminal, determinate or percurrent, cylindrical, doliiform or coronal-shaped, light brown to brown, smooth, thin-walled, with 1–3 apical conidiogenous loci. *Conidia* 13–25 × 8–11 µm ($\bar{x}$ = 20 × 9.5 µm, n = 30) acrogenous, solitary, straight, curved, subglobose to ovoid, sometimes obpyriform or obturbinate, beakless, dark brown, 2–4 transversely euseptate, with 1–2 longitudinal or oblique or Y-shaped septa in some transverse divisions, smooth, thick-walled, formed apically secondary conidiophores with 1–2 conidiogenous loci. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies growing on PDA, cottony, white to grey, reaching 5 cm in 10 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, yellow white to grey hyphae; conidia not sporulated *in vitro* within 60 days.

Material examined: THAILAND, Chiang Rai Province, Mae Fah Luang University, on rotten peel of *Citrullus lanatus* (Cucurbitaceae), 13 August 2016, J.F. Li, H-14 (MFLU XX-XXXX, holotype), ex-type living culture = MFLUCC XX-XXXX.

Notes: *Alternaria minimispora* resembles *A. breviconidiophora* due to small, subglobose to ovoid, dark brown conidia, with swollen knots conidiophores and paler brown conidiogenous cells. However, *A. minimispora* differs from *A. breviconidiophora* in having solitary, longer (129 × 10 µm vs. 45 × 9 µm) conidiophores with absent stomatic base and conidia that are rather rostrate than *A. breviconidiophora*. In multi-locus phylogenetic analyses, *A. minimispora* formed a sister lineage with *A. rostroconidia* (0.95 PP, Figure 4.6, 4.7) and distant from *A. breviconidiophora*. *Alternaria minimispora* can be

distinguished from *A. rostroconidia* in having smaller (20 × 9.5 µm vs. 66 × 22 µm), subglobose to ovoid, beakless conidia that conidia are rather short beak in *A. rostroconidia*. A comparision of RPB2 nucleotide pairwise showed that *A. minimispora* differs from *A. breviconidiophora* in 40/505 bp (7.9% difference, no gap) and differs with *A. rostroconidia* in 19/505 bp (3.8% difference, no gap). In GAPDH, *A. minimispora* differs from *A. breviconidiophora* in 12/550 bp (2.1% difference, no gap) and differs from *A. rostroconidia* in 10/545 bp (1.8% difference, no gap). In comparison, the Alt-a1 nucleotide shows that *A. minimispora* differs from *A. breviconidiophora* in 14/474 bp (3% difference, no gap) and differs with *A. rostroconidia* in 8/474 bp (1.7% difference, no gap).

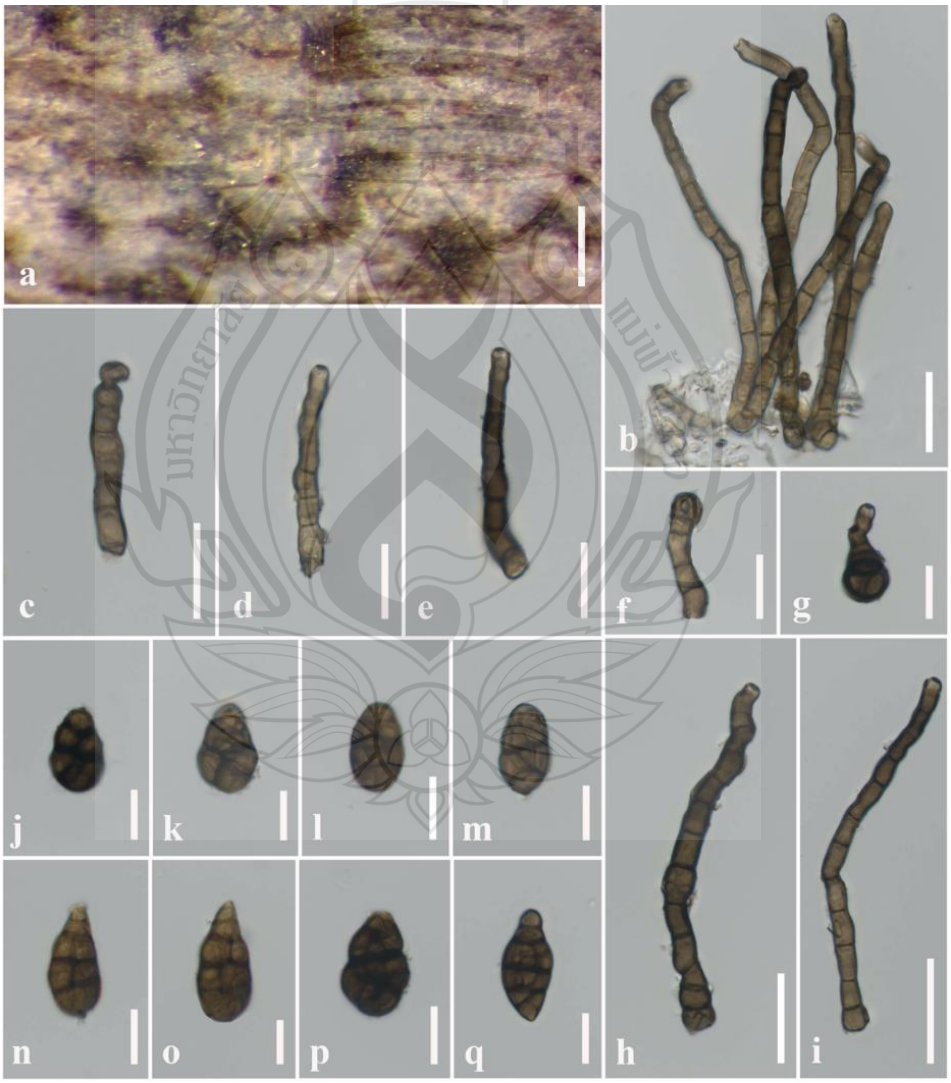


Figure 4.20 Morphology of *Alternaria minimispora*

Figure 4.20 a Colonies on rotten peel of *Citrullus lanatus*. b–e, h, i Conidiophores bearing conidiogenous cells. f Conidiogenous cells. g Secondary conidiophores arising from conidia. j–q Conidia. Scale bars: a = 300 µm, i = 50 µm, b–e, h = 30 µm, f = 15 µm, g, l–q = 10 µm, j–k = 5 µm.

4.4.1.13 *Alternaria muriformispora* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Named after its muriform conidia.

Holotype: MFLU 21-****

Saprobic on dead aerial stem of *Plantago* sp. (Plantaginaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the substrate, composed of septate, branched, smooth, thin-walled, brown hyphae. *Conidiophores* 185–201 × 12–13 µm ($\bar{x}$ = 192 × 12 µm, n = 30), macronematous, straight or flexuous, cylindrical, with swollen at the basal cell, slightly narrower towards the apex, dark brown, paler at the apex, smooth, septate, unbranched, thick-walled. *Conidiogenous cells* 4–5 × 5–7 µm ($\bar{x}$ = 4.5 × 6.2 µm, n = 20), polytretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline to light brown, smooth, thin-walled, apically doliiform with one conidiogenous locus. *Conidia* 75–88 × 23–35 µm ($\bar{x}$ = 83 × 29 µm, n = 30) acrogenous, solitary, dry, simple, straight, curved, ellipsoidal to ovoid, or obpyriform with short, narrow, paler brown, aseptate, unbranched, obtuse beak, copper brown to dark brown, 4–7 transversely euseptate, with 1–2 longitudinal or oblique or Y-shaped septa in all middle transverse divisions, without oblique or longitudinal septa at both end cells, slightly thickened and constricted at middle septa, borne in chain, verruculose to verrucose, thin-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from lateral cells. Colonies growing on PDA, hairy or cottony, brown to dark brown, reaching 5 cm in 7 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, brown to dark brown hyphae; conidia not sporulated *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Meldola, on dead aerial stem of *Plantago* sp. (Plantaginaceae), 8 September 2014, E. Camporesi, IT2101 (MFLU XX-XXXX, holotype), ex-type living culture = MFLUCC XX-XXXX.

Notes: Multi-locus phylogenetic analyses showed that *Alternaria muriformispora* is sister to *A. pseudoinfectoriae* with significant support support (86% ML, 0.99 PP) and also formed a well-resolved clade with *A. breviconidiophora* and *A. lathyri* (90% ML, 1.00 PP). *Alternaria muriformispora* differs from *A. pseudoinfectoriae* in having larger (83 × 29 µm vs. 33 × 19 µm), ovoid to ellipsoidal, or obpyriform, short beak, copper brown to dark brown conidia, with 4–7 transverse eusepta, and 1–2 longitudinal or oblique or Y-shaped septa in all middle transverse divisions, while *A. pseudoinfectoriae* has subglobose to obclavate, or obpyriform, light brown conidia, with 3–4 transverse eusepta and 1–2 longitudinal or oblique or Y-shaped septa and conidia that form long, cylindrical, septate, unbranched secondary conidiophores with one apical conidiogenous locus. A comparision of RPB2 nucleotide pairwise showed that *A. muriformispora* differs from *A. pseudoinfectoriae* in 10/559 bp (1.8% difference, no gap). In Alt-a1, *A. muriformispora* differs from *A. pseudoinfectoriae* in 9/474 bp (1.9% difference, no gap).

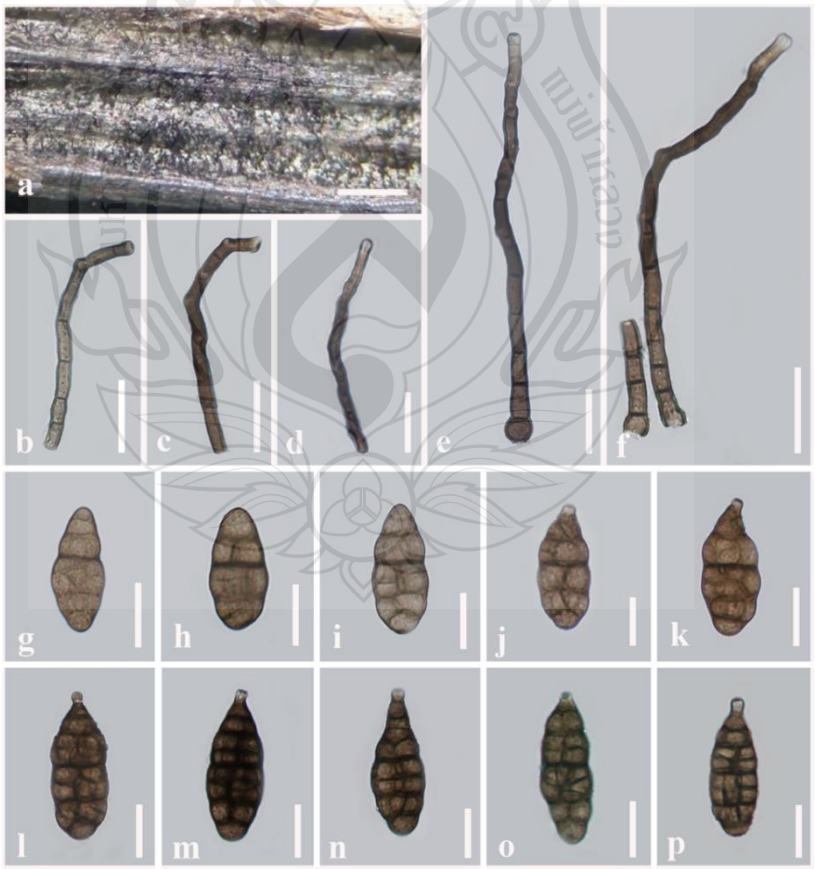


Figure 4.21 Morphology of *Alternaria muriformispora*

Figure 4.21 a Colonies on dead aerial stem of *Plantago* sp. (Plantaginaceae). b–f Conidiophores bearing conidiogenous cells. g–p Conidia. Scale bars: a = 100 µm, b–f = 50 µm, g, j–p = 30 µm, h, i = 20 µm.

4.4.1.14 *Alternaria oblongoellipsoidea* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Referring to its oblong to ellipsoidal conidia.

Holotype: MFLU 21-****

Saprobic on dead stem of *Cichorium intybus* L. (Asteraceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the substrate, composed of septate, branched, smooth, thin-walled, brown to dark brown hyphae. *Conidiophores* 80–120 × 6.5–11 µm ($\bar{x}$ = 96 × 9.5 µm, n = 30), macronematous, light brown to brown, straight or flexuous, cylindrical, septate, minutely verruculose, thick-walled, with several aggregated at the base. *Conidiogenous cells* 3.5–6 × 3–6 µm ($\bar{x}$ = 4.5 × 3.5 µm, n = 20), polytretic, swollen, integrated, terminal, determinate or percurrent, doliiform, subhyaline to pale greenish-brown, smooth, thin-walled, with 2–4 apical conidiogenous loci. *Conidia* 35–60 × 18–25 µm ($\bar{x}$ = 52 × 22 µm, n = 30) acrogenous, solitary, straight or curved, oblong to ellipsoidal, or ovoid, with short, narrow, septate beak, pale brown to brown, sectored, 4–7 transverse eusepta, with 1–2 longitudinal, or oblique or Y-shaped septa in transverse divisions, smooth to minutely verrucose, thick-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all edge cells. Colonies growing on PDA, hairy or cottony, brown to dark brown, reaching 5 cm in 7 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, dark brown hyphae; conidia not sporulated *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Meldola, on dead aerial stem of *Cichorium intybus* (Asteraceae), 8 September 2014, E. Camporesi, IT2102 (MFLU XX-XXXX, holotype), ex-type living culture = MFLUCC XX-XXXX.

Notes: In multi-locus phylogenetic analyses, *Alternaria oblongoellipsoidea* is sister to *A. phragmiticola* with significant support (77% ML, 0.99 PP in analyses 1 (Figure 4.6) and 81% ML, 1.00 PP in analyses 2 (Fig. 7)) and formed a well-resolved clade with *A. macilenta*. A comparision of RPB2 nucleotide pairwise showed that *A. oblongoellipsoidea* differs from *A. phragmiticola* in 10/560 bp (1.8% difference, no gap) and also differs from

A. phragmiticola in 8/492 bp (1.6% difference, no gap) based on a comparison of Alt-a1 nucleotide pairwise. *Alternaria oblongoellipsoidea* can be distinguished from *A. phragmiticola* in having smaller (52 × 22 µm vs. 70 × 25 µm) oblong to ellipsoidal conidia, with 4–7 transverse eusepta, and 1–2 longitudinal or oblique or Y-shaped septa in transverse divisions. While *A. phragmiticola* has obclavate to obpyriform conidia, with longer rostrate beak and less conidial septation than *A. oblongoellipsoidea.*



Figure 4.22 Morphology of *Alternaria oblongoellipsoidea*

Figure 4.22 a Colonies on dead stem of *Cichorium intybus*. b–h Conidiophores bearing conidiogenous cells. i–l Conidia. m Germinated conidium. Scale bars: a = 300 µm, d–m = 30 µm, b, c = 20 µm.

4.4.1.15 *Alternaria obpyriconidia* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Named after its obpyriform conidia.

Holotype: MFLU 21-****

Saprobic on dead stems of *Vicia faba* L. (Fabaceae). Sexual state: Undetermined. Asexual state: *Mycelium* superficial on the substrate, composed of septate, branched, smooth, thin walled, subhyaline to pale white hyphae. *Conidiophores* (130–)139.5–155 × 11.5–13 µm ($\bar{x}$ = 145.8 × 12.6 µm, n = 100), macronematous, mononematous, straight or flexuous, cylindrical, slightly swollen at the apical cell, copper brown to dark brown, septate, unbranched, smooth, thick-walled. *Conidiogenous cells* 19–23 × 9–12.5 µm ($\bar{x}$ = 19.7 × 10.8 µm, n = 100), polytretic, sympodial, integrated, terminal, determinate or percurrent, cylindrical to doliiform, subhyaline, smooth, thick-walled, apically rounded or doliiform, with 2–4 conidiogenous loci. *Conidia* (58–)62.5–68(–69) × (12.5–)22.5–28(–30) µm ($\bar{x}$ = 64 × 25.4 µm, n = 100) acrogenous, solitary, dry, simple, straight or curved, ellipsoidal to obclavate or obpyriform, with short, narrow, pale brown, aseptate, rostrate beak, pale brown to greyish brown, 3–4 transversely eusepta, with 1–2 longitudinal, or oblique or Y-shaped septa at the middle cells, constricted at the central septum, borne in chain, verruculose or verrucose, thin-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 12 hours and germ tubes produced from all cells. Colonies growing on PDA, hairy or cottony, pale to dark brown, reaching 5 mm in 7 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, subhyaline to brown hyphae; conidia not sporulated *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Bagno di Romagna, Vagianna, on dead aerial stem of *Vicia faba* L. (Fabaceae), 29 January 2014, E. Camporesi, IT1688 (MFLU XX-XXXX, holotype), ex-type living culture = MFLUCC14-0435.

Notes: In the phylogenetic analyses, *Alternaria obpyriconidia* formed a separate lineage basal to *A. macroconidia*, *A. vitis* and *A. salicicola* with significant support

in Bayesian inference analysis (1.00 PP; Figure 4.7). *Alternaria obpyriconidia* differs from *A. macroconidia* in having smaller (64 × 25.4 µm vs. 75.6 × 27.7 µm), pale brown to greyish brown conidia, with 3–4 transversely eusepta, while *A. macroconidia* has olivaceous brown to golden brown or brown conidia, with 3–5 transversely disto- or eusepta and conidia that are not constricted in *A. macroconidia*. *Alternaria vitis* differs from *A. obpyriconidia* in having rather obpyriform to subglobose conidia, with less transversely conidial septation, while *A. salicicola* differs from *A. obpyriconidia* in having rather subglobose to obclavate, lighter brown conidia, with sectored 1–6 transversely disto- or eusepta and 1–3 longitudinal or oblique or Y-shaped distosepta. A comparision of ITS nucleotide pairwise showed that *A. obpyriconidia* differs from *A. salicicola* in 9/490 bp (1.8% difference, no gap), and differs from *A. vitis* in 9/490 bp (1.8% difference, no gap). A comparision of GAPDH nucleotide showed that *A. obpyriconidia* differs from *A. salicicola* in 21/575bp (3.7% difference, no gap), and the species also differs from *A. salicicola* in 22/500 bp (4.4% difference, no gap) in Alt-a1 nucleotide pairwise comparison.



Note a Colonies on dead stems of *Vicia faba*. b–d Conidiophores. e–h Conidiophores bearing conidiogenous cells. i–r Conidia. Scale bars: a = 1000 µm, e = 50 µm, b–d, f–h, n–q = 30 µm, i–m, r = 20 µm.

Figure 4.23 Morphology of *Alternaria obpyriconidia*

4.4.1.16 *Alternaria orobanches* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Named after the host occurrence "genus Orobanche"

Holotype: MFLU 21-****

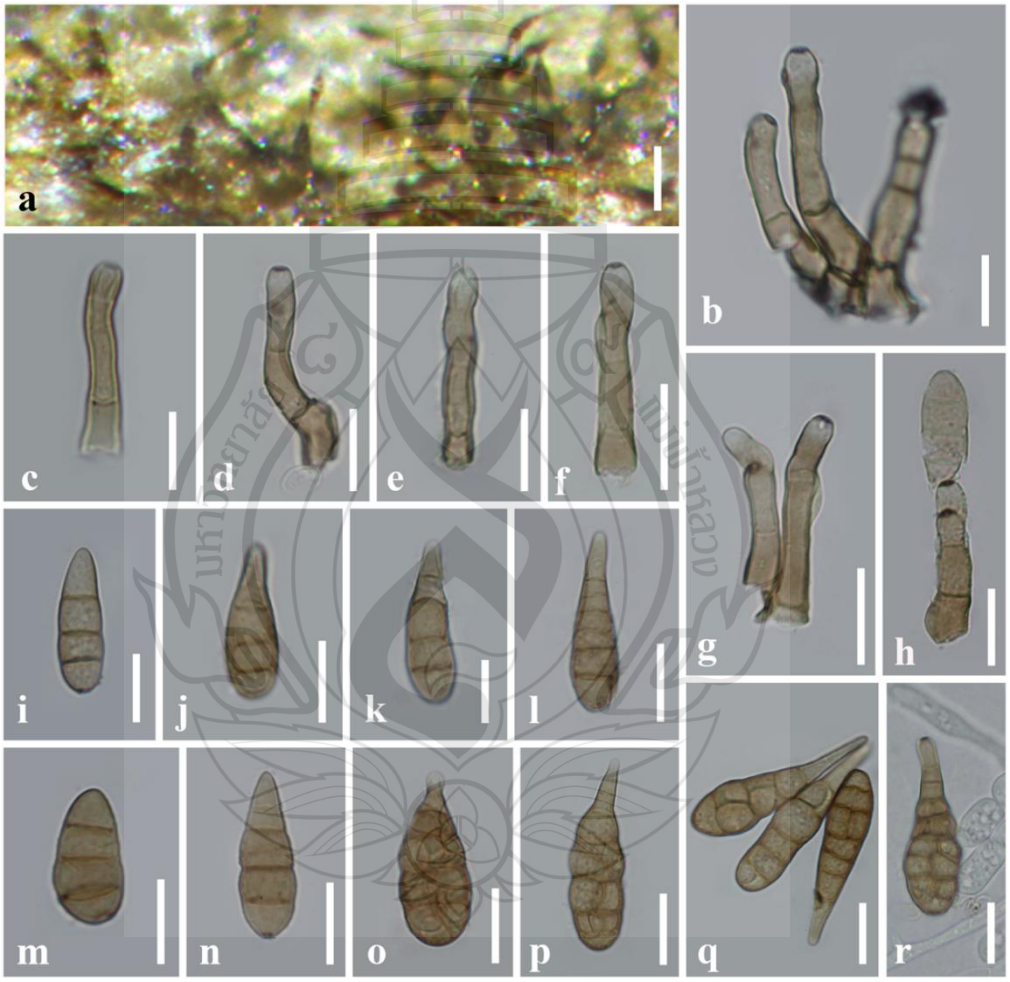
Saprobic on dead hanging stem of *Orobanche* sp. (Orobanchaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the host substrate, with dark hyphae. *Conidiophores* 50–90 × 15–18 µm ($\bar{x}$ = 75 × 17 µm, n = 30), macronematous, straight or flexuous, cylindrical, yellowish brown to pale green-brown, indistinct septate, unbranched, smooth to minutely verrucose, thick-walled, aggregated, arising from stromatic base. *Conidiogenous cells* 13–18 × 10–12 µm ($\bar{x}$ = 15 × 11 µm, n = 20), mono- to polytretic, integrated, terminal, determinate or percurrent, subglobose to doliiform, subhyaline to pale brown, minutely verrucose, thin-walled, with two apical conidiogenous loci. *Conidia* 20–50 × 10–20 µm ($\bar{x}$ = 38 × 16 µm, n = 30) acrogenous, solitary, straight or curved, obclavate to ovoid, sometimes rostrate apex or formed short, narrow, subhyaline, septate, unbranched beak, pale yellowish-brown to pale brown, 3–6 transverse septa, with 0–1 longitudinal, or oblique or Y-shaped septum in some transverse divisions, slightly constricted at the central septum, minutely verrucose, thick-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 12 hours and germ tubes produced from lateral cells. Colonies growing on PDA, hairy fluffy, pale white to white, reaching 5 cm in 14 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, white hyphae; conidia not sporulated *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Predappio, Monte Mirabello, on dead hanging stem of *Orobanche* sp. (Orobanchaceae), 16 July 2014, E. Camporesi, IT1997 (MFLU XX-XXXX, holotype), ex-type living culture, MFLUCC XX-XXXX.

Notes: *Alternaria orobanches* resembles *A. arctoseptata* in having short, colorless conidiophores arising from a stomatic base. However, *A. orobanches* differs from *A. arctoseptata* due to its shorter, wider and less septate conidiophores (75 × 17 µm, 1–3-septate vs. 82 × 9 µm, 2–4-septate). Conidia of *A. orobanches* are obclavate to ovoid, pale yellowish-brown to pale brown while *A. arctoseptata* has subglobose to ovoid or pyriform,

yellowish brown to dark brown conidia. On the other hand, a comparison of GAPDH nucleotide pairwise showed that *A. orobanches* differs from *A. arctoseptata* in 10/490 bp (2% difference, no gap) and also differs from *A. arctoseptata* in 13/480 bp (2.7% difference, no gap) based on Alt-a1 nucleotide pairwise comparison. Multi-locus phylogeny also supports the distinctiveness of these two species. *Alternaria orobanches* constitutes an independent lineage basal to other species in section *Alternaria* with high support in analysis 1 (100% ML, 0.99 PP; Figure 4.6), but not support in analysis 2.



Notes a Colonies on dead stem of *Orobanche* sp. b–g Conidiophores bearing conidiogenous cells. h Conidiophores bearing conidiogenous cells with attached conidia. i–q Conidia. r Germinated conidium. Scale bars: a = 100 µm, g = 50 µm, b–f, h–l, n, p, q = 30 µm, m, o, r = 20 µm.

Figure 4.24 Morphology of *Alternaria orobanches*

4.4.1.17 *Alternaria ovoidea* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Referring to its ovoid (droplets-like) conidia.

Holotype: MFLU 21-****

Saprobic on stem of *Dactylis glomerata* L. (Poaceae). Sexual morph Undetermined. Asexual morph *Mycelium* partly superficial on host substrate, composed of septate, branched, smooth, thin-walled, pale brown hyphae. *Conidiophores* 270–300 × 6.5–11 µm ($\bar{x}$ = 280 × 8 µm, n = 100), macronematous, mononematous, copper brown to dark brown, erect, flexuous or sigmoid, cylindrical, septate, branched, smooth to verrucose thick-walled. *Conidiogenous cells* 9–13 × 8.5–15 µm ($\bar{x}$ = 9.7 × 11.4 µm, n = 100), mono- to polytretic, integrated, terminal, determinate or percurrent, subcylindrical, pale brown to light brown, smooth, thick-walled, apically doliiform with conidiogenous loci cicatrized on conidial secession. *Conidia* 48–65 × 15.5–30 µm ($\bar{x}$ = 55.4 × 27.2 µm, n = 100) acrogenous, solitary, ovoid, orangish brown to copper brown, sectored, with 1–3 indistinct transverse septa, and 1 longitudinal or oblique or Y-shaped septum in transverse divisions, verruculose, thick-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies growing on PDA, cottony, brown to dark brown, reaching 5 cm in 7 days at 25ºC, mycelium superficial, effuse, radially striated, with irregular edge; conidia not sporulated *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Fiumicello di Premilcuore, on dead aerial stem of *Dactylis glomerata* (Poaceae), 19 January 2014, E. Camporesi, IT1656 (MFLU XX-XXXX, holotype), ex-type living culture = MFLUCC 14-0427.

Notes: Phylogenetic analyses showed that *Alternaria oviodea* constitutes an independent lineage clustered with *A. baoshanensis* and *A. arctoseptata* (81% ML, 1.00 BYPP; Figure 4.7). Morphologically *A. oviodea* differs from *A. baoshanensis* and *A. arctoseptata* in having non-beak, oviod conidia and solitary, flexuous or sigmoid, copper brown to dark brown conidiophores, with a non-stomatic base, while the conidiophores are arising from a stomatic base both in *A. arctoseptata* and *A. baoshanensis.* Conidia of *A. arctoseptata* are rather constricted at the septa and colorless than *A. oviodea*, while

A. baoshanensis has light brown to yellowish brown, non-beak, subglobose to ellipsoidal conidia.



Note a Colonies on dead stem of *Dactylis glomerata*. b–h Conidiophores bearing conidiogenous cells. i–q Conidia. r Culture on PDA. Scale bars: r = 2 cm, a = 0.1 cm, b–d, g = 50 µm, e, f, h = 30 µm, i–q = 20 µm.

Figure 4.25 Morphology of *Alternaria ovoidea*

4.4.1.18 *Alternaria phragmiticola* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Named after the host "*Phragmites*", of which the species was collected.

Holotype: MFLU 21-****

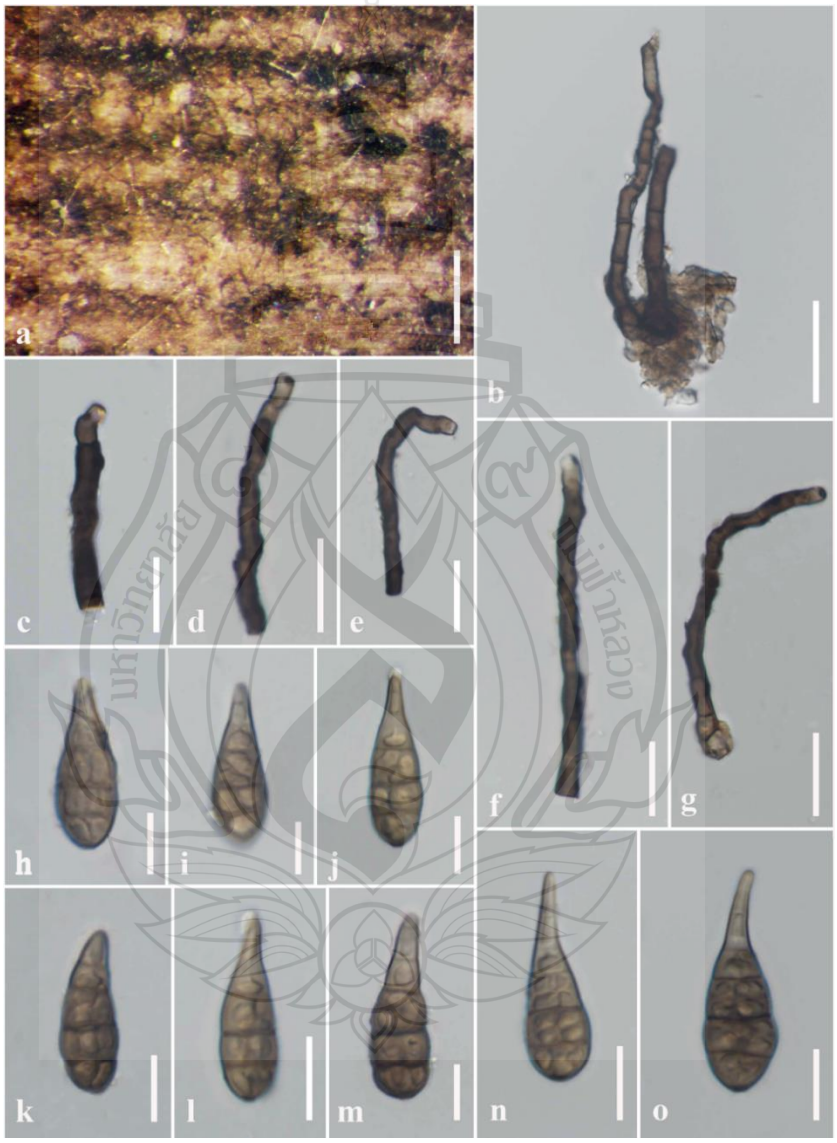
Saprobic on stem of *Phragmites* sp. (Poaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the substrate, with dark hyphae. *Conidiophores* 50–108 × 4–8 µm ($\bar{x}$ = 90 × 6.5 µm, n = 30), macronematous, straight or flexuous, cylindrical, dark brown, septate, smooth, thick-walled. *Conidiogenous cells* 6–8 × 8–10 µm ($\bar{x}$ = 6.5 × 9.5 µm, n = 20), polytretic, integrated, terminal, determinate or percurrent, cylindrical, pale brown, smooth, thin-walled, apically doliiform, with 1–3 conidiogenous loci. *Conidia* 42–88 × 21–30 µm ($\bar{x}$ = 70 × 25 µm, n = 30) acrogenous, solitary, straight to curved, obclavate to obpyriform, with short, narrow, rostrate paler brown, septate apex, pale yellowish-brown to brown, 3–4 transversely disto- or eusepta, with 1–2 longitudinal or oblique or Y-shaped septa in some transverse divisions, borne in chain, smooth to verruculose thick-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from lateral cells. Colonies growing on PDA, hairy or cottony, brown to dark brown, reaching 5 mm in 20 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, yellow white to grey hyphae; conidia not sporulated *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Meldola, San Colombano, on dead aerial stem of *Phragmites* sp. (Poaceae), 28 September 2015, E. Camporesi, IT2630 (MFLU XX-XXXX, holotype), ex-type living culture, MFLUCC XX-XXXX.

Notes: Multi-locus phylogenetic analyses demonstrated that three strains of *Alternaria phragmiticola* formed a robust clade (100% ML, 1.00 PP; Figure 4.6, 4.7) and clustered with *A. macilenta* and *A. oblongoellipsoidea*. These three species formed a well-resolved clade together in both analysis 1 (83% ML, 0.99 PP; Figure 4.6) and analysis 2 (82% ML, 1.00 PP; Figure 4.7) with significant support. *Alternaria phragmiticola* can be distinguished from *A. macilenta* and *A. oblongoellipsoidea* in having solitary, dark brown,

conidiophores and obclavate to obpyriform, pale yellowish-brown to brown, conidia with a rostrate apex, while *A. macilenta* has brown, aggregated conidiophores, arising from a stomatic base and subcylindrical to obclavate, beakless, pale brown to light greyish brown, distoseptate conidia. *Alternaria oblongoellipsoidea* has more twisted apex conidiophores and oblong to ellipsoidal conidia, with more conidial eusepta.



Note (MFLU XX-XXXX, holotype). a Colonies on dead stem *Phragmites* sp. b–g Conidiophores bearing conidiogenous cells. h–o Conidia. Scale bars: a = 200 µm, b, k–o = 30 µm, c–j = 20 µm.

Figure 4.26 Morphology of *Alternaria phragmiticola*

4.4.1.19 *Alternaria pseudoinfectoriae* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Referring to the conidial structures resemble *Alternaria* section *infectoriae*.

Holotype: MFLU 21-****

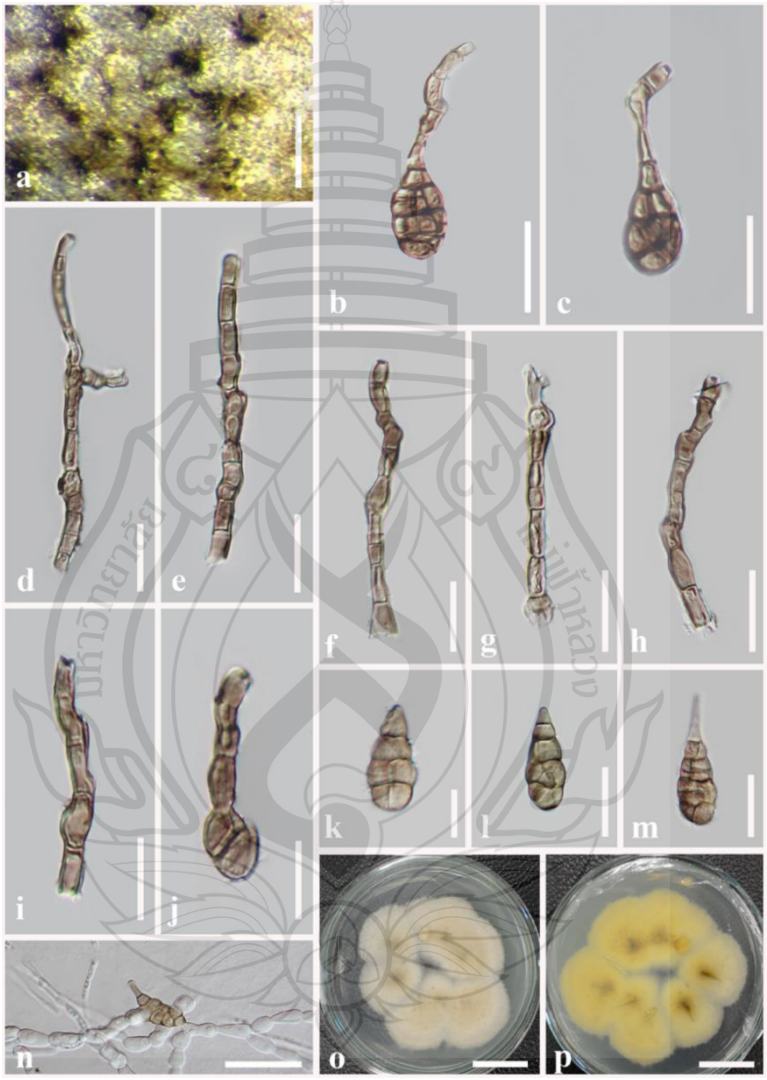
Saprobic on stem *Chenopodium* sp. (Chenopodiaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on host substrate, composed of septate, branched, smooth, thin-walled, brown hyphae. *Conidiophores* 55–68 × 12–14 µm ($\bar{x}$ = 62 × 13 µm, n = 30), macronematous, mononematous, straight or flexuous, cylindrical, light brown to brown, septate, branched, smooth, thick-walled. *Conidiogenous cells* 11–12 × 10–14 µm ($\bar{x}$ = 11.5 × 12 µm, n = 20), monotretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline to light brown, smooth, thin-walled, apically doliiform with one conidiogenous locus. *Conidia* 25–40 × 13–25 µm ($\bar{x}$ = 33 × 19 µm, n = 30) acrogenous, holoblastic, solitary, straight, subglobose to obclavate, or obpyriform, sometimes with short, narrow, rostrate, paler brown, septate beak, light brown, 3–4 transverse eusepta, with 1 longitudinal or oblique or Y-shaped septum in some transverse divisions, borne in chain, smooth to minutely verrucose, thin-walled, formed apically secondary conidiophores with one conidiogenous locus. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies immersed in PDA, cottony, white to grey, reaching 5 cm in 7 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, white hyphae; conidia not sporulated *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Forlì, Via Nenni, on dead aerial stem of *Chenopodium* sp. (Chenopodiaceae), 17 October 2014, E. Camporesi, IT2181 (MFLU XX-XXXX, holotype), ex-type living culture = MFLUCC XX-XXXX.

Notes: *Alternaria pesudoinfectoriae* resembles species in section *Infactoriae* due to its conidia often developing long secondary conidiophores. Although species in section *Panax* also formed long secondary conidiophores, conidiogenous loci on secondary conidiophores are rather monotretic in *A. pesudoinfectoriae*, which more resemble structures of species in section *Infectoriae* (Pryor & Michailides 2002; Lawrence et al. 2013). However, *A. pesudoinfectoriae* corresponds with section *Alternaria* in having

straight or curved primary conidiophores, simple to branched, with one apical conidiogenous locus, and conidia born in chain (Woudenberg et al., 2013). In phylogenetic analyses, *A. pesudoinfectoriae* formed a well-resolved clade with *A. breviconidiophora*, *A. lathyri* and *A. muriformispora* with significant support in both analysis 1 (99% ML, 1.00 PP; Figure 4.6) and analysis 2 (90% ML, 1.00 PP; Figure 4.7).



Note a Colonies on dead stem of *Chenopodium* sp. b, c, j Conidia formed apical secondary conidiophores. d–i Conidiophores. k–m Conidia. n Germinated conidium. o, p Colonies on PDA. Scale bars: o, p = 2 cm, a = 300 µm, b–f, h, n = 20 µm, g, i, j = 15 µm, k–m = 10 µm.

Figure 4.27 Morphology of *Alternaria pseudoinfectoriae*

4.4.1.20 *Alternaria rostroconidia* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****.

Etymology: Referring to the rostrate conidia.

Holotype: MFLU 21-****

Saprobic on dead stem of *Arabis* sp. (Brassicaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on host substrate, with dark hyphae. *Conidiophores* 105–120 × 11–15 µm ($\bar{x}$ = 112 × 13 µm, n = 30), macronematous, solitary or 2–5 aggregated at the base, straight or flexuous, cylindrical, light brown to dark brown, septate, geniculate, smooth or sometimes semi-verrucose, thick-walled. *Conidiogenous cells* 12–18 × 5–8 µm ($\bar{x}$ = 15 × 6 µm, n = 20), mono- to polytretic, normally sympodial proliferations, integrated, terminal, determinate or percurrent, cylindrical, subhyaline or semi-colored, smooth, thin-walled, apically doliiform, with 1–2 conidiogenous loci, with swollen knots near conidiogenous loci. *Conidia* 50–80 × 25–30 µm ($\bar{x}$ = 66 × 22 µm, n = 30) acrogenous, solitary, straight or curved, ellipsoidal or ovoid to obpyriform, with short, narrow, rostrate, paler brown, aseptate beak, with distinct hilum at the apex, dark brown, 3–4 transverse eusepta, with 1 longitudinal or oblique or Y-shaped septum in some transverse divisions, sometimes sectored, slightly constricted at the septa, borne in chain, smooth, thick-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 12 hours and germ tubes produced from lateral cells. Colonies growing on PDA, cottony, brown to dark brown, reaching 5 cm in 10 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, white to grey hyphae; conidia not sporulated *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Premilcuore, on dead aerial stem of *Arabis* sp. (Brassicaceae), 8 October 2017, E. Camporesi, IT3515 (MFLU XX-XXXX, holotype), ex-type living culture, MFLUCC XX-XXXX.

Notes: *Alternaria rostroconidia* morphologically corresponds with species in section *Alternaria* in having obypriform, born in chain conidia with several transverse and longitudinal septa (Woudenberg et al., 2013). In multi-locus phylogenetic analyses, *A. rostroconidia* has a close relationship with *A. minimispora* with significant support in BI analyses (0.95 PP; Figure 4.6, 4.7) but no support in ML analyses both analysis 1 and

analysis 2. Morphological and nucleotide base comparisons between *A. rostroconidia* and *A. minimispora* were mentioned in the notes of *A. minimispora.*



Note a Colonies on dead stem of *Arabis* sp. b–f Conidiophores bearing conidiogenous cells. g–m Conidia. Scale bars: a = 200 µm, e = 50 µm, b, d, f = 30 µm, c, g–m = 20 µm.

Figure 4.28 Morphology of *Alternaria rostroconidia*

4.4.1.21 *Alternaria salicicola* J.F. Li, Bulgakov & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Named after the host genus *Salix*, of which the species was found.

Holotype: MFLU 21-****

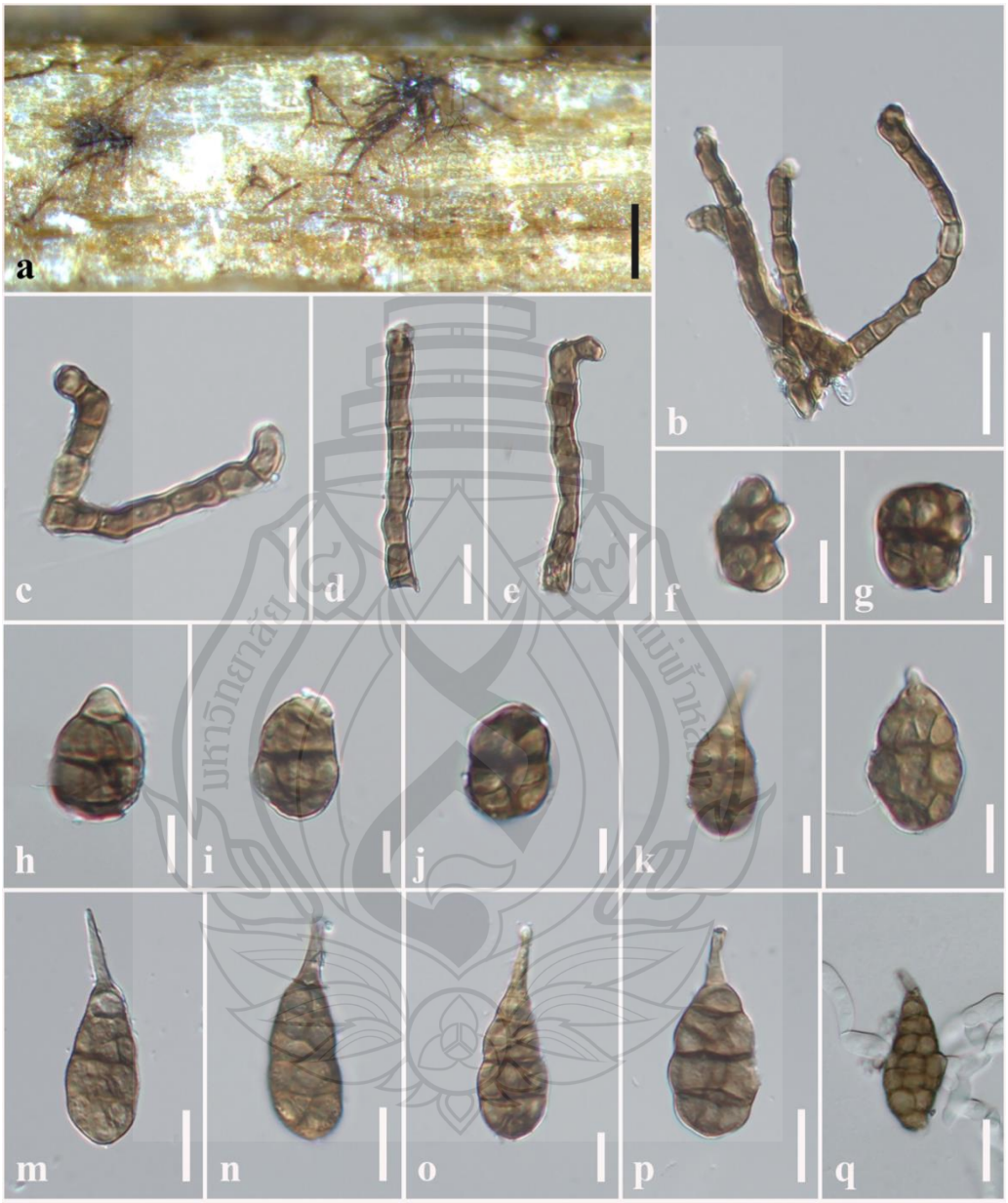
Saprobic on dead twig of *Salix alba* L. (Salicaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on host substrate, with dark hyphae. *Conidiophores* 110–150 × 10–15 µm ($\bar{x}$ = 135 × 13 µm, n = 30), macronematous, straight or flexuous, cylindrical, light brown to brown, septate, branched at the base, smooth to verruculose, thick-walled. *Conidiogenous cells* (5–)10–13 × 8–10(–14) µm ($\bar{x}$ = 12.3 × 8.5 µm, n = 20), polytretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline to paler light brown, smooth, thick-walled, apically doliiform, with 1–3 conidiogenous loci. *Conidia* 10–50 × 12–38 µm ($\bar{x}$ = 45 × 32 µm, n = 30) acrogenous, solitary, straight or curved, subglobose to obclavate or obpyriform, with short to long, narrow, acicular to doliiform, septate beak when mature, light yellowish-brown to light brown, sectored of 1–6 transversely disto- or eusepta, with 1–3 longitudinal or oblique or Y-shaped distosepta, borne in chain, smooth to verruculose, thick-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from lateral cells. Colonies growing on PDA, hairy or cottony, brown to dark brown, reaching 5 cm in 10 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, light brown to brown hyphae; conidia not sporulated *in vitro* within 60 days.

Material examined: RUSSIA, Rostov Region, Krasnosulinsky District, trees near Kudryuchya River, on dead twig of *Salix alba* (Salicaceae), 18 June 2015, T.S. Bulgakov, T-504 (MFLU 21-XXXX, holotype), living culture, MFLUCC XX-XXXX.

Notes: *Alternaria salicicola* is the only species collected from Russia in this study. In multi-locus phylogenetic analyses, two strains of *A. salicicola* formed a robust clade (100% ML, 1.00 PP; Figure 4.7) sister to *A. vitis* (MFLUCC 17-1109) with significant support in BI analyses (0.95 PP; Figure 4.7) and also clustered with *A. macroconidia* and *A. obpyriconidia*. *Alternaria salicicola* can be distinguished from *A. vitis* (MFLUCC 17-1109) in having larger conidia (45 × 32 vs. 39 × 15 µm), that are more distoseptate and borne in chain, whereas *A. vitis* usually produces a single conidium (Jayawardena et al.,

2018). *Alternaria vitis* has been reported as a common pathogen on grape causing fruit rots and leaf blight (Jayawardena et al., 2018). While *A. salicicola* was found as a saprobe on *Salix alba*, the pathogenic capacity of the species has not yet been investigated.



Note a Colonies on dead twig of *Salix alba*. b–e Conidiophores bearing conidiogenous cells. f–p Conidia. q Germinated conidium. Scale bars: a = 150 µm, b, d, e, q = 30 µm, c, k–p = 20 µm, f–j = 10 µm.

Figure 4.29 Morphology of *Alternaria salicicola*

4.4.1.22 *Alternaria torilis* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Named after the host genus "*Torilis*".

Holotype: MFLU 21-****

Saprobic on dead aerial stem of *Torilis arvensis* (Huds.) Link (Apiaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on host substrate, composed of septate, branched, smooth, thin-walled, brown to light brown hyphae. *Conidiophores* (155–)177–185(–191) × (7.5–)8–10(–11) µm ($\bar{x}$ = 175.2 × 8.8 µm, n = 100), macronematous, mononematous, straight or flexuous, cylindrical, dark brown, unbranched, septate, unbranched, sometimes branched, smooth, thick-walled. *Conidiogenous cells* 7–9(–10) × (6.5–)7.5–10 µm ($\bar{x}$ = 8.2 × 8.9 µm, n = 100), mono- to polytretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline, smooth, thin-walled, apically doliiform, with two conidiogenous loci cicatrized on conidial secession. *Conidia* (55–)60–75(–82) × (23–)25–31.5 (–32) µm ($\bar{x}$ = 68.5 × 28.5 µm, n = 100) acrogenous, solitary, dry, straight, fusiform to ovoid, or obturbinate to obpyriform, sometimes with short, narrow, pale brown to light brown, aseptate beak, brown to dark brown, 2–4 transverse eusepta, with 1 longitudinal or oblique or Y-shaped distoseptum in some transverse divisions, borne in chain, minutely verruculose, thin-walled, formed apically secondary conidiophores with one conidiogenous locus. *Conidial secession* schizolytic.

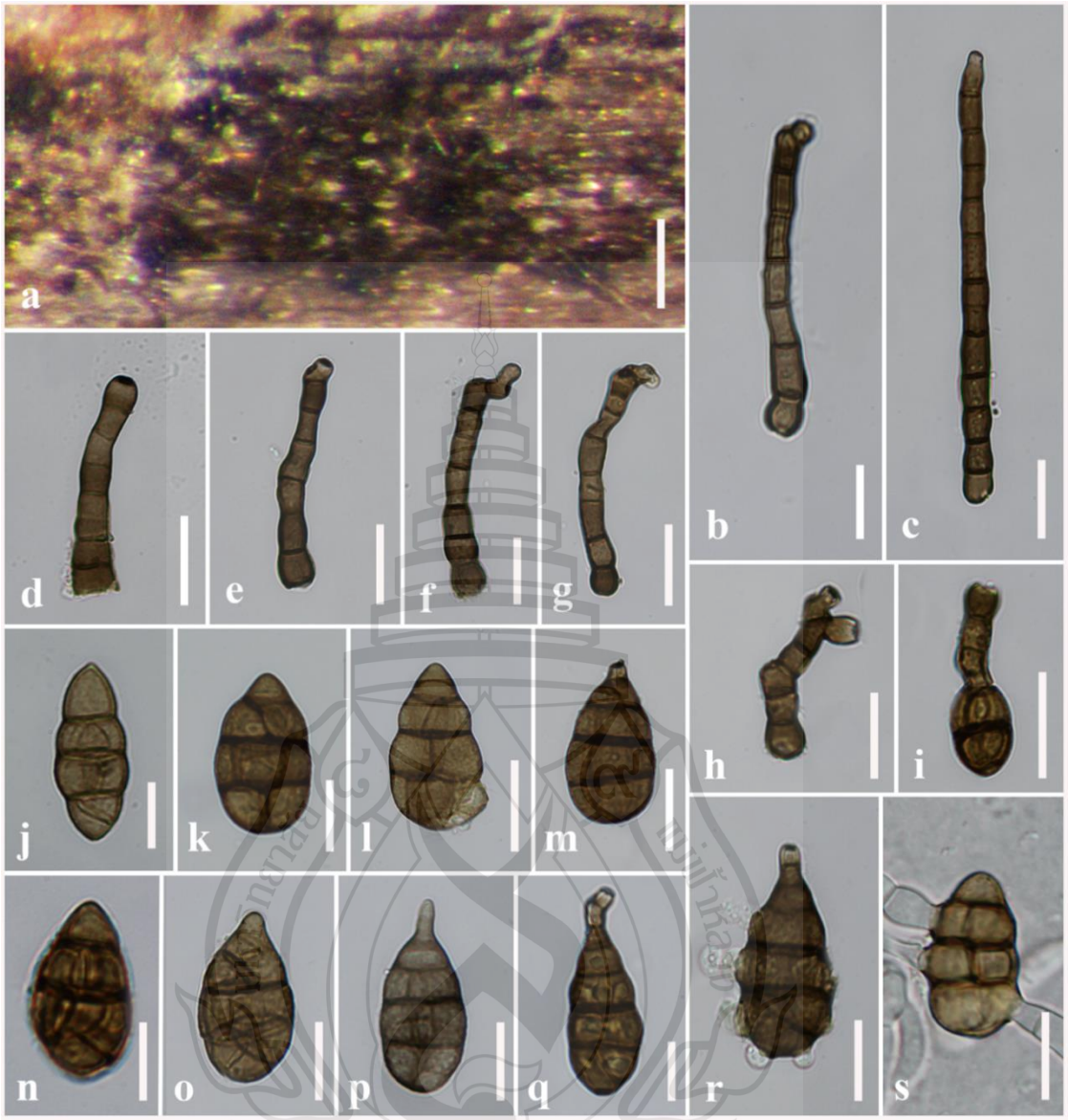
Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from lateral cells. Colonies growing on PDA, hairy or cottony, light brown to brown, reaching 5 cm in 14 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, colorless hyphae. Conidia sporulated on OA within 15 days, phragmosporous to muriform, oblong to ovoid, brown to dark brown, with short, doliiform, apical beak, formed apically or laterally, short, branched or unbranched secondary conidiophores with 1–2 conidiogenous loci at apex, 1–3 transversely septa, with 1–2 longtudinal or Y-shape septa in transverse division, smooth to minutely verrucose, thin-walled.

Material examined: ITALY, Province of Forlì-Cesena, Forlì, San Lorenzo in Noceto, on dead aerial stem of *Torilis arvensis* (Apiaceae), 23 January 2014, E. Camporesi, IT1667 (MFLU XX-XXXX, holotype), ex-type living culture = MFLUCC14-0433.

Notes: *Alternaria torilis* resembles *A. alternata* in having a brown to dark brown short beak, 2–4-transversely septate conidia and forming secondary conidiophores. The conidium body can narrow gradually into a tappered beak or secondary conidiophore, curved primary conidiophores and solitary conidiophores with mono- to polytretic conidiophores with conidiogenous loci at apex. Morphologically, *A. torilis* differs from *A. alternata* by its darker, ovoid to obturbinate, or obpyriform which are rather ovoid to chiefly obclavate or obpyriform in *A. alternata*, and conidiophores of *A. torilis* normally have two conidiogenous cells and are rostrate at the apex. In the phylogenetic analyses, *A. torilis* constitutes an independent lineage basal to the clade contains *A. muriformispora, A. pseudoinfectoriae, A. lathyri* and *A. breviconidiophora* with significant support in Bayesion inference analyses (0.98 PP; Figure 4.7) and distant from *A. alternata*. *Alternaria torilis* can be distinguished from *A. breviconidiophora* in having much larger (68.5 × 28.5 µm vs. 10 × 8 µm), ovoid to obturbinate, or obpyriform conidia, with more conidial septation that are rather globose to subglobose in *A. breviconidiophora*. The species is similar to *A. lathyri* in having ovoid to obpyriform, dark brown conidia, with 2–4 transverse eusepta, but differs in having larger conidia (68.5 × 28.5 µm vs. 55× 25 µm), branched or unbranched conidiophores and conidia also form apically secondary conidiophores with one conidiogenous locus. *Alternaria torilis* differs from *A. muriformispora* in having less conidial septation. Meanwhile, the species forms apically secondary conidiophores similar to *A. pseudoinfectoriae* but can be distinguished from *A. pseudoinfectoriae* due to its dark brown conidia that are light brown in *A. pseudoinfectoriae*.

A comparision of ITS nucleotide pairwise showed that *A. torilis* differs from *A. alternata* (CBS 916.96, ex-type) in 9/485 bp (1.9% difference, no gap), differs from *A. brevconidiophora* in 8/490 (1.6% difference, no gap) and differs from *A. muriformispora* in 8/490 bp (1.6% difference, no gap). A comparision of RPB2 nucleotide pairwise showed that *A. torilis* differs from *A. alternata* (CBS 916.96, ex-type) in 42/558 bp (7.5 % difference, no gap), differs from *A. breviconidiophora* in 27/560 bp (4.8 % diference, no gap), differs from *A. lathyri* in 30/560 bp (5.6% difference, no gap), differes from *A. muriformispora* in 28/560 bp (5% difference, no gap), and differs from

A. pseudoinfectoriae 32/560 bp (5.7% difference, no gap). A comparision of GAPDH nucleotide pairwise showed that *A. torilis* differs from *A. alternata* (CBS 916.96, ex-type) in 31/590 bp (5.3% difference, no gap), differs from *A. breviconidiophora* in 16/590 bp (2.7% difference, no gap), differs from *A. lathyri* in 20/590 bp (3.6% difference, no gap), differs from *A. muriformispora* in 21/590 bp (3.6% difference, no gap), and differs from *A. pseudoinfectoriae* in 19/590 bp (3.2% difference, no gap). A comparision of Alt-a1 nucleotide pairwise showed that *A. torilis* differs from *A. alternata* (CBS 916.96, ex-type) in 25/465 bp (5.4% difference, no gap), differs from *A. breviconidiophora* in 13/465 bp (2.8% difference, no gap), differs from *A. lathyri* in 12/465 bp (2.6% difference, no gap), differs from *A. muriformispora* in 10/465 bp (2.2% difference, no gap), and differes from *A. pseudoinfectoriae* in 8/465 bp (1.7% difference, no gap).



Note (MFLU XX-XXXX, holotype). a Colonies on stem of *Torilis arvensis*. b–h Conidiophores bearing conidiogenous cells. i Secondary conidiophores arising from conidium. j–q Conidia. r, s Germinated conidia. Scale bars: a = 200 µm, c, g = 30 µm, b, d–f, h–s = 20 µm.

Figure 4.30 Morphology of *Alternaria torilis*

4.4.2 Section *Infectoriae* Woudenb. & Crous, Study in Mycology 75: 194 (2013)

Type species: *Alternaria infectoria* E.G. Simmons.

Notes: Section *Infectoriae* is one of the largest and most complicated sections in *Alternaria*, containing approximately 50 species (Woudenburg et al., 2013; Gannibal & Lawrence, 2016; Marin-Felix et al., 2019; Iturrieta-González et al., 2020). Species in this section often form white or nearly white, floccose colonies on nutrient-rich media and sporulated several conidial chains on low sugar media as extensive three-dimensional branching patterns, and the conidia usually produce long secondary conidiophores (Pryor & Michailides, 2002; Poursafar et al., 2017). Although, members of sect. *Infectoriae* are commonly known as saprobes, many species such as *A. infectoria* and *A. triticina* have been reported as important pathogens on various plant hosts as well as human infections (Andersen et al., 2009; Lawrence et al., 2013, 2016; Poursafar et al., 2017; Somma et al., 2019; Iturrieta-González et al., 2020). Additionally, species in this section produce secondary metabolites such as novae-zelandins and infectopyrone, rather than mycotoxins, which are unique to this section (Poursafar et al., 2017; Somma et al., 2019). The latest updated account of species number in sect. *Infectoriae* was carried out by Marin-Felix et al. (2019), who introduced six new species mostly collected from herbivore dung and plant litter and Iturrieta-González et al. (2020) who introduced three novel species causing human cutaneous infections. In this study, we introduce two other novel saprobic species collected from dead plants in Italy based on typical morphology and multi-locus phylogeny.

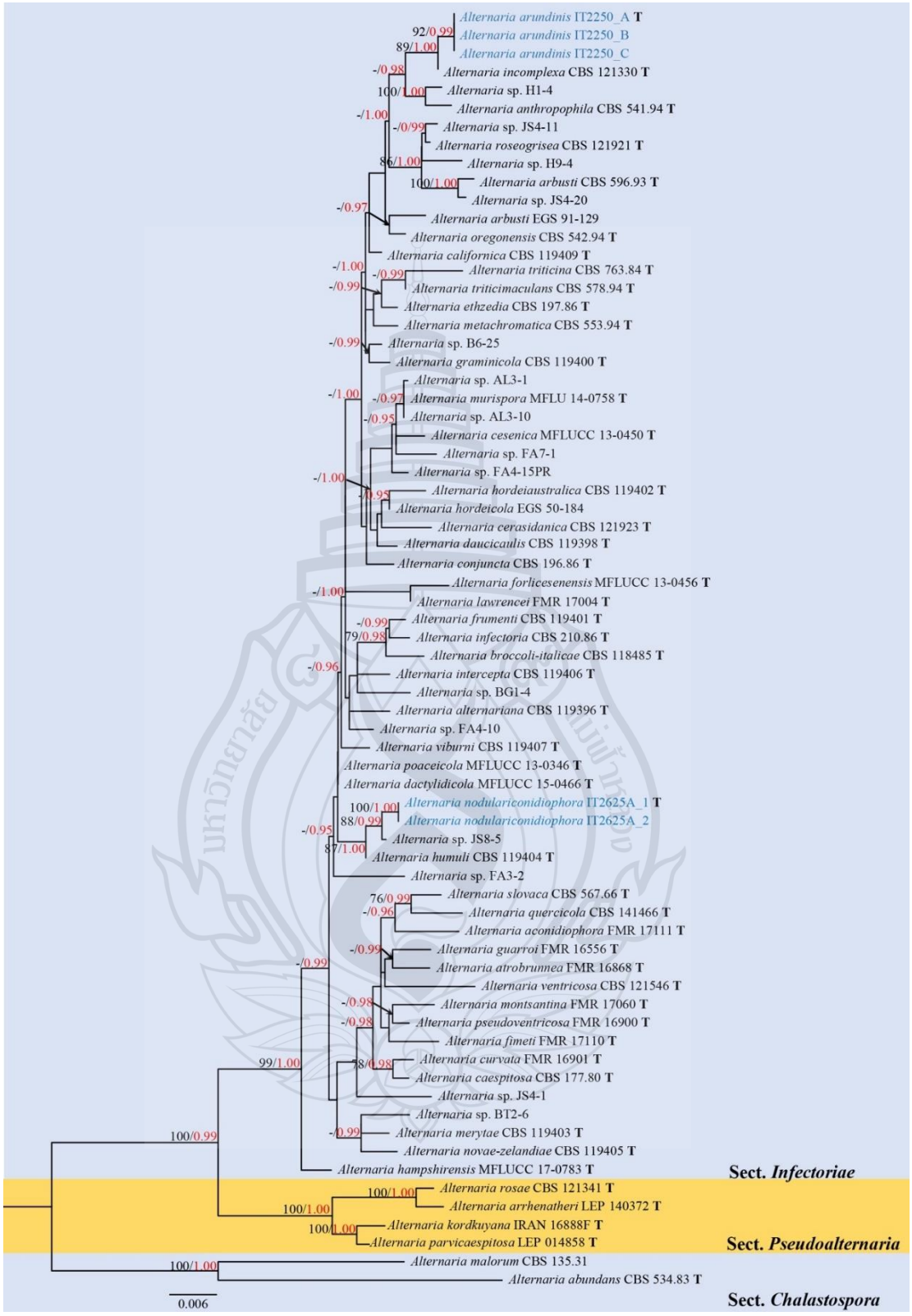


Figure 4.31 Phylogenetic construction of *Alternaria* sect. *Infectoriae* using RAxML-based analysis of a combined multigene

Figure 4.31 Phylogenetic construction of *Alternaria* sect. *Infectoriae* using RAxML-based analysis of a combined ITS, GAPDH and ATPase DNA sequence dataset. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 70% and Bayesian posterior probabilities (PP, red) equal to or greater than 0.95 are shown above the nodes. The tree is rooted with taxa in sect. *Chalastospora* (*A. gossypii* CBS 135.31 and *A. abundans* CBS 534.83). Newly generated strains are in blue and the ex-type strains are indicated by "T".

4.4.2.1 *Alternaria arundinis* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: ****; Facesoffungi number: ****

Etymology: Named after the host genus *Arundo*, of which the species was collected.

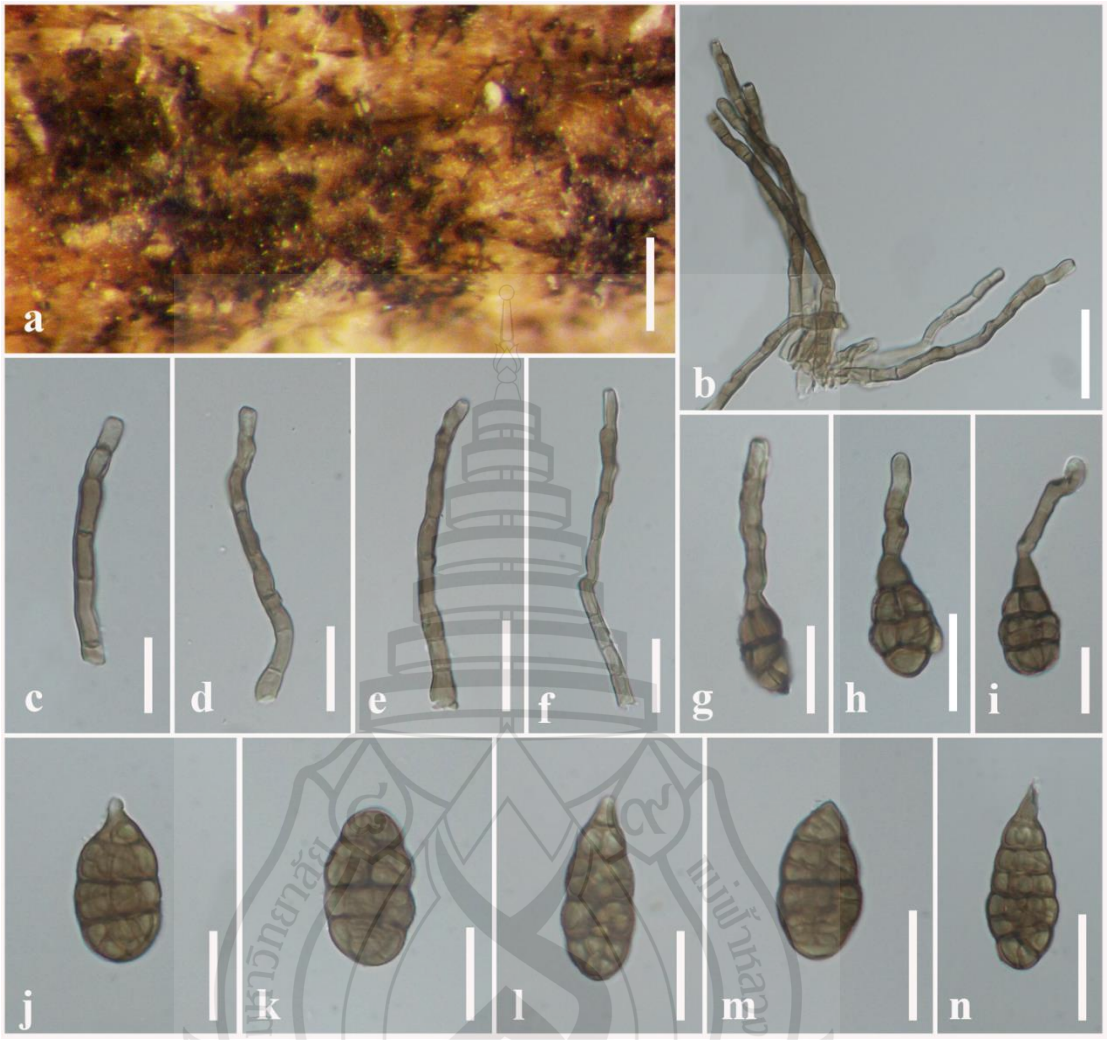
Holotype: MFLU 21-****

Saprobic on dead leaf of *Arundo donax* L. (Poaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on the host substrate, with dark hyphae. *Conidiophores* 50–85 × 5–8 µm ($\bar{x}$ = 70 × 7 µm, n = 30), macronematous, straight or flexuous, cylindrical, pale brown to pale yellowish-brown, septate, smooth, thick-walled, plexiform on base. *Conidiogenous cells* 5–6 × 3–4 µm ($\bar{x}$ = 5.5 µm × 3.6 µm, n = 20), polytretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline to pale brown or light brown, smooth, thin-walled, apically rounded to doliiform, with 1–2 conidiogenous loci. *Conidia* 20–45 × 15–25 µm ($\bar{x}$ = 38 × 20 µm, n = 30) acrogenous, solitary, straight or curved, ellipsoidal to ovoid, or obclavate, pale greyish-brown to pale yellowish-brown, 3(–5) transverse eusepta, with 1–2 longitudinal or oblique or Y-shaped disto- or eusepta in some transverse divisions, becoming sectored, slightly constricted near some the septa, borne in chain, beakless or with short, acicular rostrum, apically formed long secondary conidiophores, with 1–3 apical or lateral conidiogenous loci, smooth, thick-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies growing on PDA, cottony, white to dark grey, reaching 5 cm in 10 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, white to grey hyphae; conidia not sporulated *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Predappio, Rocca delle Caminate, on dead aerial leaf of *Arundo donax* (Poaceae), 17 November 2014, E. Camporesi, IT2250 (MFLU XX-XXXX, holotype), ex-type living culture, MFLUCC XX-XXXX.

Notes: *Alternaria arundinis* is typical of sect. *Infectoriae* in forming secondary conidiophores, with white to dark grey colony on PDA. Multi-locus phylogeny demonstrated that *A. arundinis* formed an independent lineage sister to *A. incomplexa* with significant support (89% ML, 1.00 PP; Figure 4.31) and clustered with *Alternaria* sp. (H1-4) and *A. anthropophila* (CBS 541.94). *Alternaria arundinis* resembles *A. incomplexa* in having ellipsoidal to ovoid, or obclavate conidia, with 3(–5) transverse eusepta, and 1–2 longitudinal septa. However, *A. arundinis* can be distinguished from *A. incomplexa* in having shorter conidiophores (70 × 7 µm vs. 100 × 4 µm) and slightly larger conidia (38 × 20 µm vs. 18–25 × 5–8 µm), which formed secondary conidiophores with several conidiogenous loci, while the conidia can raise up to 30–40 × 8–13 µm, with 5–8 transverse septa and 0–4 longitudinal septa in *A. incomplexa* (Simmons, 1996). *Alternaria arundinis* differs from *A. anthropophila* in having shorter conidiophores (50–85 × 5–8 µm vs. 26–120 × 4–7 µm) with laterally and apically geniculate conidiogenous loci, while conidiophores are apically geniculate in *A. anthropophila*, the conidia of *A. arundinis* are shorter and wider (20–45 × 15–25 µm vs. 11–63 × 6–11 µm), and more longitudinal or oblique euseptate (1–2 vs. 0–1) (Iturrieta-González et al., 2020). A comparision of ITS nucleotide pairwise showed that *A. arundinis* differs from *A. incomplexa* in 8/546 bp (1.5% difference, no gap) and differs from *A. anthropophila* in 10/546 bp (1.8% difference, no gap). In GAPDH, *A. arundinis* differs from *A. incomplexa* in 9/521 bp (1.7% difference, no gap) and differs from *A. anthropophila* in 14/521 bp (2.7% difference, no gap). In ATPase, *A. arundinis* differs from *A. incomplexa* in 16/1000 bp (1.6% difference, no gap) and differs from *A. anthropophila* in 21/1000 bp (2.1% difference, no gap).



Note a Colonies on dead stem of *Arundo donax*. b–f Conidiophores bearing conidiogenous cells. g–i Conidia formed secondary conidiophores. j–n Conidia. Scale bars: a = 150 µm, b, f, j, l–n = 30 µm, c–e, g–i k = 20 µm.

Figure 4.32 Morphology of *Alternaria arundinis*

4.4.2.2 *Alternaria nodulariconidiophora* J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: ****; Facesoffungi number: ****

Etymology: Referring to the geniculate conidiophores.

Holotype: MFLU 21-****

Saprobic on stem of *Heracleum sphondylium* L. (Apiaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on host substrate, composed of brown to dark brown hyphae. *Conidiophores* (111–)119.5–183 × (12–)15–23 µm ($\bar{x}$ = 175.2 × 18 µm, n = 30), macronematous, aggregate on a stomatic base, straight or flexuous, nodular, cylindrical, light brown to brown, septate, smooth thick-walled. *Conidiogenous cells* (5.5–)7–9 × 8.6–10 µm ($\bar{x}$ = 8.6 × 9.5 µm, n = 20), holo- to polytretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline to pale brown, smooth, thin-walled, apically doliiform, with 1–2 conidiogenous loci on bulge. *Conidia* (36–)45–54 × 19–37 µm ($\bar{x}$ = 48 × 29 µm, n = 30) acrogenous, solitary, dry, simple, straight, curved, subglobose to ovoid, or obturbinate to obpyriform, sometimes with short, narrow, aseptate beak, greyish brown, 3(–5) transversely euseptate, with 1 longitudinal or oblique septum in some transverse divisions, smooth or minutely verucose, borne in chain, thin-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies growing on PDA, hairy or cottony, brown to dark brown, reaching 5 cm in 5 days at 25ºC, mycelium superficial, effuse, with irregular edge, brown to dark brown hyphae. Conidia sporulated in PDA after 60 days, phragmosporous to muriform, subglobose to ellipsoidal, or fusiform, yellowish brown, 1–4 transverse euseptate, with 1–2 longitudinal or oblique or Y-shaped septa in some transverse divisions, constricted at the septa, verrucose, formed apically or laterally geniculated secondary conidiophores, with 1–2 apical or lateral conidiogenous loci.

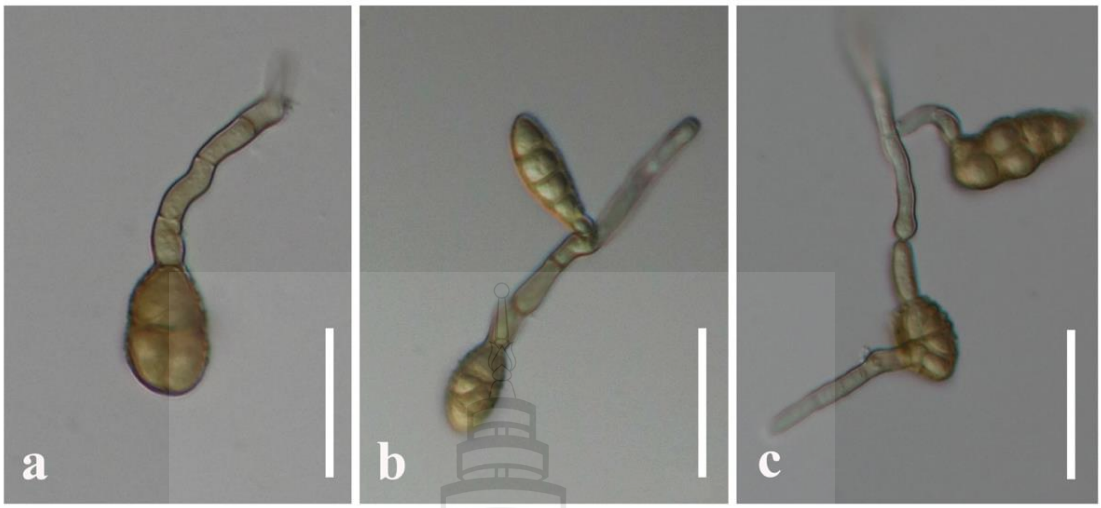
Material examined: ITALY, Province of Forlì-Cesena, Meldola, Piandispino, on dead aerial stem of *Heracleum sphondylium* (Apiaceae), 10 October 2017, E. Camporesi, IT2625A (MFLU XX-XXXX, holotype), ex-type living culture, MFLUCC XX-XXXX.

Notes: In multi-locus phylogeny, *A. nodularconidiophora* formed a separate lineage and clustered with *Alternaria* sp. (JS8-5) and *A. humuli* (CBS 119404) with significant support (87% ML, 1.00 PP; Figure 4.31). *Alternaria nodularconidiophora* is characterized by subglobose to ovoid, or obturbinate to obpyriform conidia, with a rostrate apex, geniculate conidiophores with swollen knots, arising from a stomatic base, and subhyaline to pale brown conidiogenous cells, with 1–2 conidiogenous loci on bulge, while *A. humuli* (CBS 119404) is characterized by subhyaline to arachnoid to loosely wooly colonies, simple to moderately, branched primary conidiophores, with 1–3 geniculate conidiogenous loci, elliptical or ovoid, 5–8 transversely septa, with or without one longitudinal septum (Simmons, 2002). A comparision of ITS nucleotide pairwise showed that *A. nodularconidiophora* differs from *A. humuli* in 8/545 bp (1.5% difference, no gap). In GAPDH, the species differs from *A. humuli* in 8/521 bp (1.5% diference, no gap) and differs from *A. humuli* in 11/900 bp (1.5% diference, no gap) in ATPase nucleotide pairwise comparison.



Note a Colonies on dead stem of *Heracleum sphondylium*. b–e Geniculate conidiophores bearing conidiogenous cells. f–m Conidia. n Germinated conidium. Scale bars: a = 100 µm, e, h–l, n = 30 µm, b–d, f, g, m = 20 µm.

Figure 4.33 Morphology of *Alternaria nodulariconidiophora*



Note a Conidium formed a geniculate secondary conidiophore. b, c Secondary conidiophores bearing conidia with three-dimensional branching patterns. Scale bars: a = 20 µm, b, c = 50 µm.

Figure 4.34 *Alternaria nodulariconidiophora* sporulated on PDA

4.4.3 Section *Porri* D.P. Lawr., Gannibal, Peever & B.M. Pryor, Mycologia 105: 541. 2013.

Type species: *Alternaria porri* (Ellis) Cif.

Notes: The section *Porri* is characterized by medium to large conidia with a simple or branched, filamentous long beak, which is the largest section in *Alternaria* (Woudenberg et al., 2013, 2014). This section includes many important phytopathogens, such as *A. bataticola*, *A. porri*, *A. solani*, and *A. tomatophila*. In our survey, a new species *A. brevirostra* is described on dead grass from Italy based on both morphological and phylogenetic evidence.

Alternaria brevirostra J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Referring to the short rostrate conidia in sect. *Porri*.

Holotype: MFLU 21-****

Saprobic on stems of *Erysimum* sp. (Brassicaceae) and *Plantago* sp. (Plantagineae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on host substrate, composed of septate, branched, brown to dark brown, smooth, thin-walled hyphae. *Conidiophores* 55–72 × 10–15 µm ($\bar{x}$ = 68 × 13 µm, n = 30), macronematous,

aggregated at the base, flexuous, cylindrical, pale yellowish-brown to light brown, septate, unbranched, smooth, thick-walled. *Conidiogenous cells* 5–6 × 7–7.5 µm ($\bar{x}$ = 5.5 × 7.2 µm, n = 20), polytretic, integrated, terminal, determinate or percurrent, spathulate or dolabriform, subhyaline to pale brown, smooth, thin-walled. *Conidia* 75–84 × 15–20 µm ($\bar{x}$ = 80 × 17 µm, n = 30) acrogenous, solitary, straight or curved, subcylindrical to obclavate, with slightly long, narrow, colorless, acicular, aseptate rostrum, light yellowish brown to brown, distoseptate, 5–7 transversely septa, with 1–2 longitudinal or oblique septa in some transverse divisions, borne in chain, smooth, thin-walled. *Conidial secession* schizolytic.

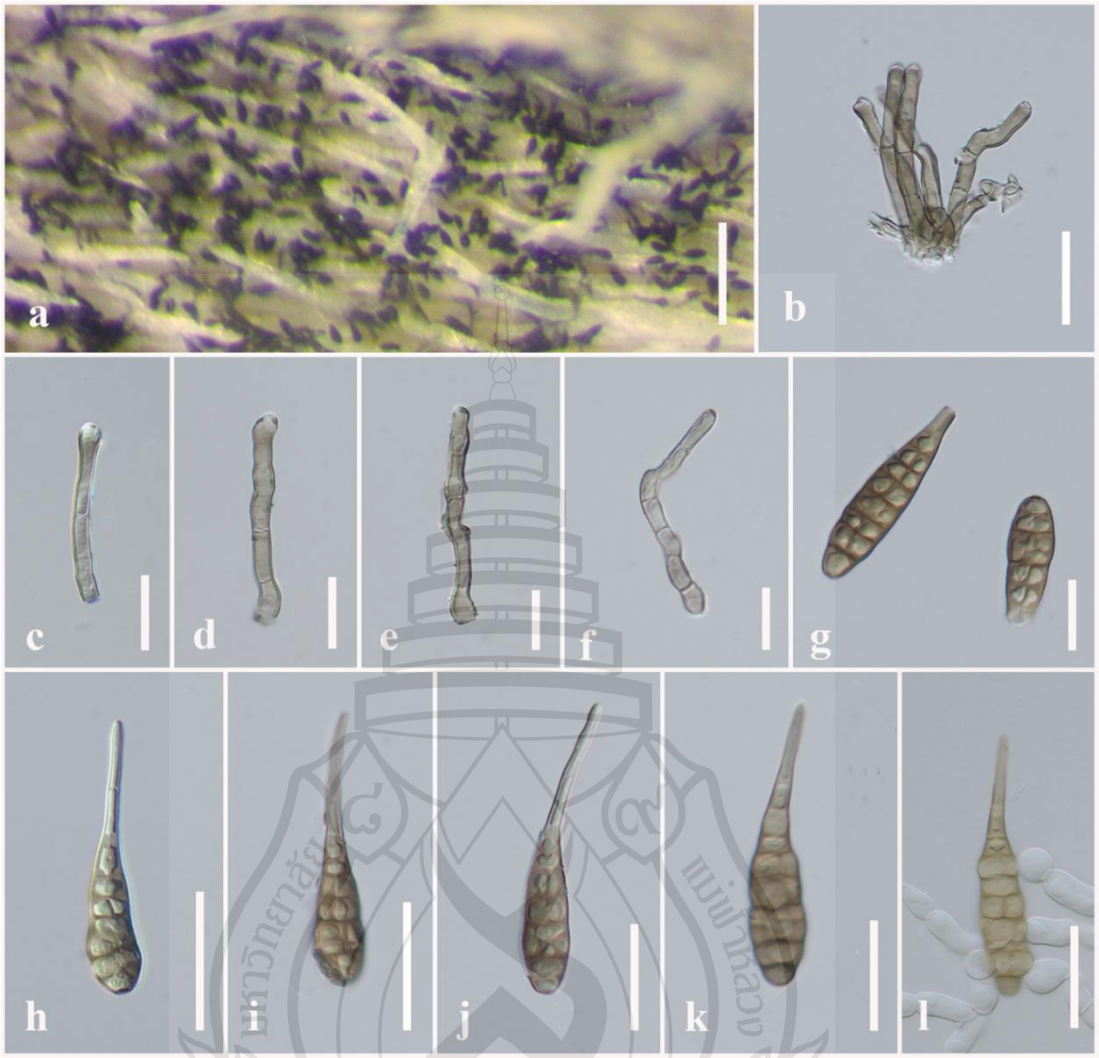
Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies growing on PDA, hairy or cottony, brown to dark brown, reaching 5 mm in 20 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, brown to dark brown hyphae; conidia not sporulated *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Tontola di Predappio, on dead aerial stem of *Plantago* sp. (Plantagineae), 20 October 2014, E. Camporesi, IT2195 (MFLU XX-XXXX, holotype), ex-type living culture, MFLUCC XX-XXXX; *ibid.*, Forlì, Via Nenni, on dead aerial stem of *Erysimum* sp. (Brassicaceae), 28 July 2014, E. Camporesi, IT2028 (MFLU XX-XXXX, paratype), living culture = MFLUCC XX-XXXX.

Notes: *Alternaria brevirostra* morphologically corresponds to species in section *Porri* via being born in chains, long and filamentous rostrate, multiple transverse and longitudinal septa conidia (Woudenberg et al., 2013). Morphologically *A. brevirostra* resembles *A. macrospora* by its obpyriform, multi-septate conidia with a beak, but differs in having a shorter beak (40–50 µm vs. 100–200 µm) and distoseptate conidia (Simmons, 2007). In phylogenetic analyses, *A. brevirostrum* formed a sister lineage with *A. macrospora* (CBS 117228) with significant support (74% ML, 0.99 PP; Figure 4.35) and clustered with *A. cucumerina* (CBS 116114), *A. venezuelensis* (CBS 116121) and *A. aragakii* (CBS 594.93).

A comparision of ITS nucleotide pairwise showed that *A. brevirostra* differs from *A. macrospora* in 10/530 bp (1.9% difference, no gap), differs from *A. cucumerina* in

9/530 bp (1.7% difference, no gap), differs from *A. venezuelensis* in 9/530 bp (1.7% difference, no gap), and differs from *A. aragakii* in 11/530 bp (2% difference, no gap). In GAPDH, the species differs from *A. macrospora* in 11/577 bp (1.9% difference, no gap), differs from *A. cucumerina* (CBS 116114) in 6/570 bp (1.1% difference, no gap), differs from *A. venezuelensis* in 8/577 bp (1.4% difference, no gap), and differs from *A. aragakii* in 9/577 bp (1.6% difference, no gap). In TEF1-α, *A. brevirostra* differs from *A. macrospora* in 10/337 bp (3% difference, no gap), differs from *A. cucumerina* in 7/337 bp (2% difference, no gap), differs from *A. venezuelensis* in 6/337 bp (1.8% difference, no gap), and differs from *A. aragakii* in 10/337 bp (3% difference, no gap). In RPB2 nucleotide pairwise comparison, *Alternaria brevirostra* differs from *A. macrospora* (CBS 117228) in 11/539 bp (2% difference, no gap), differs from *A. cucumerina* (CBS 116114) in 12/539 bp (2.2% difference, no gap), differs from *A. venezuelensis* (CBS 116121) in 14/539 bp (2.6% difference, no gap), and differs from *A. aragakii* (CBS 594.93) 11/539 bp (2% difference, no gap).



Note a Colonies on dead stem of *Plantago* sp. b–f Conidiophores bearing conidiogenous cells. g–k Conidia. l Germinated conidium. Scale bars: a = 100 µm, h–l = 50 µm, b–f = 30 µm, g = 20 µm.

Figure 4.35 Morphology of *Alternaria brevirostra*

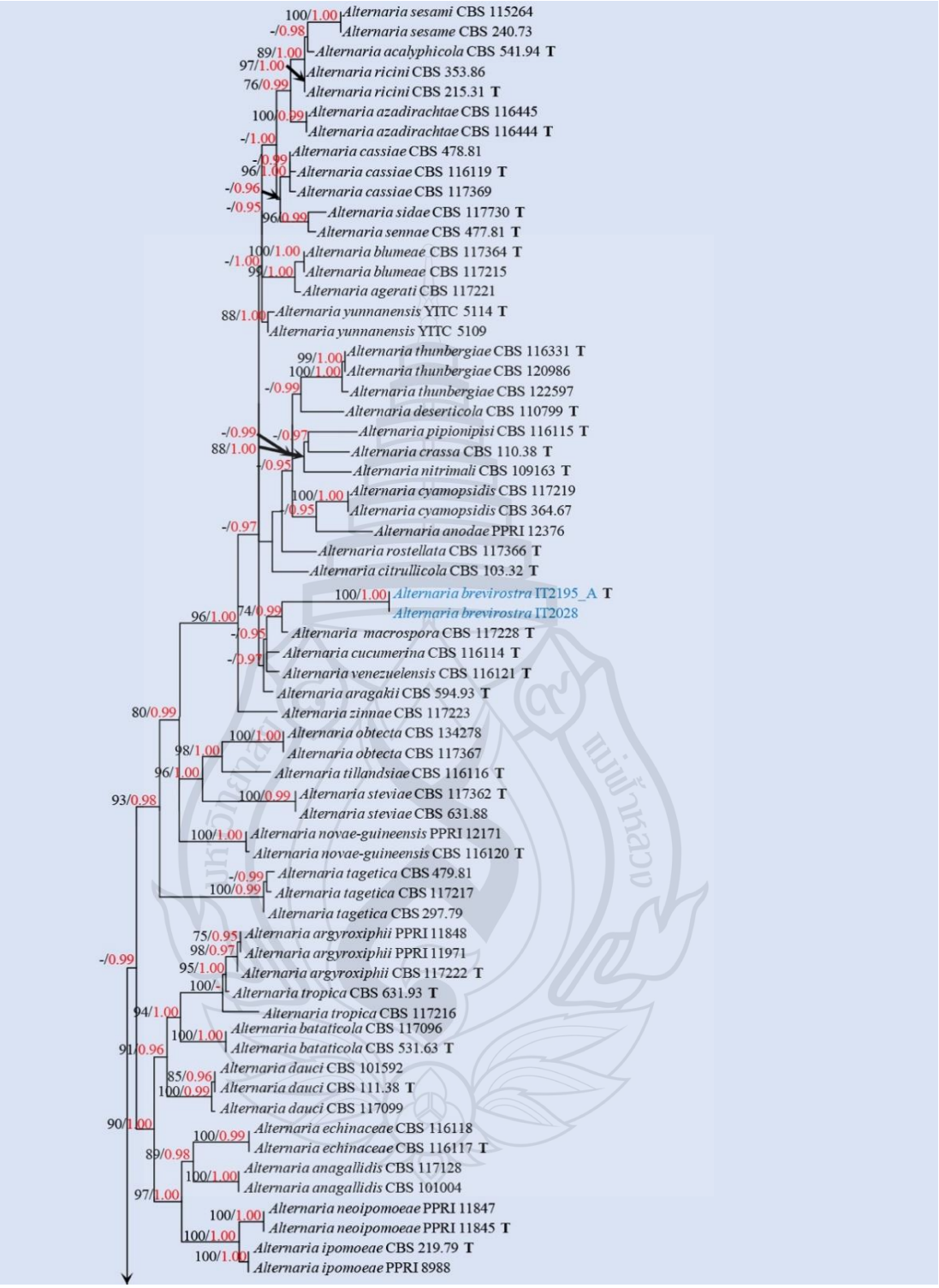
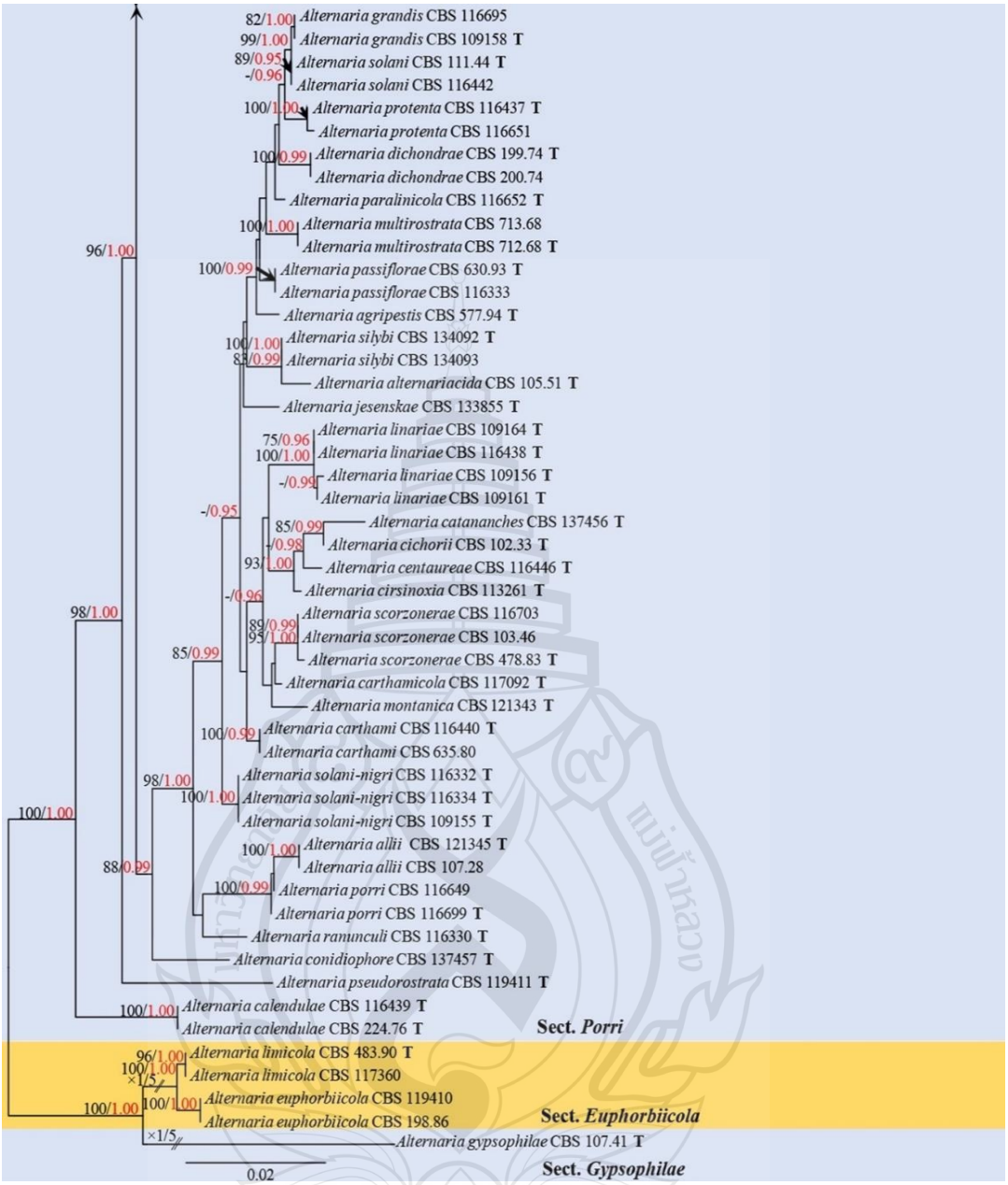


Figure 4.36 Phylogenetic construction of *Alternaria* sect. *Porri* using RAxML-based analysis of a combined multigene



Note Phylogenetic construction of *Alternaria* sect. *Porri* using RAxML-based analysis of a combined ITS, GAPDH, TEF1-α, RPB2 and Alt-a1 DNA sequence dataset. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 70 % and Bayesian posterior probabilities (PP, red) equal to or greater than 0.95 are shown above the nodes. The tree is rooted to Sect. *Gypsophilae* (*A. gypsophilae* CBS 107.41). Newly generated strains are in blue and the type strains are indicated by superscript "T".

Figure 4.36 (continued)

4.4.4 Section *Radicina* D.P. Lawr., Gannibal, Peever & B.M. Pryor, Mycologia 105: 541. 2013.

Type species: *Alternaria radicina* Meier, Drechsler & E.D. Eddy

Notes: Section *Radicina* is a small section in *Alternaria* comprising only eight phylogenetic species (Marin-Felix et al., 2019; He et al., 2021b). Most species in this section were reported as pathogens of Apiaceae (Lawrence et al., 2016; Marin-Felix et al., 2019; He et al., 2021b). In this study, we introduced a novel species, *A. phytolaccae* as a saprobe on *Phytolacca* sp. (Phytolaccaceae) which is reported from different host family for the first time.

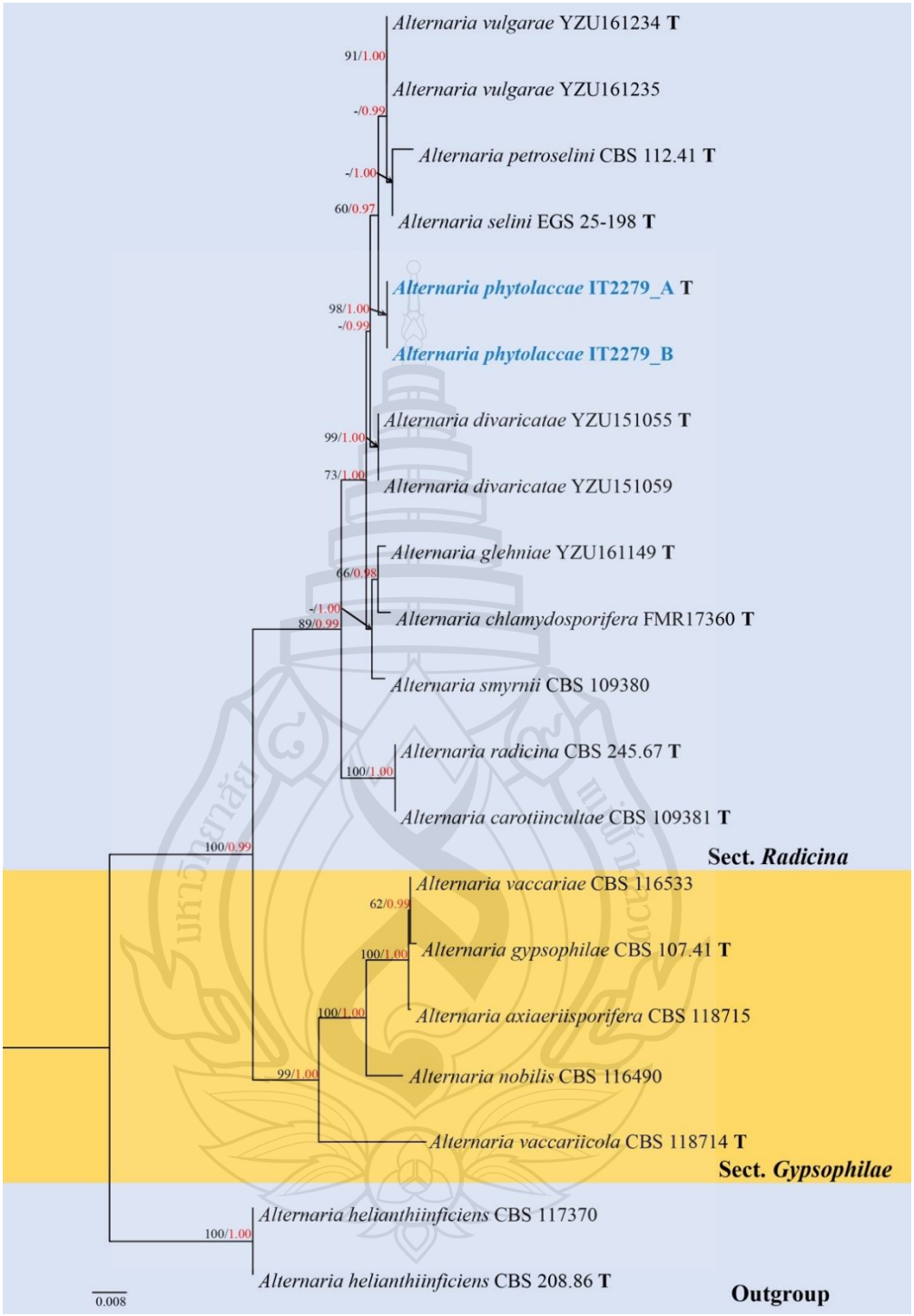


Figure 4.37 Phylogenetic construction of *Alternaria* sect. *Radicina* using RAxML-based analysis of a combined multigene

Figure 4.37 Phylogenetic construction of *Alternaria* sect. *Radicina* using RAxML-based analysis of a combined ITS, TEF1-α, GAPDH and Alt-a1 DNA sequence dataset. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 60% and Bayesian posterior probabilities (PP, red) equal to or greater than 0.95 are shown above the nodes. The tree is rooted to *A. helianthiinficiens* (CBS 208.86 and CBS 117370). Newly generated strains are in blue and the type strains are indicated by superscript "T".

Alternaria phytolaccae J.F. Li, Camporesi, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF****; Facesoffungi number: FoF****

Etymology: Name after the host genus *Phytolacca*.

Holotype: MFLU 21-****

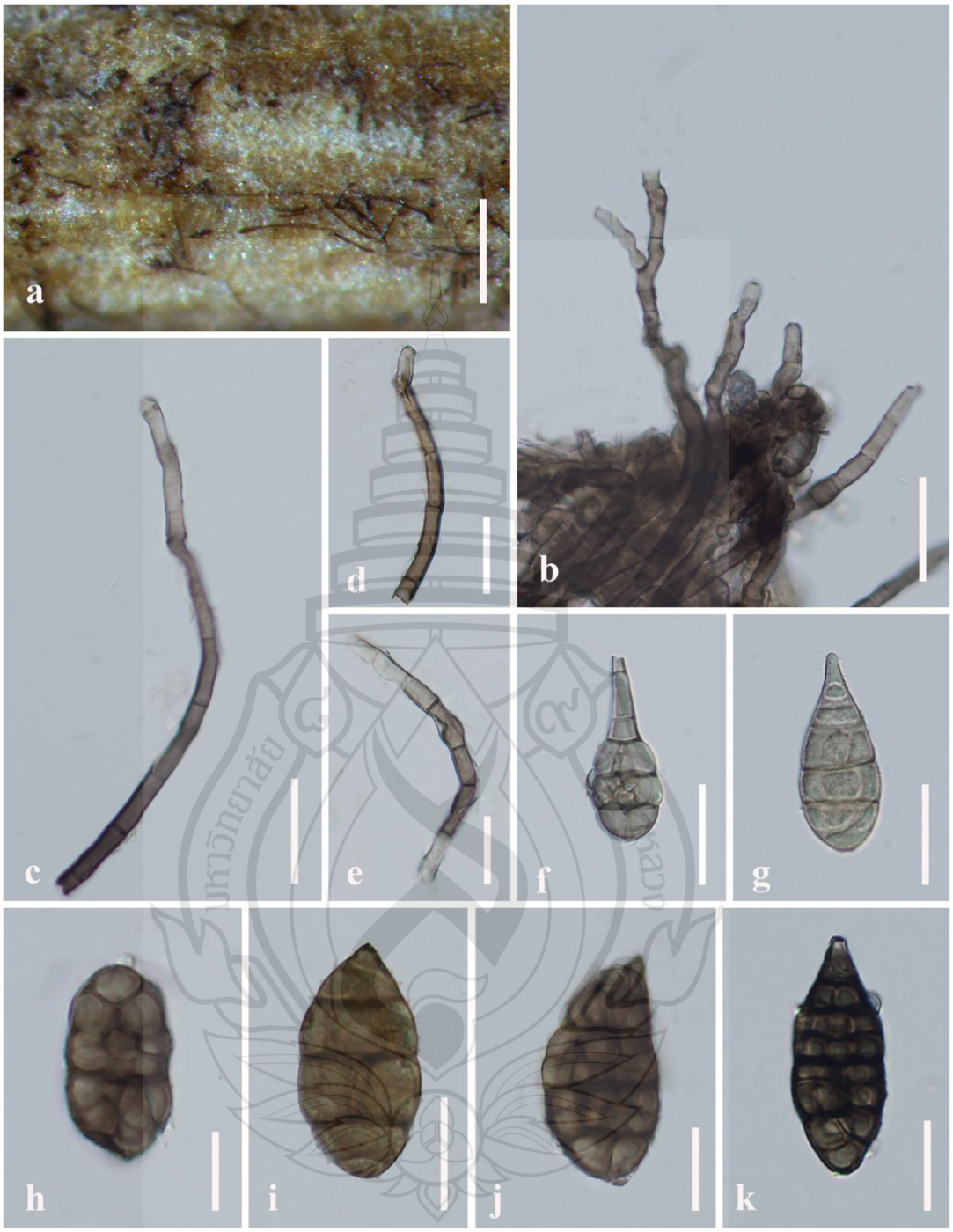
Saprobic on dead hanging stem of *Phytolacca* sp. (Phytolaccaceae). Sexual morph Undetermined. Asexual morph *Mycelium* superficial on host substrate, with dark hyphae. *Conidiophores* 160–200 × 8–11 µm ($\bar{x}$ = 174 × 9 µm, n = 30), macronematous, straight or flexuous, cylindrical, pale brown to dark greyish brown, geniculate, normally sympodial proliferations with several conidiogenous loci at the apex, branched, clumped, septate, smooth, thick-walled, arising from stomatic base. *Conidiogenous cells* 10–16 × 8–9 µm ($\bar{x}$ = 14 × 8.6 µm, n = 20), polytretic, integrated, terminal, determinate or percurrent, dolabriform to spathulate, subhyaline, smooth, thin-walled, apically doliiform, with 2–3 conidiogenous loci on side of cell. *Conidia* 30–65 × 20–30 µm ($\bar{x}$ = 45 × 24 µm, n = 30) acrogenous, solitary, clustered or clumps, curved, ellipsoidal to obpyriform, with acute apex, beakless, sometimes with short, narrow, septate beak, pale yellowish- brown to dark greyish-brown, 3–7 transversely eusepta, with 1–2 longitudinal or oblique or Y-shape septa in some transverse divisions, becoming sectored, slightly constricted at the septa, smooth to minutely verrucose, thick-walled. *Conidial secession* schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies growing on PDA, cottony, brown to dark brown, reaching 5 cm in 7 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, grey to dark grey hyphae; conidia not sporulated *in vitro* within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Forl', San Lorenzo in Noceto, on dead hanging stem of *Phytolacca* sp. (Phytolaccaceae), 4 December 2014,

E. Camporesi, IT2279 (MFLU XX-XXXX, holotype), ex-type living culture, MFLUCC XX-XXXX.

Notes: *Alternaria phytolaccae* morphologically corresponds with section *Radicina* in having branched, clumped, geniculate, sympodial proliferations conidiophores with several conidiogenous loci at the beakless apex. In multi-locus phylogenetic analyses, *A. phytolaccae* formed a separate lineage and clustered with *A. petroselini*, *A. selini* and *A. vulgarae* in section *Radicina* with significant support (60% ML, 0.97 PP, Figure 4.37). A comparision of ITS nucleotide pairwise showed that *A. phytolaccae* differs from *A. petroselini* in 8/520 bp (1.5% diference, no gap), differs from *A. selini* in 8/520 bp (1.5% diference, no gap) and differs from *A. vulgarae* 9/520 bp (1.7% difference, no gap). In GAPDH, *A. phytolaccae* differs from *A. selini* in 9/567 bp (1.6% difference, no gap) and differs from *A. vulgarae* in 9/567 bp (1.6% difference, no gap).



Note a Colonies on dead hanging stem of *Phytolacca* sp. b–e Conidiophores bearing conidiogenous cells, with 2–3 conidiogenous loci on side of cell. f, g Immature conidia. h–k Conidia. Scale bars: a = 150 µm, c = 50 µm, b, d–f = 30 µm, g–k = 20 µm.

Figure 4.38 Morphology of *Alternaria phytolaccae*

4.5 Discussion

4.5.1 Diversity of *Alternaria* Species

The diversity of *Alternaria* species has been reported on varied plant hosts, mainly consisting of monotyledon and dicotyledon plants from globoal studies (Ellis, 1971, 1976; Simmons, 2007; Woudenberg et al., 2013, 2014, 2015; Lawrence et al., 2013, 2014, 2016; Marin-Felix et al., 2019). In our fungal diversity survey of China, Italy, Russia and Thailand, 70 collections were made from sundry plant host species. Morphological examinations revealed that these species belonged to the genus *Alternaria*, and interestingly, 24 taxa could not be ascribed to any known taxa within the *Alternaria*, according to recent classification provided in many recent studies. We also noted morphological differences among extant species, and hence 24 new species, viz. *Alternaria arctoseptata*, *A. arundinis*, *A. baoshanensis*, *A. breviconidiophora*, *A. brevirostra*, *A. ellipsoconidia*, *A. eupatoriicola*, *A. falcata*, *A. lathyri*, *A. macilenta*, *A. macroconidia*, *A. minimispora*, *A. muriformispora*, *A. nodulariconidiophora*, *A. oblongoellipsoidea*, *A. obpyriconidium*, *A. orobanches*, *A. ovoidea*, *A. phragmiticola*, *A. phytolaccae*, *A. pseudoinfectoriae*, *A. rostroconidia*, *A. salicicola*, and *A. torilis* as well as new host and geographical records on *A. alternata* and *A. doliconidium* are described and illustrated which have been proposed and classified in sects. *Alternaria*, *Infectoriae*, *Porri* and *Radicina*. To justify our new fungal species by following the recommendations outlined by Jeewon and Hyde (2016), we noted morphological similarities and also some remarkable differences. For instance, our phylogenies reveal that strains of *A. eupatoriicola* (IT3518_A and IT3518_B) from Italy and cultures H-22 and MFLUCC 14-0436 from Thailand are positioned in a well-supporting subclade (Figure 4.7), though these strains morphologically may indicate they are the same species. Due to missing specimens and data for H-22 and MFLUCC 14-0436, we can only state these two cultural strains as *Alternaria* sp. presently. On the other hand, we note *Alternaria oblongoellipsoidea* (IT2102_A and IT2102_B) and *A. ellipsoconidia* (IT2086_A, IT2086_B and IT2086_C) share a meandering conidiophore apex with confidential hollow conidiogenous loci, which rarely occurs in sect. *Alternaria*. However, the multi-locus phylogenetic analyses (analyses 1, 2), additional morphological characterisitics that differ in *A. oblongoellipsoidea* from *A. ellipsoconidia* via having rather

common and short conidiophores (96 µm × 9.5 µm vs. 145 µm × 6.5 µm), and rather polytretic, swollen, doliiform conidiogenous cells, prompt *A. oblongoellipsoidea* and *A. ellipsoconidia* to be different species residing in sect. *Alternaria*. Interestingly, *A. arctoseptata* and *A. lathyri* were isolated from same host genus *Lathyrus* (Fabaceae). However, *A. lathyri* morphologically differs from *A. arctoseptata* in having darker brown, solitary, non-stomatic base conidiophores with less swollen knots, and dark brown conidia, as well as in phylogenetic analyses combined seven gene loci (ITS, LSU, SSU, TEF1-α, RPB2, GAPDH and Alt-a1), *A. lathyri* is positioned in a subclade that is distinct from *A. arctoseptata*. Furthermore, we also note that *A. orobanches* (IT1997_A and IT1997_B) formed a single lineage basal to sect. *Alternaria*. Ultimately, *A. orobanches* morphologically share clonal structures having born in chains, phragmosporous to muriform small-spored conidia and conidiophores with monotretic or polytretic conidiogenous loci at apex, that correspond the morphological features of sect. *Alternaria*, forming a single lineage placed in a clade of sect. *Alternaria* in phylogrames of section *Alternaria* (analyses 1). Thus, *A. orobanches* is considered to be placed in sect. *Alternaria* based on morphology and phylogeny. The remaining comprehensive justifications of novel species refer to the notes of species.

This study also reveals new host records for *Alternaria doliconidium* and diverse hosts species for *A. alternata*. While *A. doliconidium* have previously been collected from *Rosa* sp. in Italy (Wanasinghe et al., 2018), we recovered *A. doliconidium* from *Reseda* sp. (Resedaceae) in China, which is the first record of both host species and location for this species. Another particular culture noted *A. doliconidium* sporulated on OA media within 30 days, which was produced born in chains, oblong to subglobose, verruculose to verrucose conidia, with a short, branched or unbranched, aseptate apical beak, as well as apical or lateral secondary conidiophores were formed, which is the first description of sporulation for *A. doliconidium*. Moreover, 45 new collections of *A. alternata* were isolated from diverse plant hosts (Figure 4.8) in China, Italy and Thailand. Comparing these 45 collections with other *A. alternata* strains (mainly from CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands) in phylogenetic analyses, revealed our *A. alternata* complex could be phylogenetically summarized as several internal subclade (Figure 4.8), such as strains HWP-01, IT2144, IT2145, IT3598, and IT3651 forming an internal subclade with strains CBS 965.95 and CBS 966.95 (pathogen and saprobe) in subclade A; the

saprobic strains H-71, IT2143, and IT3556 forming an internal subclade with strain CBS 130254 (isolated from human sputum in India), and CBS 911.97 (isolated from *Artemisia brevifolia* (Asteraceae) in India) and clustered with CBS 130262, CBS 130265 (isolated from human sputum in India) and CBS 121492 (isolated from *Cucumis melo* (Cucurbitaceae) in China) (pathogen and saprobe, subclade A). Hence, these results prove that *A. alternata* and *A. doliconidium* are not host-specific and may not be phylogenetically distinctive on or in the same/similar host or habitat. It is foreseeable that as more complementary sections and taxa as novel *Alternaria* species are discovered from nature environments in future fieldwork, understanding *Alternaria* species relatives on hosts, unique genetic loci and chemical properties will be important topics in future works (Bock et al., 2002, 2005; Stewart et al., 2013; Tsuge et al., 2013).

In conclusion, the *Alternaria* species obtained in our survey identified using combined multi-locus gene analyses, based on the ITS, LSU, SSU, TEF1-α, RPB2, GAPDH and Alt-a1 genes, which showed a higher diversity on same specific host, such as *A. arctoseptata* and *A. lathyri* on *Lathyrus* sp.; as well a higher diversity of host species for one *Alternaria* species, such as *A. doliconidium* on *Rosa* sp. and *Reseda* sp., *A. alternate* from diverse plant hosts (Figure 4.8); at the same time, with the study of *A. doliconidium* and *A. hampshirensis* on *Rosa* sp. by Wanasinghe et al. (2018), and *A. alternate, A. italica* and *A. vitis* on *Vitis* sp. by Jayawardena et al. (2018); while over 30% (25 out of 72 specimens) in our present survey from diverse host plants are new to science. It is indicate that the discovering of one/more species from one/more host is inevitably, and foreseeable that *Alternaria* species with host association is essential, which are, the explore on more complementary sections and taxa as novel *Alternaria* species relative on diverse hosts, are expected in future occupations.

4.5.2 Phylogenetic Concepts of *Alternaria*

The method of use of DNA sequence data is very important in resolving *Alternaria* taxonomy. Characters of conidia in some sections are not peculiar, such as, conidia are mostly dictyosporous in sects. *Alternaria* and *Japonicae*; while some are mostly phragmosporous, such as sects. *Alternantherae* and *Nimbya*. Species in sections *Alternantherae*, *Dianthicola* and *Porri* have long apical narrow beaks or secondary conidiophores and these characters are absent in sections *Chalastospora*, *Gypsophilae* and

Ulocladium, while dictyospores and phragmospores can be found in the same section, such as sect. *Infectoriae* and *Phragmosporae* (Ellis, 1971, 1976; Simmons, 2007). The *Alternaria* complex analyses have been investigated by Woudenberg et al. (2013, 2014, 2015), Lawrence et al. (2016), Ghafri et al. (2019) and Jayawardena et al. (2019), with recent 28 phylogenetic sections of genus *Alternaria*. In our phylogenetic analyses combined ITS, LSU, SSU, TEF1-α, RPB2, GAPDH and Alt-a1, the topology of phylogenetic tree for *Alternaria* complex are respond the results with previous studies. However, many sections that share common structures are usually distinct in their molecular genetic relationship (Lawrence et al., 2016; Jayawardena et al., 2019, present study). The single gene analyses show that the ITS, RPB2 and Alt-al genes could indicate most of *Alternaria* section positions; while TEF1-α and GAPDH genes could be inducted species well but not for section positions; at last, LSU and SSU genes could not indicated both species and section position for *Alternaria* complex, which is not informative for most sections of *Alternaria*, unless in a higher fungal rank. It is interesting to note that RPB2 gene reveals most phylogenetical distinct lineage as well as most nucleotide differences in comparison between different species.

The *Alternaria* section *Alternaria* has been studied by Woudenberg et al. (2013, 2015) and Lawrence et al. (2013, 2016) based on both phylogeny and morphology. Although Woudenberg et al. (2015) assigned 35 morphospecies which cannot be reliably distinguished based on the multi-locus phylogeny to synonymized under *A. alternata* while notes the necessities of further research of sect. *Alternaria* due to the inconsistencies in morphology and molecular, which mainly based on comparison of single nucleotide polymorphisms, but less morphology compared. However, several later studies based on combined multi-locus phylogeny showed that recent *A. alternata* species may not form single lineage in molecular analyses (Jayawardena et al., 2018; Chethana et al., 2019, present study). In addition, we compared our recent collections with both aspect of morphology and phylogeny. Interestingly, our phylogenetic analyses show that the phylogenetic strains of *A. alternata* can separated to minimum five different clade (analyses 3, Figure 4.8). While the novel taxa from sundry host species are both morphologically and phylogenetically distinct from *A. alternata* complex and other species in *Alternaria* sect. *Alternaria* (Figure 4.6, 4.7).

The plasma membrane ATPase and cmdA loci are suggested to be the most informative markers for delimitation of *Alternaria* species in sect. *Infectoriae* by Lawrence et al. (2013, 2016), while the Alt-a1 locus unreliably amplifies some species within this section (Lawrence et al., 2013; Woudenberg et al., 2013; Marin-Felix et al., 2019). However, the cmdA locus has been less used for the phylogeny of most *Alternaria* sections and species (Marin-Felix et al., 2019). ITS barcode as well considered a good phylogenetic marker to define the *Alternaria* sections. However, it has limited discriminatory power to distinguish species (Lawrence et al., 2012, 2013, 2016; Woudenberg et al., 2013, 2015; Marin-Felix et al., 2019). In our study, two new taxa are indicated in *Alternaria* sect. *Infectoriae*, based on phylogenetic analyses combined ITS, GAPDH and ATPase nucleotide sequences. Our new taxa *A. arundinis* (IT2625A_1, IT2625A_2 and IT2625A_3) and *A. nodularconidiophora* (IT2250_A and IT2250_B) have oblong-ellipsoidal or obclavate conidia can apically or laterally formed long, geniculate, multi-locus secondary conidiophores. These morphological structures are confirmed the conidial characters in sect. *Infectoriae*. However, *A. nodularconidiophora* was observed it less develop secondary conidiophores on the natural substrate but heavily produced on culture incubated on PDA media, the mechanism to cause generated secondary conidiophores should be explored in through study. On the other hand, some sexual morph species in sect. *Infectoriae* are known, while no sexual morph were observed in this survey. The sexual morph link to asexual species is expected in future works.

Lawrence et al. (2013) redefined the molecular data of *Porri* species-group in *Alternaria* which first reported by Pryor and Gilbertson (2000) and established *Alternaria* sect. *Porri*. This section is morphologically charactorized as apical long filamentous rostrum in most of mature conidia (Lawrence et al., 2013; Woudenberg et al., 2013, 2014). Woudenberg et al. (2014) indicated that ITS, Alt-a1, GAPDH, RPB2 and TEF1-α are the effective phylogenetic markers to determine relationships and species delineation for *Alternaria* sect. *Porri*. However, many strains of species in this section could not well resolved their phylogenetic status (Lawrence et al., 2013; Woudenberg et al., 2013, 2014, present study). In our study, more than 40 phylogenetic identified species in sect. *Porri* with one new taxon are confirmed base on phylogenetic analyses combined ITS, Alt-a1, GAPDH, RPB2 and TEF1-α. Our new taxon *A. brevirostra* (IT2195_A and IT2028), which morphologically produce a relatively short filamentous beak on conidia, it is need further

verification whether *A. brevirostra* could grow longer apical beak if maturity or media cultured and discuss the appearance that short conidial filamentous beak occur on species in sect. *Porri*. Besides, more effective nucleotide markers for this section are expected.

Species-group of *Alternaria radicina* was first morphologically described by Simmons (2007), and the *Alternaria* sect. *Radicina* was first recognized by Pryor and Gilbertson (2000) based on sequence data of the ITS and SSU nucleotide. Lawrence et al (2013) and Woudenberg et al. (2013) confirmed this section by using multi-locus phylogenetic analyses, which include GAPDH, RPB2, TEF1-α and Alt-a1 loci. Uniformly, species in *Alternaria* sect. *Racidina* are less phylogenetic confirmed and morphologically share some similar morphological characters, such as conidiophores, sporulation, conidial shape and others. The recent phylogenetic studies of this section were reported by Marin-Felix et al. (2019), Tao et al. (2019) and He et al. (2021b), when they described the novel species in this section, which based on the multi-locus phylogenetic analyses of ITS, GAPDH, RPB2 and TEF1-α nucleotide sequences. In our survey, based on the phylogenetic analyses of combined ITS, GAPDH, RPB2 and TEF1-α nucleotide sequences, a new species *A. phytolaccae* (IT2279_A and IT2279_B) is justified to be respectively correspond the morphological and phylogenetic features in sect. *Radicina* in Woudenberg et al. (2013) and Lawrence et al. (2016). Furthermore, species are few phylogenetic confirmations in this section, more investigation and explore on fresh samples are expect for the *Alternaria* sect. *Radicina*.

On the other hand, we note the analyses clearly show that species are phylogenetically diverse in *Alternaria* complex (Figure 4.6, 4.7, 4.8). The studies of *Alternaria* from various samples as well show that genus *Alternaria* is speciose (Ellis, 1971, 1976; Simmons, 1983, 1986, 1989, 2002, 2007; Lawrence et al., 2012, 2013, 2014, 2016; Woudenberg et al., 2013, 2014, 2015; Thambugala et al., 2017; Wanasinghe et al., 2018; Jayawardena et al., 2018, 2019; Chethana et al., 2019; Ghafri et al., 2019; Marin-Felix et al., 2019; Tao et al., 2019; He et al., 2021b, present study), the novel species/section are expected if more hosts/habitats are explored. However, due to the phylogenetic diversity of *Alternaria* species, to justify the species complex in section level (novel section) should species in same section should share at least three or more unique conidial structures which differ from other sections, while phylogenetically formed independent distinct clade in *Alternaria* complex; to justify species at the intraspecific level (novel

species) should compare the differences with one or two morphologically similar species, and three or more phylogenetically close species, while compare the nucleotide differences on informative genes, which follow the outline of Jeewon and Hyde (2016). Moreover, genus *Alternaria* could not totally well solved by any recent gene sequence, more unique genetic loci and chemical properties of genus *Alternaria* are expected in future works (Bock et al., 2002, 2005; Stewart et al., 2013; Tsuge et al., 2013; Woudenberg, 2013, 2014, 2015).

4.5.3 Application of Molecular Dating to Solve Taxonomy of *Alternaria*

Divergence time estimation of fungi in high rank were explored based on multi-locus analyses which mainly combined LSU, SSU and TEF1-α genes (Hyde et al., 2017; Liu et al., 2017, 2018, 2019; Zhang et al., 2019). However, to resolve divergence time estimation of genus *Alternaria* with high rank fungal strains, we combined ITS and RPB2 genes into our divergence analyses (Figure 4.4, 4.5), which are informative markers for *Alternaria* species. The species in *Alternaria* complex could well solved in intraspecific level, and RPB2 genes well-solved most recent sections in *Alternaria* (Lawrence et al., 2014, 2016; Wodenberg et al., 2013, 2014, 2015). The divergence time estimation in our study shows that *Alternaria* diverged approximately at 62 (42–85) Mya, while the crown age of *Alternaria* is around 53 (36–72) Mya as well as the divergence time of sect. *Crivellia* in Late Paleocene to early Eocene. Although this divergence time estimate corresponds with the related discourse reported in Kalgutkar and Sigler (1995) whereas a fossil species *Piriurella alternariata* obtained from late Palaeocene or early Eocene (56±5 Mya) affinity with *Alternaria* based on comprised the morphological structures of modern *Alternaria*, the lacking of credible molecular and more comparable evidences could not verified that *Piriurella alternariata* affinity with *Alternaria*. On the other hand, *Alternaria* sections diverged in early age, such as species in sect. *Crivellia*, sect. *Phragmosporae*, sect. *Ulocladium* and sect. *Undifilum* are morphologically beakless, round, mostly with transverse eusepta, rarely constricted at the septa, while the type section, sect. *Alternaria* with mostly distinct characters of *Alternaria* occurred with a late divergence time as 13 (6–20) Mya (Inderbitzin et al., 2006; Woudenberg et al., 2013). Moreover, the sect. *Phragmosporae* diverged with other sections in age as 43 (28–58) Mya in Eocene, and crown age of this section is around 28 Mya which is in an early crown age of *Alternaria*, while all species within this section were known from soil and seawater environments

(Woudenberg et al., 2013) and less reported as pathogens or endophytes. In conclusions, the *Alternaria* species in sections diverged in early age mostly morphologically have beakless conidia with transverse septate with rare constricted, which may be the primitive structures of ancient *Alternaria* species.

Both phylogenetic and evolutionary estimate analyses base on multigene data are shown that some *Alternaria* sections in close molecular relationship and share same divergence time as well. Sect. *Porri* and sect. *Euphorbiicola* diverged from a clade with divergence time at 10 (5.6–17.4) Mya. However, species in sect. *Euphorbiicola* morphologically conidia with beak not distinct from the spore body, while differ from the characters in sect. *Porri*. *Alternaria cumini* E.G.Simmons in Sect. *Eureka* formed a separated lineage from Sect. *Eureka* in both phylogenetic and divergence analyses (Figure 4.4 and 4.5), while Sect. *Eureka* and sect. *Embellisioides* share a divergence time as 24 (14–35) Mya, these two sections and *A. cumini* share common morphological structures in having ovoid to subcylindrical, straight to inequilateral, transseptate and less longitudinal septa (Simmons, 2007; Woudenberg et al., 2013) which can be summarized as one expect section. *Alternaria thalictrigena*, sect. *Panax*, and sect. *Teretispora* share a divergence time as 27 (17–40) Mya, species in this two sections and *A. thalictrigena* share same characters in having paler and lanky conidia, which *A. thalictrigena*, sect. *Panax*, and sect. *Teretispora* can be recommended as a expect section. Strains in Sect. *Pseudoalternaria* in our divergence analyses are lacking of informative sequences, we use only LSU gene of the type strain *A. arrhenatheri* (CBS 133068) which ITS, SSU, TEF 1-α and RPB2 genes are absent (GenBank, 2021) for this section. Thus, the divergence result of Sect. *Pseudoalternaria* may not convinced in this study. Species in sect. *Crivellia* share the structures as cylindrical, straight to curved to inequilateral, with transverse eusepta, rarely constricted at septa (Woudenberg et al., 2013), which are morphologically distinct with species in other *Alternaria* sections, while sect. *Crivellia* is not well-resolved in ML and BI analysis in genus *Alternaria* (analyses 1) as well formed a separated lineage from other *Alternaria* sections in divergence time estimation analyses, this section may consider as an expected phylogenetic genus. The further analyses and more informative gene sequences are expected in future works.

We note that the age for the crown of most sections (24 out of 28, 85.7%) in *Alternaria* are between 0.1 to 20 Mya, while the genus *Alternaria* (53 Mya), *Alternaria* section *Nimbya* (24 Mya) and *Alternaria* sect. *Phragmosporae* (28 Mya) evolved in the range for family (20–100 Mya, crown age) which recommended by Liu et al. (2017), However, the length of branch between stem ancestor and crown clades could be affected by species richness of the group, net diversification rate, timescale and model setup (Zhao et al., 2016; Liu et al., 2017), the evolutionary result in this study only to explore divergence time of *Alternaria* and each sections in this genus while not support to establish family. On the other hand, the divergence time (stem age) of most sections in *Alternaria* are between 10 and 50 Mya. We suggest that these times range can be the supplementary evidence for acceptance of sections in *Alternaria*, which is: *Alternaria* sections should evolved in the range between 0.1 to 20 Mya (crown age) and 10 to 50 Mya (stem age).

Table 4.7 Taxa used for phylogenetic analyses and divergence time estimation of Pleosporales in this study (excluding Alteraria species)

Species name	Strains/Voucher no.	GenBank accession number				
		ITS	LSU	SSU	TEF1-α	RPB2
Aigialus grandis	BCC 18419	-	GU479774	GU479738	GU479838	GU479813
Aigialus mangrovei	BCC 33563	-	GU479776	GU479741	GU479840	GU479815
Aigialus parvus	BCC 18403	-	GU479778	GU479744	GU479842	GU479817
Aigialus rhizophorae	BCC 33572	-	GU479780	GU479745	GU479844	GU479819
Aliquandostipite khaoyaiensis	CBS 118232	-	GU301796	-	GU349048	-
Amniculicola immersa	CBS 123083	-	FJ795498	GU456295	GU456273	NG_056964
Amorosia littoralis	NN 6654	AM292047	AM292055	AM292056	-	-
Angustimassarina populi	MFLUCC 13–0034	KP899137	KP888642	KP899128	KR075164	-
Anteaglonium abbreviatum	GKM219N	-	GQ221881	-	GQ221916	-
Anteaglonium parvulum	GKM 1029	MN582759	GQ221878	-	GQ221915	-
Arthonia dispersa	UPSC2583	-	AY571381	AY571379	-	-
Ascochyta pisi	AFTOL-ID 1583	-	DQ678070	DQ678018	DQ677913	DQ677967
Ascocratera manglicola	BCC 09270	-	GU479782	GU479747	GU479846	GU479821
Astrosphaeriella fusispora	MFLUCC 10–0555	-	KT955462	KT955443	KT955425	KT955413
Bahusandhika indica	GUFCC 18001	KF460273	KF460274	-	-	-
Berkleasmium micronesicum	BCC 8141	DQ280262	DQ280272	DQ280268	-	-
Berkleasmium nigroapicale	BCC 8220	DQ280261	DQ280273	DQ280269	-	-
Bimuria novae-zelandiae	CBS 107.79	MH861181	NG_058623	NG_061017	DQ471087	DQ471159
Boeremia exigua	CBS 431.74	FJ427001	JX681074	EU754084	KY484687	KT389569

Table 4.7 (continued)

Species name	Strains/Voucher no.	GenBank accession number				
		ITS	LSU	SSU	TEF1-α	RPB2
Botryosphaeria dothidea	CBS 115476	KF766151	NG_027577	DQ677998	DQ767637	GU357802
Capnodium salicinum	CBS 131.34	MH855469	DQ678050	DQ677997	-	KT216553
Caryospora minima	-	-	EU196550	EU196551	-	-
Cladosporium cladosporioides	CBS 170.54	MH857281	AY213694	DQ678004	FJ936162	GU357790
Coelodictyosporium pseudodictyosporium	MFLUCC 13–0451	KR025858	KR025862	-	-	-
Corynespora cassiicola	CBS 100822	-	GU301808	GU296144	GU349052	GU371742
Corynespora smithii	CABI 5649b	-	GU323201	-	GU349018	GU371783
Cyclothyriella rubronotata	CBS 141486	NR_147651	KX650544	KX650507	KX650519	KX650574
Decaisnella formosa	BCC 25617	NR_147651	GQ925847	GQ925834	GU479850	KX650574
Decaisnella formosa	BCC 25616	-	GQ925846	GQ925833	GU479851	GU479825
Delitschia chaetomioides	SMH 3253.2	-	GU390656	-	GU327753	-
Delitschia winteri	CBS 225.62	-	DQ678077	DQ678026	DQ677922	DQ677975
Dendrographa leucophaea	AFTOL-ID 355	DQ842015	AF279382	AF279381	DQ842007	GU561849
Dendryphion comosum	CBS 208.69	MH871026	-	-	-	MH859293
Dissoconium aciculare	CBS 204.89	AY725520	GU214419	GU214523	-	KX288435
Exosporium stylobatum	CBS 160.30	JQ044428	JQ044447	-	-	-
Gloniopsis calami	MFLUCC 15–0739	KX669036	KX646363	KX669034	KX671965	-
Gloniopsis praelonga	CBS 112415	-	FJ161173	FJ161134	FJ161090	FJ161113
Guttulispora crataegi	MFLUCC 13–0442	KP899134	KP888639	KP899125	KR075161	-

Table 4.7 (continued)

Species name	Strains/Voucher no.	GenBank accession number				
		ITS	LSU	SSU	TEF1-α	RPB2
Halotthia posidoniae	BBH 22481	-	GU479786	GU479752	-	-
Herpotrichia diffusa	CBS 250.62	-	DQ678071	DQ678019	DQ677915	DQ677968
Hypsostroma caimitalense	GKM 1165	-	GU385180	-	-	-
Hypsostroma saxicola	SMH 5005	-	GU385181	-	-	-
Hysterium angustatum	CBS 236.34	MN608547	FJ161180	GU397359	FJ161096	FJ161117
Jahnula seychellensis	SS2113	-	EF175665	EF175644	-	-
Katumotoa bambusicola	KT 1517a	LC014560	AB524595	AB524454	AB539108	AB539095
Lentimurispora urniformis	MFLUCC 18–0497	-	MH179144	MH179160	MH188055	-
Leptosphaeria doliolum	CBS 505.75	MH860947	GQ387576	GQ387515	GU349069	KT389640
Leptoxyphium cacuminum	MFLUCC 10–0049	-	JN832602	JN832587	-	-
Ligninsphaeria jonesii	MFLUCC 15–0641	-	KU221037	-	-	-
Ligninsphaeria jonesii	GZCC 15–0080	-	KU221038	-	-	-
Lignosphaeria fusispora	MFLUCC 11–0377	KP899140	KP888646	-	-	-
Lignosphaeria thailandica	MFLUCC 11–0376	KP899139	KP888645	-	-	-
Lindgomyces ingoldianus	ATCC 200398	JF419898	AB521736	AB521719	-	-
Lindgomyces rotundatus	KT 1096	-	AB521740	AB521723	-	-
Lophiostoma macrostomum	KT 508	-	AB619010	AB618691	LC001751	-
Lophiotrema lignicola	CBS 122364	MH863205	GU301836	GU296166	GU349072	LC194424
Lophiotrema nucula	CBS 627.86	LC194497	GU301837	GU296167	GU349073	GU371792

Table 4.7 (continued)

Species name	Strains/Voucher no.	GenBank accession number				
		ITS	LSU	SSU	TEF1-α	RPB2
Massaria anomia	CBS 591.78	-	GU301839	GU296169	-	GU371769
Massaria inquinans	M19	MH863726	HQ599402	HQ599444	HQ599342	HQ599460
Massarina corticola	CBS 154.93	-	FJ795448	FJ795491	-	FJ795465
Massarina eburnea	CBS 473.64	-	MH877786	GU296170	GU349040	FJ795466
Massariosphaeria phaeospora	CBS 611.86	-	GU301843	GU296173	-	GU371794
Mauritiana rhizophorae	BCC 28866	-	GU371824	GU371832	GU371817	-
Melanomma pulvis-pyrius	CBS 124080	MH863349	GU456323	GU456302	GU456265	GU456350
Murilentithecium clematidis	MFLUCC 14–0562	KM408756	KM408759	NG_061185	KM454445	KM454447
Murispora rubicunda	IFRD 2017	-	FJ795507	GU456308	GU456289	-
Neoastrosphaeriella krabiensis	MFLUCC 11–0025	NR_120004	JN846729	JN846739	-	-
Neoroussoella bambusae	MFLUCC 11–0124	KJ474827	KJ474839	-	KJ474848	KJ474856
Neotrematosphaeria biappendiculata	KTC 1124	-	GU205227	GU205256	-	-
Nigrograna fuscidula	CBS 141476	KX650547	KX650547	KX650509	KX650522	KX650576
Nigrograna mackinnonii	CBS 110022	KF015653	GQ387614	GQ387553	KF407985	KF015704
Occultibambusa bambusae	MFLUCC 13–0855	KU940123	KU863112	KU872116	KU940193	KU940170
Occultibambusa chiangraiensis	MFLUCC 16–0380	-	KX655546	KX655551	KX655561	KX655566
Ohleria modesta	MGC	KX650562	KX650562	-	KX650533	KX650582
Ohleria modesta	OM	KX650563	KX650563	KX650513	KX650534	KX650583
Paradictyoarthrinium diffractum	MFLUCC 13–0466	KP744455	KP744498	KP753960	-	KX437764

Table 4.7 (continued)

Species name	Strains/Voucher no.	GenBank accession number				
		ITS	LSU	SSU	TEF1-α	RPB2
Paradictyoarthrinium hydei	MFLUCC 17–2512	MG747498	MG747497	MH454349	-	MG780232
Phaeoseptum aquaticum	CBS 123113	KY940803	JN644072	-	-	-
Phaeoseptum terricola	MFLUCC 10–0102	NR_158869	MH105779	MH105780	MH105781	MH105782
Phyllosticta capitalensis	CBS 226.77	FJ538336	KF206289	KF766300	FJ538394	KY855820
Piedraia hortae	CBS 480.64	KF902289	GU214466	AY016349	-	-
Pleomassaria siparia	CBS 279.74	MH860853	DQ678078	DQ678027	DQ677923	KT216532
Pleopunctum ellipsoideum	MFLUCC 19–0390	NR_171090	MK804517	MK804514	MK828510	-
Pleopunctum pseudoellipsoideum	MFLUCC 19–0391	MT627717	MK804518	-	MK828511	MT878455
Pleospora herbarum	CBS 191.86	MH861935	MH873624	GU238232	KC584731	KC584471
Preussia funiculata	CBS 659.74	-	GU301864	GU296187	GU349032	GU371799
Prosthemium orientale	MAFF 239509	AB554084	AB553748	AB553641	-	-
Pseudoastrosphaeriella bambusae	MFLUCC 11–0205	-	KT955475	KT955455	KT955437	KT955414
Pseudoastrosphaeriella thailandensis	MFLUCC 10–0553	-	KT955477	KT955456	KT955439	KT955411
Psiloglonium araucanum	CBS 112412	-	FJ161172	FJ161133	FJ161089	FJ161112
Pteridiospora javanica	MFLUCC 11–0159	-	KJ742940	KJ739607	KJ739605	-
Racodium rupestre	L346	GU067666	EU048583	EU048575	-	-
Racodium rupestre	L424	GU067669	EU048582	EU048577	-	-
Rimora mangrovei	JK 5246A	-	GU301868	GU296193	-	GU371759
Roccella fuciformis	Tehler 8171	AJ634015	FJ638979	-	-	FJ639038

Table 4.7 (continued)

Species name	Strains/Voucher no.	GenBank accession number				
		ITS	LSU	SSU	TEF1-α	RPB2
Roussoella nitidula	MFLUCC 11–0634	KJ474834	KJ474842	-	KJ474851	KJ474858
Salsuginea ramicola	KT 2597.1	-	GU479800	GU479767	GU479861	GU479833
Salsuginea ramicola	KT 2597.2	-	GU479801	GU479768	GU479862	GU479834
Schismatomma decolorans	Ertz 5003 (BR)	AY548808	NG_027622	NG_013155	-	-
Scorias spongiosa	CBS 325.33	-	KF901821	-	MN829417	KT216542
Sigarispora ravennica	MFLUCC 14–0005	NR_148079	KP698414	KP698415	-	-
Sporormia fimetaria	UPS:Dissing Gr.81.194	GQ203769	GQ203729	-	-	-
Teichospora parva	GKM 169 N	-	GU385165	-	GU327768	-
Teichospora striata	JK5678I	-	GU301813	GU296149	GU479852	GU371758
Teichospora tennesseensis	ANM 911	-	GU385207	-	GU327769	-
Tetraplosphaeria sasicola	MAFF 239677	MN937236	AB524631	AB524490	AB524838	-
Thyridaria acaciae	CBS 138873	KP004469	KP004497	-	-	-
Thyridaria broussonetiae	CBS 141481	KX650568	KX650568	KX650515	KX650539	KX650586
Thyridaria macrostomoides	GKM 1033	-	GU385190	-	GU327776	-
Thyridaria macrostomoides	GKM 1159	-	GU385185	-	GU327778	-
Thyridaria macrostomoides	GKM 224N	-	GU385191	-	GU327777	-
Torula herbarum	CBS 111855	KF443409	KF443386	KF443391	KF443403	KF443396
Torula herbarum	CPC 24114	KR873260	KR873288	-	-	-
Torula hollandica	CBS 220.69	KF443406	KF443384	KF443389	KF443401	KF443393

Table 4.7 (continued)

Species name	Strains/Voucher no.	GenBank accession number				
		ITS	LSU	SSU	TEF1-α	RPB2
Triplosphaeria maxima	MAFF 239682	NR_119407	AB524637	AB524496	AB524843	-
Tubeufia chiangmaiensis	MFLUCC 11–0514	KF301530	KF301538	KF301543	KF301557	-
Tubeufia javanica	MFLUCC 12–0545	KJ880034	KJ880036	KJ880035	KJ880037	-
Westerdykella ornata	CBS 379.55	MH857522	GU301880	GU296208	GU349021	GU371803
Wicklowia aquatica	F76-2	-	GU045445	GU266232	-	GU371813
Zopfia rhizophila	CBS 207.26	-	DQ384104	DQ384086	-	-

Note Abbreviations: AFTOL-ID: Assembling the Fungal Tree of Life; ANM: A.N. Miller; ATCC: American Type Culture Collection, Virginia, U.S.A; BBH: BIOTEC Bangkok Herbarium, Thailand; BCC: BIOTEC Culture Collection, Bangkok, Thailand; CABI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; CBS: Westerdijk Fungal Biodiversity Institute, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture Collection of Pedro Crous, Netherlands; Ertz: D. Ertz; GKM: G.K. Mugambi; GUFCC: Goa University Fungal Culture Collection, Goa, India; MTCC Microbial Type Culture Collection and Gene Bank, Chandigarh, India; GZCC: Guizhou Culture Collection, Guizhou Academy of Agricultural Sciences, Guiyang, China; IFRD: International Fungal Research and Development Centre, Chinese Academy of Forestry, Kunming, China; JK: J. Kohlmeyer; KT: K. Tanaka; KUMCC: Kunming Institute of Botany Culture Collection, Chinese Science Academy, Kunming, China; MAFF: Ministry of Agriculture, Forestry and Fisheries, Japan; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; NN: NovoNordisk culture collection (now Novozymes, Bagsvaerd, Denmark); SQUCC: Sultan Qaboos University Culture Collection, Sultan Qaboos University, Al-Khod 123, Oman; SMH: S.M. Huhndorf; Tehler: A. Tehler.

Table 4.8 *Alternaria* taxa used in the evolutionary and phylogenetic analyses and their corresponding GenBank numbers, including two outgroup taxa (*Pleospora tarda* CBS 714.68 and *Stemphylium herbarum* CBS 191.86)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria abundans	CBS 534.83 T	KC584581	KC584323	KC584448	JN383485	KC584154	KC584707	JN383503
Alternaria anthropophila	CBS 145587 T	-	-	LR537040	LR595906	LR537034	LR537046	-
Alternaria aconidiophora	FMR 17111 T	-	-	LR133967	NR_166229	LR133965	LR133968	-
Alternaa agerati	CBS 117221	-	-	KJ718272	KJ718098	KJ717953	KJ718447	KJ718618
Alternaria agripestis	CBS 577.94 T	-	-	JQ905196	NR_136070	JQ905169	KJ718448	JQ905112
Alternaria allii	CBS 107.28	-	-	KJ718274	NR_136071	KJ717954	KJ718449	KJ718620
Alternaria allii	CBS 121345 T	-	-	KJ718278	KJ718104	KJ717958	KJ718453	KJ718624
Alternaria alstroemeriae	CBS 118808	KP124917	KP124447	KP124764	KP124296	KP124153	KP125071	KP123845
Alternaria alstroemeriae	CBS 118809 T	NG_063029	NG_069882	KP124765	NR_163686	KP124154	KP125072	MH084526
Alternaria alternantherae	CBS 124392	KC584506	KC584251	KC584374	KC584179	KC584096	KC584633	KP123846
Alternaria alternariacida	CBS 105.51 T	-	-	KJ718279	NR_136072	KJ717959	KJ718454	KJ718625
Alternaria alternariae	CBS 126989 T	KC584604	KC584346	KC584470	AF229485	AY278815	KC584730	-
Alternaria alternariana	CBS 119396 T	-	-	-	-	JQ646289	JQ672419	JQ646376
Alternaria alternata	AFTOL-ID 1610	DQ678031	DQ678082	DQ677980	-	-	DQ677927	-
Alternaria alternata	CBS 102595	-	MH874391	-	MH862795	-	-	-
Alternaria alternata	CBS 102596	KP124950	MH874392	KP124796	MH862796	KP124183	KP125104	KP123877
Alternaria alternata	CBS 102599	KP124952	MH874395	KP124798	MH862799	KP124185	KP125106	KP123879
Alternaria alternata	CBS 102600	KP124953	MH874396	KP124799	MH862800	KP124186	KP125107	KP123880
Alternaria alternata	CBS 102602	KP124954	MH877754	KP124800	KP124332	KP124187	KP125108	KP123881
Alternaria alternata	CBS 102603	KP124955	KP124485	KP124801	KP124333	KP124188	KP125109	KP123882

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria alternata	CBS 102604	KP124956	MH874399	KP124802	MH862803	-	KP125110	-
Alternaria alternata	CBS 113013	KP124963	KP124493	KP124809	KP124341	KP124195	KP125117	KP123889
Alternaria alternata	CBS 113014	KP124964	KP124494	KP124810	KP124342	KP124196	KP125118	KP123890
Alternaria alternata	CBS 113015	KP124965	KP124495	KP124811	KP124343	KP124197	KP125119	KP123891
Alternaria alternata	CBS 113054	KP124968	KP124498	KP124814	KP124346	KP124200	KP125122	KP123894
Alternaria alternata	CBS 117130	KP124976	KP124506	KP124822	KP124354	KP124208	KP125130	KP123902
Alternaria alternata	CBS 117143	KP124977	KP124507	KP124823	KP124355	KP124209	KP125131	KP123903
Alternaria alternata	CBS 118812	-	MH877759	-	-	-	-	KP123905
Alternaria alternata	CBS 119399	KP124983	KP124513	KP124829	KP124361	-	KP125137	KP123910
Alternaria alternata	CBS 120829	KP124986	KP124516	KP124832	KP124364	KP124216	KP125140	KP123912
Alternaria alternata	CBS 121336	KP124987	KP124517	KP124833	MH863106	-	KP125141	-
Alternaria alternata	CBS 121344	KP124988	KP124518	KP124834	KP124365	KP124217	KP125142	KP123913
Alternaria alternata	CBS 121346	KP124989	KP124519	KP124835	KP124366	KP124218	KP125143	KP123914
Alternaria alternata	CBS 121492	KP124994	KP124524	KP124840	KP124370	KP124222	KP125148	KP123918
Alternaria alternata	CBS 124277	KP124997	KP124527	KP124843	KP124373	KP124225	KP125151	KP123921
Alternaria alternata	CBS 124278	KP124998	KP124528	KP124844	KP124374	KP124226	KP125152	KP123922
Alternaria alternata	CBS 175.52	KC584227	KC584320	KC584577	KC584703	KC584445	KC584151	KP123857
Alternaria alternata	CBS 916.96 T	KC584507	DQ678082	KC584375	AF347031	AY278808	KC584634	-
Alternaria alternata	EGS 34-015	-	-	-	-	-	-	KP275690
Alternaria alternata	YL 1	MN072922	MN072918	MN072920	MK860132	MN072916	MN072924	-
Alternaria alternata	YL 2	MN072923	MN072919	MN072921	MN044764	MN072917	MN072925	-

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria anagallidis	CBS 101004	-	-	KJ718281	KJ718107	KJ717960	KJ718455	KJ718627
Alternaria anagallidis	CBS 117128	-	-	KJ718282	KJ718108	KJ717961	KJ718456	KJ718628
Alternaria anodae	PPRI 12376	-	-	KJ718284	KJ718110	KJ717963	KJ718458	KJ718630
Alternaria aragakii	CBS 594.93 T	-	MH874093	JQ905187	MH862445	JQ646359	KJ718459	JQ905107
Alternaria arborescens	CBS 101.13	KP125016	KP124546	KP124862	KP124392	KP125170	KP124244	KP123940
Alternaria arborescens	CBS 105.24	KP125017	KP12454	KP124863	KP124393	KP125171	KP124245	KP123941
Alternaria arborescens	CBS 105.49	KP125020	KP124550	KP124866	KP124396	KP125174	KP124248	KP123944
Alternaria arborescens	CBS 108.41	KP125018	KP124548	KP124864	KP124394	KP125172	KP124246	KP123942
Alternaria arborescens	CBS 113.41	KP125019	KP124549	KP124865	KP124395	KP125173	KP124247	KP123943
Alternaria arborescens	CBS 750.68	KP125021	KP124551	KP124868	KP124398	KP125176	KP124250	KP123945
Alternaria arborescens	CBS 102605 T	KC584509	KC584253	KC584377	AF347033	AY278810	KC584636	AY563303
Alternaria arborescens	CBS 109730	KP125022	KP124552	KP124869	KP124399	KP125177	KP124251	KP123946
Alternaria arborescens	CBS 119544	NG_063030	NG_069254	KP124878	MH863062	KP125186	JQ646321	KP123955
Alternaria arborescens	CBS 119545	KP125032	KP124562	KY392798	KP124409	KP125187	KP124260	KP123956
Alternaria arborescens	CBS 123267	KP125035	KP124565	KP124882	KP124412	KP125190	KP124263	KP123959
Alternaria arbusti	CBS 596.93 T	-	MH874095	-	MH862447	FJ214806	FJ214902	-
Alternaria arbusti	EGS 91-129	-	-	-	JQ693643	JQ693621	-	-
Alternaria arctoseptata	IT2088_A T	MZ621874	MZ621948	OK236655	-	0K236608	OK236702	OK236755
Alternaria arctoseptata	IT2088_B	MZ621875	MZ621949	OK236656	-	OK236609	OK236703	OK236756
Alternaria argyranthemi	CBS 116530 T	KC584510	KC584254	KC584378	KC584181	KC584098	KC584637	MG647616
Alternaria argyroxiphii	CBS 117222 T	KJ718112	-	KJ718286	NR_136074	-	KJ718460	KJ718632

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria argyroxiphii	PPRI 11848	-	-	KJ718287	KJ718113	KJ717965	KJ718461	KJ718633
Alternaria argyroxiphii	PPRI 11971	-	-	KJ718288	KJ718114	KJ717966	KJ718462	KJ718634
Alternaria arrhenatheri	CBS 133068 T	-	MH877516	-	-	-	-	-
Alternaria arrhenatheri	LEP 140372	-	-	-	JQ693680	JQ693629	-	-
Alternaria arundinis	IT2250_A T	MZ621884	MZ621958	-	MZ622009	-	OK236712	-
Alternaira arundinis	IT2250_B	MZ621891	MZ621965	-	MZ622016	-	OK236713	-
Alternaira arundinis	IT2250_C	MZ621892	MZ621966	-	MZ622017	-	OK236714	-
Alternaria aspera	CBS 115269 T	KC584607	KC584349	KC584474	KC584242	KC584166	KC584734	KF533899
Alternaria atra	CBS 195.67 T	KC584608	KC584350	KC584475	AF229486	KC584167	KC584735	MG025842
Alternaria atrobrunnea	FMR 16868 T	-	-	LR537044	LR537033	LR537051	LR537039	-
Alternaria axiaeriisporifera	CBS 118715 T	NG_062874	NG_069127	KC584381	NR_136037	KC584101	KC584640	-
Alternaria azadirachtae	CBS 116444	-	-	KJ718289	KJ718115	KJ717967	KJ718463	KJ718635
Alternaria azadirachtae	CBS 116445 T	-	-	KJ718290	NR_136075	KJ717968	KJ718464	KJ718636
Alternaria bataticola	CBS 117096	-	-	KJ717971	KJ718294	KJ718120	KJ718468	KJ718639
Alternaria bataticola	CBS 531.63 T	-	-	KJ718291	NR_136076	JQ646349	KJ718465	JQ646433
Alternaria betae-kenyensis	CBS 118810 T	NG_063032	NG_069256	JQ905180	NR_136118	JQ905161	KP125197	JQ905104
Alternaria carthamicola	CBS 117092 T	-	-	KJ718308	NR_136079	KJ717984	KJ718482	KJ718652
Alternaria cassiae	CBS 116119 T	-	-	KJ718310	KJ718136	KJ717986	KJ718484	KJ718654
Alternaria cassiae	CBS 117369	-	-	KJ718312	KJ718138	KJ717988	KJ718486	KJ718656
Alternaria cassiae	CBS 478.81	-	MH873122	KJ718309	MH861368	KJ717985	KJ718483	KJ718653
Alternaria cerealis	CBS 119544 T	NG_063030	MH874614	KP124878	MH863062	-	KP125186	KP123955

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria blumeae	CBS 117215	-	-	KJ718299	KJ718125	KJ717976	KJ718473	KJ718644
Alternaria blumeae	CBS 117364 T	-	-	KJ718300	NR_136077	-	KJ718474	-
Alternaria baoshanensis	H-50_A T	MZ621878	MZ621952	OK236659	MZ622003	OK236613	OK236706	OK236760
Alternaria baoshanensis	H-50_B	MZ621879	MZ621953	OK236660	MZ622004	OK236612	OK236707	OK236759
Alternaria bornmuelleri	DAOM231361 T	KC584624	KC584366	KC584491	FJ357317	FJ357305	KC584751	JN383516
Alternaria botryospora	CBS 478.90 T	KC584594	KC584336	KC584461	AY278844	AY278831	KC584720	AY563324
Alternaria botryospora	EGS 39-099	-	-	-	-	-	JQ672401	-
Alternaria botrytis	CBS 197.67 T	KC584609	KC584351	KC584476	KC584243	KC584168	KC584736	LC481637
Alternaria brassicae	CBS 116528	KC584514	KC584258	KC584382	KC584185	KC584102	KC584641	-
Alternaria brassicae-pekinensis	CBS 121493 T	KC584611	KC584353	KC584478	KC584244	KC584170	KC584738	KF533892
Alternaria brassicicola	CBS 118699	KC584515	KC584259	KC584383	JX499031	KC584103	KC584642	-
Alternaria breviconidiophora	IT3308_A T	MZ621870	MZ621944	OK236651	MZ621997	OK236604	OK236698	OK236751
Alternaria breviconidiophora	IT3308_B	MZ621871	MZ621945	OK236652	MZ621998	OK236605	OK236699	OK236752
Alternaria breviramosa	CBS 121331 T	KC584574	KC584318	KC584442	FJ839608	KC584148	KC584700	-
Alternaria brevirostra	IT2028	MZ621890	MZ621963	OK236667	MZ022012	OK236620	OK236718	
Alternaria brevirostra	IT2195_A T	MZ621889	-	OK236666	MZ622015	OK236619	OK236717	-
Alternaria broccoli-italicae	CBS 118485 T	-	-	LR134194	-	-	LR134262	-
Alternaria burnsii	CBS 107.38 T	NG_063033	NG_069257	JQ646457	NR_136119	JQ646305	KP125198	JQ646388
Alternaria burnsii	CBS 110.50	KP125044	KP124574	KP124890	KP124421	KP124271	KP125199	KP123968
Alternaria burnsii	CBS 879.95	KP125045	KP124575	KP124891	KP124422	KP124272	KP125200	KP123969
Alternaria burnsii	CBS 118816	KP125046	KP124576	KP124892	KP124423	KP124273	KP125201	KP123970

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria burnsii	CBS 118817	KP125047	KP124577	KP124893	KP124424	KP124274	KP125202	KP123971
Alternaria burnsii	CBS 130264	KP125048	KP124578	KP124894	KP124425	KP124275	KP125203	KP123972
Alternaria caespitosa	CBS 177.80 T	KC584625	KC584367	KC584492	MH861255	KC584178	KC584752	-
Alternaria calendulae	CBS 116439	-	-	KJ718303	KJ718129	KJ717979	KJ718477	KJ718646
Alternaria calendulae	CBS 224.76 T	-	-	JQ646518	NR_136078	JQ646330	KJ718475	JQ646414
Alternaria calycipyricola	CBS 121545 T	KC584516	KC584260	KC584384	KC584186	KC584104	KC584643	JQ646381
Alternaria californica	CBS 119409 T	-	-	LR134181	NR_136021	-	KY352500	-
Alternaria capsici-annui	CBS 504.74	KC584517	KC584261	KC584385	KC584187	KC584105	KC584644	-
Alternaria caricis	CBS 480.90 T	KC584600	KC584342	KC584467	AY278839	AY278826	KC584726	AY563321
Alternaria carotiincultae	CBS 109381 T	KC584518	KC584262	KC584386	KC584188	KC584106	KC584645	AY563287
Alternaria carthami	CBS 116440 T	-	-	KJ718306	KJ718132	KJ717982	KJ718480	KJ718650
Alternaria carthami	CBS 635.80	-	MH873065	KJ718305	KJ718131	KJ717981	KJ718479	KJ718649
Alternaria catananches	CBS 137456 T	-	-	KJ718313	NR_136080	KJ717989	KJ718487	KJ718657
Alternaria centaureae	CBS 116446 T	-	-	KJ718314	NR_136081	KJ717990	KJ718488	KJ718658
Alternaria cerasidanica	CBS 121923 T	-	-	LR135746	LR135744	LR135747	LR135745	-
Alternaria cesenica	MFLUCC 13-0450 T	KP711385	KP711384	-	KP711383	-	KP711386	-
Alternaria cetera	CBS 121340 T	KC584573	KC584317	KC584441	JN383482	AY562398	KC584699	-
Alternaria chartarum	CBS 200.67 T	KC584614	KC584356	KC584481	AF229488	KC584172	KC584741	LC481629
Alternaria cheiranthi	CBS 109384	KC584519	KC584263	KC584387	AF229457	KC584107	KC584646	JQ905106
Alternaria cheiranthi	CBS 96830	KC584519	KC584263	KC584387	MW487228	KC584646	KC584107	AY563290
Alternaria chlamydospora	CBS 491.72 T	KC584520	KC584264	KC584388	KC584189	KC584108	KC584647	JQ646367

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria chlamydosporifera	FMR17360 T	-	-	LR133926	NR_166228	LR133927	LR133929	-
Alternaria chlamydosporigena	CBS 341.71	KC584584	KC584326	KC584451	KC584231	KC584156	KC584710	-
Alternaria cichorii	CBS 102.33 T	-	-	JQ646504	NR_136082	JQ646340	KJ718489	JQ646424
Alternaria cinerariae	CBS 116495	KC584521	KC584265	KC584389	KC584190	KC584109	KC584648	-
Alternaria cirsinoxia	CBS 113261 T	-	-	JQ646499	NR_136083	JQ646351	KJ718491	JQ646435
Alternaria citrullicola	CBS 103.32 T	-	-	KJ718318	NR_136084	KJ717994	KJ718492	KJ718662
Alternaria concatenata	CBS 120006 T	KC584613	KC584355	KC584480	KC584246	AY762950	KC584740	EU684149
Alternaria conidiophore	CBS 137457 T	-	-	-	KJ718145	KJ718493	KJ717995	KJ718663
Alternaria conjuncta	CBS 196.86 T	KC584522	KC584266	KC584390	FJ266475	AY562401	KC584649	AY563281
Alternaria conoidea	CBS 132.89	KC584585	KC584327	KC584452	AF348226	FJ348227	KC584711	FJ348228
Alternaria consortialis	CBS 104.31 T	KC584615	KC584357	KC584482	KC584247	KC584173	KC584742	FJ266509
Alternaria crassa	CBS 110.38 T	-	-	KJ718320	NR_136086	KJ717997	KJ718495	KJ718665
Alternaria cucumerina	CBS 116114 T	-	-	KJ718326	KJ718153	KJ718000	KJ718501	KJ718668
Alternaria cucurbitae	CBS 483.81	KC584616	KC584358	KC584483	FJ266483	AY562418	KC584743	-
Alternaria cumini	CBS 121329 T	KC584523	KC584267	KC584391	KC584191	KC584110	KC584650	-
Alternaria curvata	FMR 16901 T	-	-	LR133901	LR133898	LR133899	LR133902	-
Alternaria cyamopsidis	CBS 117219	-	-	KJ718330	KJ718157	KJ718004	KJ718505	KJ718672
Alternaria cyamopsidis	CBS 364.67	-	MH870694	KJ718329	MH858995	KJ718003	KJ718504	KJ718671
Alternaria dactylidicola	MFLUCC 15-0466 T	NG_063635	NG_069434	KY750720	NR_151852	-	-	-
Alternaria dauci	CBS 101592	-	-	KJ718335	KJ718162	KJ718009	KJ718510	KJ718677

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria dauci	CBS 111.38 T	-	-	KJ718331	NR_136087	KJ718005	KJ718506	KJ718673
Alternaria dauci	CBS 117099	-	-	KJ718337	KJ718164	KJ718011	KJ718512	KJ718679
Alternaria daucicaulis	CBS 119398 T	-	-	LR134177	NR_136027	JQ646294	LR134241	-
Alternaria dennisii	CBS 110533	KC584586	KC584328	KC584453	KC584232	KC584157	KC584712	-
Alternaria dennisii	CBS 476.90 T	KC584587	KC584329	KC584454	JN383488	JN383469	KC584713	JN383505
Alternaria deserticola	CBS 110799 T	-	-	KJ718424	NR_136107	KJ718077	KJ718595	KJ718755
Alternaria dianthicola	CBS 116491	KC584526	KC584270	KC584394	KC584194	KC584113	KC584653	-
Alternaria dichondrae	CBS 199.74 T	-	-	JQ646493	NR_136088	JQ646357	KJ718514	JQ646441
Alternaria dichondrae	CBS 200.74	-	-	KJ718340	KJ718167	KJ718012	KJ718515	KJ718681
Alternaria didymospora	CBS 766.79	KC584588	KC584330	KC584455	FJ357312	FJ357300	KC584714	-
Alternaria divaricatae	YZU151055 T	-	-	MW579316	-	-	MW579314	-
Alternaria divaricatae	YZU151059	-	-	MW579317	-	-	MW579315	-
Alternaria doliconidium	MFLUCC 14-0020	MZ632684	OK333361	-	OK332869	-	-	-
Alternaria doliconidium	HKAS 100840	NG_065142	NG_069551	-	NR_158361	-	-	-
Alternaria doliconidium	MFLUCC 17-0263 T	MG829094	MG828980	-	MG828864	-	-	-
Alternaria echinaceae	CBS 116117 T	-	-	KJ718343	NR_136089	KJ718015	KJ718518	KJ718684
Alternaria echinaceae	CBS 116118	-	-	KJ718344	KJ718171	KJ718016	KJ718519	KJ718685
Alternaria eichhorniae	CBS 119778	KP125050	KP124580	KP124896	KP124426	KP124277	KP125205	-
Alternaria eichhorniae	CBS 489.92 T	NG_063034	KP124579	KP124895	-	KP124276	KP125204	KP123973
Alternaria elegans	CBS 109159 T	KC584527	KC584271	KC584395	KC584195	KC584114	KC584654	GQ180092
Alternaria elegans	EGS 45.072	-	-	-	-	GQ180076	-	GQ180092

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria ellipsoiconidia	IT2086_A T	MZ621862	MZ621936	OK236643	MZ621989	OK236596	OK236690	OK236743
Alternaria ellipsoiconidia	IT2086_B	MZ621863	MZ621937	OK236644	MZ621990	OK236597	OK236691	OK236744
Alternaria ellipsoiconidia	IT2086_C	MZ621864	MZ621938	OK236645	MZ621991	OK236598	OK236692	OK236745
Alternaria embellisia	CBS 339.71	KC584582	KC584324	KC584449	KC584230	KC584155	KC584708	-
Alternaria eryngii	CBS 121339	KC584529	KC584273	KC584397	JQ693661	AY562416	KC584656	-
Alternaria ethzedi	CBS 197.86 T	NG_062882	MH873630	KC584398	MH861941	-	KC584657	-
Alternaria eureka	CBS 193.86 T	KC584589	KC584331	KC584456	JN383490	JN383471	KC584715	JN383507
Alternaria eupatoriicola	IT3518_A T	MZ621855	MZ621929	OK236636	MZ621982	OK236589	OK236683	OK236736
Alternaria eupatoriicola	IT3518_B	MZ621856	MZ621930	OK236637	MZ621983	OK236589	OK236684	OK236737
Alternaria euphorbiicola	CBS 119410	-	-	KJ718346	KJ718173	KJ718018	KJ718521	-
Alternaria euphorbiicola	CBS 133874	-	-	KJ718347	KJ718174	KJ718019	KJ718522	KJ718687
Alternaria euphorbiicola	CBS 198.86	-	-	KJ718345	KJ718172	KJ718017	KJ718520	KJ718686
Alternaria falcata	IT2079_A T	MZ621865	MZ62139	OK236649	MZ621992	OK236599	OK236693	OK236746
Alternaria falcata	IT2079_B	MZ621866	MZ621940	OK236650	MZ621993	OK236600	OK236694	OK236747
Alternaria fimeti	FMR 17110 T	-	-	LR133923	LR133920	LR133921	LR133922	-
Alternaria forlicesenensis	MFLUCC 13-0456 T	NG_063639	NG_069454	-	NR_151853	-	-	-
Alternaria frumenti	CBS 119401 T	-	-	LR134172	NR_136028	JQ646295	LR134370	JQ646378
Alternaria gaisen	CBS 118488 T	KP125051	KP124581	KP124897	KP124427	KP124278	KP125206	KP123975
Alternaria gaisen	CBS 632.93	KC584531	KC584275	KC584399	KC584197	KC584116	KC584658	KP123974
Alternaria gaisen	CPC 25268	KP125052	KP124582	KP124898	KP124428	KP124279	KP125207	KP123976
Alternaria geophila	CBS 101.13 T	KP125016	KP124546	KP124862	KP124392	KP124244	KP125170	KP123940

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria glehniae	YZU161149 T	-	-	MK279394	MK279385	MK279392	-	-
Alternaria gossypina	CBS 100.23	KP125053	KP124583	KP124899	KP124429	KP124280	KP125208	KP123977
Alternaria gossypina	CBS 104.32 T	KP125054	KP124584	KP124900	KP124430	JQ646312	KP125209	JQ646395
Alternaria gossypina	CBS 107.36	KP125055	KP124585	KP124901	KP124431	-	KP125210	-
Alternaria gossypina	CBS 102597	KP125056	MH874393	KP124902	MH862797	KP124281	KP125211	KP123978
Alternaria gossypina	CBS 102601	KP125057	MH874397	KP124903	MH862801	KP124282	KP125212	KP123979
Alternaria graminicola	CBS 119400 T	-	-	LR134180	NR_136024	-	LR134249	-
Alternaria grandis	CBS 109158 T	-	-	JQ646500	-	JQ646341	-	JQ646425
Alternaria grandis	CBS 116695	-	-	KJ718416.	KJ718241	KJ718070	KJ718587	KJ718748
Alternaria guarroi	FMR 16556 T	-	-	LR537045	LR537031	LR537050	LR53703	-
Alternaria gypsophilae	CBS 107.41 T	KC584533	KC584277	KC584401	KC584199	KC584118	KC584660	KJ718688
Alternaria hampshirensis	MFLUCC 17-0783 T	NG_063671	NG_069552	MG829247	NR_157503	-	-	-
Alternaria helianthiinficiens	CBS 117370	KC584534	KC584278	KC584402	KC584200	KC584119	KC584661	-
Alternaria helianthiinficiens	CBS 208.86 T	KC584535	KC584279	KC584403	JX101649	KC584120	EU130548	-
Alternaria heterospora	CBS 123376 T	KC584621	KC584363	KC584488	KC584248	KC584176	KC584748	-
Alternaria hordeiaustralica	CBS 119402 T	-	-	JQ646497	NR_136018	JQ646283	LR134243	-
Alternaria hordeicola	EGS 50-184	-	-	JQ693642	NR_136019	JQ672425	JQ646284	JQ646372
Alternaria humuli	CBS 119404 T	-	-	LR134174	NR_136026	JQ646293	LR134199	-
Alternaria hyacinthi	CBS 416.71 T	KC584590	KC584332	KC584457	KC584233	KC584158	KC584716	FJ266506
Alternaria incomplexa	CBS 121330 T	-	-	LR134185	NR_136031	JQ646287	LR134250	JQ646374
Alternaria indefessa	CBS 536.83 T	KC584591	KC584333	KC584458	KC584234	KC584159	KC584717	AY563323

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria infectoria	CBS 210.86 T	KC584536	KC584280	KC584404	DQ323697	AY278793	KC584662	FJ266502.1
Alternaria inflata	FMR 16477	-	-	-	MT109376	-	LR133938	MT108481
Alternaria intercepta	CBS 119406 T	-	-	LR134170	NR_135957	FJ214831	FJ214927	JQ646380
Alternaria ipomoeae	CBS 219.79 T	-	-	KJ718348	NR_136090	KJ718020	KJ718523	KJ718689
Alternaria ipomoeae	PPRI 8988	-	-	KJ718349	KJ718176	KJ718021	KJ718524	KJ718690
Alternaria iridiaustralis	CBS 118404	KP125058	KP124588	KP124904	KP124434	KP124283	KP125213	KP123980
Alternaria iridiaustralis	CBS 118486 T	NG_063035	NG_069258	KP124905	NR_136120	KP124284	KP125214	KP123981
Alternaria iridiaustralis	CBS 118487	KP125060	KP124590	KP124906	KP124436	KP124285	KP125215	KP123982
Alternaria italica	KUMCC 17-0090	-	-	-	MG764018	-	-	-
Alternaria italica	MFLUCC 14-0421 T	-	MG818319	MG859737	MG764017	-	-	-
Alternaria jacinthicola	CBS 133751 T	KP125062	KP124592	KP124908	KP124438	KP124287	KP125217	KP123984
Alternaria jacinthicola	CBS 878.95	KP125061	KP124591	KP124907	KP124437	KP124286	KP125216	KP123983
Alternaria jacinthicola	CPC 25267	KP125063	KP124593	KP124909	KP124439	KP124288	KP125218	KP123985
Alternaria jesenskae	CBS 133855 T	-	-	KJ718350	NR_136091	KJ718022	KJ718525	KJ718691
Alternaria jonica	CBS 118390	KC584537	KC584281	KC584405	KC584201	KC584121	KC584663	-
Alternaria kordkuyana	IRAN 16888F T	-	-	-	MF033843	MF033826	-	-
Alternaria kulundii	M313 T	KJ443087	KJ443132	KJ443176	KJ443262	KJ649618	-	-
Alternaria lathyri	IT1640_A T	MZ621847	MZ621921	OK236628	MZ621974	OK236581	OK236675	OK236728
Alternaria lathyri	IT1640_B	MZ621848	MZ621922	OK236629	MZ621975	OK236582	OK236676	OK236729
Alternaria lawrencei	FMR 17004 T	-	-	LR133911	NR_166227	LR133908	LR133912	-
Alternaria leucanthemi	CBS 421.65 T	KC584605	KC584347	KC584472	KC584240	KC584164	KC584732	-

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria leucanthemi	CBS 422.65	KC584606	KC584348	KC584473	KC584241	KC584165	KC584733	-
Alternaria limaciformis	CBS 481.81 T	KC584539	KC584283	KC584407	KC584203	KC584123	KC584665	JQ646368
Alternaria limicola	CBS 117360	-	-	KJ718352	KJ718179	KJ718023	KJ718527	-
Alternaria limicola	CBS 483.90 T	-	-	KJ718351	KJ718178	JQ646329	KJ718526	JQ646413
Alternaria linariae	CBS 109156	-	-	KJ718356	KJ718183	-	KJ718531	-
Alternaria linariae	CBS 109161	-	-	KJ718357	KJ718184	-	KJ718532	-
Alternaria linariae	CBS 109164	-	-	KJ718358	KJ718185	-	-	-
Alternaria linariae	CBS 116438	-	-	KJ718359	KJ718186	KJ718027	KJ718533	KJ718695
Alternaria longipes	CBS 113.35	KP125064	KP124594	KP124910	KP124440	KP124289	KP125219	KP123986
Alternaria longipes	CBS 121332	KP125067	KP124597	KP124913	KP124443	KP124292	KP125222	KP123989
Alternaria longipes	CBS 121333	KP125068	KP124598	KP124914	KP124444	KP124293	KP125223	KP123990
Alternaria longipes	CBS 539.94	KP125065	KP124595	KP124911	KP124441	KP124290	KP125220	KP123987
Alternaria longipes	CBS 540.94	KC584541	KC584285	KC584409	-	-	KC584667	-
Alternaria longipes	CBS 917.96	KP125066	KP124596	KP124912	KP124442	KP124291	KP125221	KP123988
Alternaria macilenta	IT2076_A T	MZ621845	MZ621919	OK236626	MZ621972	OK236579	OK236673	OK236726
Alternaria macilenta	IT2076_B	MZ621846	MZ621920	OK236627	MZ621973	OK236580	OK236674	OK236727
Alternaria macroconidia	IT1756_A T	MZ621876	MZ621950	OK236657	MZ622001	OK236610	OK236704	OK236757
Alternaria macroconidia	IT1756_B	MZ621876	MZ621951	OK236658	MZ622002	OK236611	OK236705	OK236758
Alternaria macrospora	CBS 117228 T	KC584542	KC584286	KC584410	KC584204	KC584124	KC584668	KJ718702
Alternaria malorum	CBS 135.31	-	-	JQ646481	-	JQ646278	-	JQ646369
Alternaria merytae	CBS 119403 T	-	-	LR134119	NR_136025	JQ646292	LR134198	-

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria metachromatica	CBS 553.94 T	-	-	-	NR_077212	FJ214835	FJ214931	-
Alternaria minimispora	H-14_A T	MZ621853	MZ621927	OK236634	MZ621980	OK236587	OK236681	OK236734
Alternaria minimispora	H-14_B	MZ621854	MZ621928	OK236635	MZ621981	OK236588	OK236682	OK236735
Alternaria montanica	CBS 121343 T	-	-	KJ718367	NR_136093	KJ718033	KJ718541	KJ718703
Alternaria montsantina	FMR 17060 T	-	-	LR133918	LR133913	LR133915	LR133919	-
Alternaria multirostrata	CBS 712.68 T	-	NG_069737	KJ718368	NR_136094	JQ646362	-	JQ646446
Alternaria multirostrata	CBS 713.68	-	MH870930	KJ718369	KJ718196	KJ718034	KJ718542	KJ718705
Alternaria muriformispora	IT2101_A T	MZ621849	MZ621923	OK236630	MZ621976	OK236583	OK236677	OK236730
Alternaria muriformispora	IT2101_B	MZ621850	MZ621924	OK236631	MZ621977	OK236584	OK236678	OK236731
Alternaria murispora	MFLU 14-0758 T	NG_065060	NG_069261	-	NR_137964	-	-	-
Alternaria neoipomoeae	PPRI 11845 T	-	-	KJ718371	NR_136095	KJ718036	KJ718544	KJ718707
Alternaria neoipomoeae	PPRI 11847	-	-	KJ718372	KJ718199	KJ718037	KJ718545	KJ718708
Alternaria nepalensis	CBS 118700 T	KC584546	KC584290	KC584414	KC584207	KC584126	KC584672	-
Alternaria nitrimali	CBS 109163 T	-	-	KJ718374	NR_136096	JQ646358	KJ718547	JQ646442
Alternaria nobilis	CBS 116490	KC584547	KC584291	KC584415	KC584208	KC584127	KC584673	JQ646385
Alternaria nodulariconidiophora	IT2625A_1 T	MZ621885	MZ621959	-	MZ622010	-	OK236715	-
Alternaria nodulariconidiophora	IT2625A_2	MZ621886	MZ621960	-	MZ622011	-	OK236716	-
Alternaria novae-guineensis	CBS 116120 T	-	-	KJ718375	NR_136097	KJ718039	KJ718548	KJ718711
Alternaria novae-guineensis	PPRI 12171	-	-	KJ718376	KJ718203	KJ718040	KJ718549	KJ718712
Alternaria novae-zelandiae	CBS 119405 T	-	-	LR134120	NR_136029	JQ646296	LR134197	JQ646379
Alternaria obclavata	CBS 124120 T	KC584575	FJ839651	KC584443	KC584225	KC584149	KC584701	-

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria oblongoellipsoidea	IT2102_A T	MZ621840	MZ621914	OK236621	MZ621967	OK236574	OK236668	OK236721
Alternaria oblongoellipsoidea	IT2102_B	MZ621841	MZ621915	OK236622	MZ621968	OK236575	OK236669	OK236722
Alternaria obpyriconidia	IT1688_A T	MZ621851	MZ621925	OK236633	MZ621978	OK236585	OK236680	OK236732
Alternaria obpyriconidia	IT1688_B	MZ621852	MZ621926	OK236632	MZ621979	OK236586	OK236679	OK236733
Alternaria obtecta	CBS 117367	-	-	KJ718377	KJ718204	KJ718041	KJ718550	KJ718713
Alternaria obtecta	CBS 134278	-	-	KJ718378	KJ718205	KJ718042	KJ718551	KJ718714
Alternaria omanensis	SQUCC13580 T	MK878559	MK878556	MK880893	MK878562	MK880899	MK880896	-
Alternaria omanensis	SQUCC15560	MK878560	MK878557	MK880894	MK878563	MK880900	MK880897	-
Alternaria omanensis	SQUCC15561	MK878561	MK878558	MK880895	MK878564	MK880901	MK880898	-
Alternaria oregonensis	CBS 542.94 T	NG_062894	NG_069137	KC584416	NR_135935	FJ214849	KC584674	-
Alternaria orobanches	IT1997_A T	MZ621882	MZ621956	-	MZ622007	-	OK236710	OK236763
Alternaria orobanches	IT1997_B	MZ621883	MZ621957	-	MZ622008	-	OK236711	OK236764
Alternaria oudemansii	CBS 114.07 T	KC584619	KC584361	KC584486	FJ266488	KC584175	KC584746	FJ266514
Alternaria ovoidea	IT 1656_A T	MZ621880	MZ621954	OK236661	MZ622005	OK236614	OK236708	OK236761
Alternaria ovoidea	IT 1656_B	MZ621881	MZ621955	OK236662	MZ622006	OK236615	OK236709	OK236762
Alternaria panax	CBS 482.81	KC584549	KC584293	KC584417	KC584209	KC584128	KC584675	-
Alternaria panax	EGS 48.149	LC440600	-	JQ585701	JX418343	KP877993	AB862972	KF516492
Alternaria papavericola	CBS 116606 T	KC584579	KC584321	KC584446	FJ357310	FJ357298	KC584705	JN383501
Alternaria paralinicola	CBS 116652 T	-	-	KJ718379	NR_136098	KJ718043	KJ718552	KJ718715
Alternaria parvicaespitosa	LEP 014858 T	-	-	-	MF033859	-	MF033842	KJ920981
Alternaria passiflorae	CBS 116333	-	-	KJ718384	KJ718211	KJ718046	KJ718557	KJ718719

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria passiflorae	CBS 630.93 T	-	-	KJ718383	KJ718210	KJ718556	JQ646352	KJ718718
Alternaria penicillata	CBS 116608 T	KC584572	KC584316	KC584440	FJ357311	FJ357299	KC584698	-
Alternaria penicillata	CBS 116607	KC584580	KC584322	KC584447	KC584229	KC584153	KC584706	-
Alternaria perpunctulata	CBS 115267 T	KC584550	KC584294	KC584418	KC584210	KC584129	KC584676	JN383513
Alternaria petroselini	CBS 112.41 T	KC584551	KC584295	KC584419	KC584211	KC584130	KC584677	-
Alternaria petroselini	EGS 25-198 T	NG_064862	NG_069140	KC584426	AF229455	JQ672453	AY278800	FJ266504
Alternaria petuchovskii	M304 T	KJ443079	KJ443124	KJ443170	KJ443254	KJ649616	-	-
Alternaria photistica	CBS 212.86 T	KC584552	KC584296	KC584420	KC584212	KC584131	KC584678	AY563282
Alternaria phragmiticola	IT2630_A T	MZ621867	MZ621941	OK236649	MZ621994	OK236602	OK236696	OK236749
Alternaria phragmiticola	IT2630_B	MZ621868	MZ621942	OK236650	MZ621995	OK236603	OK236697	OK236750
Alternaria phragmiticola	IT2630_C	MZ621869	MZ621943	OK236648	MZ621996	OK236601	OK236695	OK236748
Alternaria phragmospora	CBS 274.70 T	KC584595	KC584337	KC584462	JN383493	JN383474	KC584721	JN383509
Alternaria phytolaccae	IT2279_A T	MZ621887	MZ621961	OK236663	MZ622013	OK236616	OK236719	OK236765
Alternaria phytolaccae	IT2279_B	MZ621888	MZ621962	OK236664	MZ622014	OK236617	OK236720	OK236766
Alternaria pipionipisi	CBS 116115 T	-	-	KJ718387	NR_136099	KJ718049	KJ718560	KJ718722
Alternaria poaceicola	MFLUCC 13-0346 T	NG_068375	KY205718	KY460971	KY026587	-	-	-
Alternaria porri	CBS 116649	-	-	KJ718390	KJ718217	KJ718052	KJ718563	KJ718725
Alternaria porri	CBS 116698	KC584553	KC584297	KC584421	DQ323700	KC584132	KC584679	KJ718726
Alternaria porri	CBS 116699 T	-	-	KJ718391	NR_136100	KJ718053	KJ718564	KJ718727
Alternaria proteae	CBS 475.90 T	KC584597	KC584339	KC584464	AY278842	KC584161	KC584723	FJ266505
Alternaria protenta	CBS 116437 T	-	-	KJ718393	KJ718220	KJ718055	KJ718566	KJ718729

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria protenta	CBS 116651	KC584562	KC584306	KC584430	KC584217	KC584688	KC584139	KC584688
Alternaria pseudoinfectoriae	IT2181_A T	MZ621857	MZ621931	OK236638	MZ621984	OK236591	OK236685	OK236738
Alternaria pseudoinfectoriae	IT2181_B	MZ621858	MZ621932	OK236639	MZ621985	OK236592	OK236686	OK236739
Alternaria pseudorostrata	CBS 119411 T	KC584554	KC584298	KC584422	JN383483	AY562406	KC584680	AY563295
Alternaria pseudoventricosa	FMR 16900 T	-	-	LR133934	LR133928	LR133935	LR133936	-
Alternaria quercicola	CBS 141466 T	-	-	LR134188	-	-	LR134259	-
Alternaria radicina	CBS 245.67 T	KC584555	KC584299	KC584423	KC584213	KC584133	KC584681	AY563286
Alternaria ranunculi	CBS 116330 T	-	-	KJ718398	NR_136101	KJ718058	KJ718571	KJ718732
Alternaria ricini	CBS 215.31 T	-	-	KJ718399	NR_136102	KJ718059	KJ718572	KJ718733
Alternaria ricini	CBS 353.86	-	-	KJ718400	KJ718227	JQ646331	KJ718573	KJ718734
Alternaria rosae	CBS 121341 T	-	-	-	NR_136017	-	-	-
Alternaria roseogrisea	CBS 121921 T	-	MH874710	LR134192	MH863165	LR134103	LR134260	-
Alternaria rostellata	CBS 117366 T	-	-	KJ718402	NR_136103	JQ646332	KJ718575	KJ718736
Alternaria rostroconidia	IT3515_A T	MZ621842	MZ621916	OK236623	MZ621969	OK236576	OK236670	OK236723
Alternaria rostroconidia	IT3515_B	MZ621843	MZ621917	OK236624	MZ621970	OK236577	OK236671	OK236724
Alternaria rostroconidia	IT3515_C	MZ621844	MZ621918	OK236625	MZ621971	OK236578	OK236672	OK236725
Alternaria salicicola	T-504_A T	MZ621872	MZ621946	OK236653	MZ621999	OK236606	OK236700	OK236753
Alternaria salicicola	T-504_B	MZ621873	MZ621947	OK236654	MZ622000	OK236607	OK236701	OK236754
Alternaria saponariae	CBS 116492	KC584557	KC584301	KC584425	KC584215	KC584135	KC584683	-
Alternaria scirpicola	CBS 481.90	KC584602	KC584344	KC584469	KC584237	KC584163	KC584728	-
Alternaria scorzonerae	CBS 103.46	-	MH867651	-	-	-	-	-

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria scorzonerae	CBS 116703	-	-	KJ718365	KJ718192	KJ718539	KJ718031	KJ718700
Alternaria scorzonerae	CBS 478.83 T	-	-	-	-	JQ646334	-	JQ646418
Alternaria sennae	CBS 477.81 T	-	-	KJ718403	NR_136104	-	-	-
Alternaria septorioides	CBS 106.41 T	KC584559	KC584303	KC584427	KC584216	KC584136	KC584685	-
Alternaria septospora	CBS 109.38	KC584620	KC584362	KC584487	FJ266489	FJ266500	KC584747	FJ266515
Alternaria sesami	CBS 240.73	-	-	KJ718404	KJ718231	JQ646343	KJ718576	KJ718737
Alternaria sesami	CBS 115264	-	-	KJ718405	-	KJ718061	KJ718577	KJ718738
Alternaria shukurtuzii	M307 T	KJ443082	KJ443127	KJ443172	KJ443257	KJ649620	-	-
Alternaria sidae	CBS 117730 T	-	-	KJ718406	NR_136105	KJ718062	KJ718578	KJ718739
Alternaria silybi	CBS 134092 T	-	-	KJ718407	NR_136106	KJ718063	KJ718579	KJ718740
Alternaria silybi	CBS 134093	-	-	KJ718408	KJ718234	KJ718064	KJ718580	KJ718741
Alternaria simsimi	CBS 115265 T	KC584560	KC584304	KC584428	JF780937	KC584137	KC584686	JQ905110
Alternaria slovaca	CBS 567.66 T	KC584576	KC584319	KC584444	KC584226	KC584150	KC584702	-
Alternaria smyrnii	CBS 109380	KC584561	KC584305	KC584429	AF229456	KC584138	KC584687	-
Alternaria solani	CBS 111.44 T	-	-	KJ718412	Y17070	KJ718068	KJ718584	KJ718745
Alternaria solani	CBS 116442	-	-	KJ718415	KJ718240	KJ718069	KJ718586	KJ718747
Alternaria solani-nigri	CBS 109155	-	-	KJ718417	KJ718242	-	KJ718588	-
Alternaria solani-nigri	CBS 116332	-	-	KJ718419	KJ718244	KJ718072	KJ718590	KJ718750
Alternaria solani-nigri	CBS 116334	-	-	KJ718420	KJ718245	KJ718073	KJ718591	KJ718751
Alternaria sonchi	CBS 119675	KC584565	KC584309	KC584433	KC584220	KC584142	KC584691	-
Alternaria sp.	AL3-1	-	-	-	MF033853	MF033836	-	-

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria sp.	AL3-10	-	-	-	MF033858	MF033841	-	-
Alternaria sp.	B6-25	-	-	-	MF033852	-	-	-
Alternaria sp.	BG1-4	-	-	-	MF033855	MF033838	-	-
Alternaria sp.	BT2-6	-	-	-	MF033850	MF033833	-	-
Alternaria sp.	CBS 108.27	KC584601	KC584343	KC584468	KC584236	KC584162	KC584727	-
Alternaria sp.	CBS 115.44	KC584556	KC584300	KC584424	KC584214	KC584134	KC584682	-
Alternaria sp.	CBS 198.67	KC584610	KC584610	KC584477	-	KC584737	KC584169	-
Alternaria sp.	FA3-2	-	-	-	MF033847	MF033830	-	-
Alternaria sp.	FA4-10	-	-	-	MF033851	MF033834	-	-
Alternaria sp.	FA4-15PR	-	-	-	MF033845	MF033828	-	-
Alternaria sp.	FA7-1	-	-	-	MF033848	MF033831	-	-
Alternaria sp.	H1-4	-	-	-	MF033849	MF033832	-	-
Alternaria sp.	H9-4	-	-	-	MF033844	MF033827	-	-
Alternaria sp.	H-22	OK334124	OK333359	-	OK332867	-	-	-
Alternaria sp.	JS4-1	-	-	-	MF033856	MF033839	-	-
Alternaria sp.	JS4-11	-	-	-	MF033854	MF033837	-	-
Alternaria sp.	JS4-20	-	-	-	MF033846	MF033829	-	-
Alternaria sp.	JS8-5	-	-	-	MF033857	MF033840	-	-
Alternaria sp.	MFLUCC 14-0436	OK334125	OK333360	-	OK332868	-	-	-
Alternaria steviae	CBS 117362 T	-	-	KJ718427	NR_136108	KJ718079	KJ718598	KJ718758
Alternaria steviae	CBS 631.88	-	-	KJ718425	KJ718250	KJ718078	KJ718596	KJ718756

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria tagetica	CBS 117217	-	-	KJ718431	KJ718256	KJ718083	KJ718602	KJ718763
Alternaria tagetica	CBS 297.79	-	-	KJ718428	KJ718253	KJ718080	KJ718599	KJ718759
Alternaria tagetica	CBS 479.81	KC584566	KC584310	KC584434	KC584221	KC584143	KC584692	KJ718761
Alternaria tellustris	CBS 538.83 T	KC584598	KC584340	KC584465	FJ357316	AY562419	KC584724	MG309899
Alternaria thalictrigena	CBS 121712 T	KC584568	KC584312	KC584436	EU040211	KC584144	KC584694	-
Alternaria thunbergiae	CBS 116331 T	-	-	KJ718432	NR_136109	KJ718084	KJ718603	KJ718764
Alternaria thunbergiae	CBS 120986	-	-	KJ718433	KJ718258	KJ718085	KJ718604	KJ718765
Alternaria thunbergiae	CBS 122597	-	-	KJ718434	KJ718259	KJ718086	KJ718605	KJ718766
Alternaria tillandsiae	CBS 116116 T	-	-	KJ718435	NR_136110	KJ718087	KJ718606	KJ718767
Alternaria tomato	CBS 103.30	KP125069	KP124599	KP124915	KP124445	KP124294	KP125224	KP123991
Alternaria tomato	CBS 114.35	KP125070	KP124600	KP124916	KP124446	KP124295	KP125225	KP123992
Alternaria torilis	IT1667_A	MZ621859	MZ621933	OK236640	MZ621986	OK236593	OK236687	OK236740
Alternaria torilis	IT1667_B	MZ621860	MZ621934	OK236642	MZ621987	OK236595	OK236689	OK236742
Alternaria torilis	MFLUCC 14-0433 T	MZ621861	MZ621935	OK236641	MZ621988	OK236594	OK236688	OK236741
Alternaria triglochinicola	CBS 119676 T	KC584569	KC584313	KC584437	KC584222	KC584145	KC584695	-
Alternaria triticimaculans	CBS 578.94 T	-	-	LR134183	NR_136030	FJ214834	FJ214930	-
Alternaria triticina	CBS 763.84 T	-	MH873522	LR134186	MH861833	FJ214846	FJ214942	-
Alternaria tropica	CBS 117216	-	-	KJ718437	KJ718262	KJ718089	KJ718608	KJ718769
Alternaria tropica	CBS 631.93 T	-	MH874097	KJ718436	MH862449	JQ646354	KJ718607	JQ646438
Alternaria vaccariae	CBS 116533	KC584570	KC584314	KC584438	KC584223	KC584146	KC584696	JQ646386
Alternaria vaccariicola	CBS 118714 T	KC584571	KC584315	KC584439	KC584224	KC584147	KC584697	JQ646384

Table 4.8 (continued)

Species name	Strains/Voucher no.	GenBank accession numbers						
		SSU	LSU	RPB2	ITS	GAPDH	TEF1-α	Alt-al
Alternaria venezuelensis	CBS 116121 T	-	-	KJ718438	NR_136112	KJ718090	KJ718609	KJ718770
Alternaria ventricosa	CBS 121546 T	-	MH874671	LR134134	MH863116	-	KY352501	-
Alternaria viburni	CBS 119407 T	-	-	LR134166	FJ214876	FJ214825	FJ214921	-
Alternaria vitis	MFLUCC 17-1109	-	-	-	MG764007	-	-	-
Alternaria vulgarae	YZU161234 T	-	-	MW579312	-	MW579308	MW579310	-
Alternaria vulgarae	YZU161235	-	-	MW579313	-	MW579309	MW579311	-
Alternaria yunnanensis	YITC 5109	-	-	-	MH044725	-	MH044727	MH044726
Alternaria yunnanensis	YITC 5114 T	-	-	-	MH044728	-	MH044730	MH044729
Alternaria zinnae	CBS 117223	-	-	KJ718445	KJ718270	KJ718096	KJ718616	KJ718777
Pleospora tarda	CBS 714.68 T	KC584603	KC584345	AF107804	KC584238	AF443881	KC584729	AY563274
Stemphylium herbarum	CBS 191.86 T	GU238232	GU238160	KC584471	KC584239	AF443884	KC584731	-

Note The type strains are indicated in ”T” and the newly generated sequences are indicated in blue. Abbreviations: ATCC: American Type Culture Collection, Virginia, U.S.A; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; KUMCC: Kunming Institute of Botany Culture Collection, Chinese Science Academy, Kunming, China; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; SQUCC: Sultan Qaboos University Culture Collection, Sultan Qaboos University, Al-Khod 123, Oman.

Table 4.9 Strains used in the phylogenetic analysis of hosts and regional distribution of *Alternaria alternata* (sect. *Alternaria*) and their corresponding GenBank numbers, including two outgroup taxa (*Alternaria eichhorniae* CBS 489.92 and CBS 119778)

Strain number (Taxa name)	Origin	Host	GenBank accession numbers						
			ITS	LSU	SSU	TEF1-α	RPB2	GAPDH	Alt-al
IT1994	Italy	*Reseda* sp.	MZ960790	MZ960839	MZ961487	MZ960887	MZ961532	MZ961397	MZ961442
IT 2053	Italy	*Sonchus* sp.	MZ960758	MZ960807	MZ961455	MZ960855	MZ961500	MZ961365	MZ961410
IT 2063	Italy	*Centaurea* sp.	MZ960759	MZ960808	MZ961456	MZ960856	MZ961501	MZ961366	MZ961411
IT 2064	Italy	*Centaurea* sp.	MZ960760	MZ960809	MZ961457	MZ960857	MZ961502	MZ961367	MZ961412
IT 2087	Italy	*Agropyron* sp.	MZ960761	MZ960810	MZ981458	MZ960858	MZ961503	MZ961388	MZ961413
IT 2090	Italy	*Robinia* sp.	MZ960762	MZ960811	MZ961459	MZ960859	MZ961504	MZ961369	MZ961414
IT 2103	Italy	*Echinochloa* sp.	MZ960763	MZ960812	MZ961460	MZ960860	MZ961505	MZ961370	MZ961415
IT 2114	Italy	*Cephalaria* sp.	MZ960764	MZ960813	MZ961561	MZ960861	MZ961506	MZ961371	MZ961416
IT 2115	Italy	*Aster* sp.	MZ960765	MZ960814	MZ961462	MZ960862	MZ961507	MZ961372	MZ961417
IT 2125	Italy	*Euphorbia* sp.	MZ960766	MZ960815	MZ961463	MZ960863	MZ961508	MZ961373	MZ961418
IT 2143	Italy	*Ailanthus* sp.	MZ960767	MZ960816	MZ961464	MZ960864	MZ961509	MZ961374	MZ961419
IT 2144	Italy	*Agrostis* sp.	MZ960768	MZ960817	MZ961465	MZ960865	MZ961510	MZ961375	MZ961420
IT 2145	Italy	*Arundo* sp.	MZ960769	MZ960818	MZ961466	MZ960866	MZ961511	MZ961376	MZ961421
IT 2162	Italy	*Hedysarum* sp.	MZ960770	MZ960819	MZ961467	MZ960867	MZ961512	MZ961377	MZ961422
IT 2166	Italy	*Dipsacus* sp.	MZ960771	MZ960820	MZ961468	MZ960868	MZ961513	MZ961378	MZ961423
IT 2277	Italy	*Salsola tragus*	MZ960772	MZ960821	MZ961469	MZ960869	MZ961514	MZ961379	MZ961424
IT 2347	Italy	*Fraxinus oxycarpa*	MZ960773	MZ960822	MZ961470	MZ960870	MZ961515	MZ961380	MZ961425
IT 2427	Italy	*Wisteria* sp.	MA960774	MZ960823	MZ961471	MZ960871	MZ961516	MZ961381	MZ961426
IT 2454	Italy	*Urtica* sp.	MZ960775	MZ960824	MZ961472	MZ760872	MZ961517	MZ961382	MZ961427
IT 2900	Italy	*Pinus nigra*	MZ960776	MZ960825	MZ961473	MZ960873	MZ861518	MZ961383	MZ961428
IT 3048	Italy	*Acer opalus*	MZ960777	MZ960826	MZ961474	MZ960874	MZ961519	MZ961384	MZ961429
IT 3160	Italy	*Citrus paradisi*	MZ960778	MZ960827	MZ961475	MZ960875	MZ961520	MZ961385	MZ961430
IT 3168	Italy	*Ostrya carpinifolia*	MZ960779	MZ960828	MZ961476	MZ960876	MZ961521	MZ961386	MZ961431

Table 4.9 (continued)

Strain number (Taxa name)	Origin	Host	GenBank accession numbers						
			ITS	LSU	SSU	TEF1-α	RPB2	GAPDH	Alt-al
IT 3419	Italy	*Calamintha nepeta*	MZ960780	MZ960829	MZ961477	MZ960877	MZ961522	MZ961387	MZ961432
IT 3426	Italy	*Malva alcea*	MZ960781	MZ960830	MZ961478	MZ960878	MZ961523	MZ961388	MZ961433
IT 3439	Italy	*Reseda luteola*	MZ960782	MZ960831	MZ961479	MZ960879	MZ961524	MZ961389	MZ961434
IT 3442	Italy	*Valeriana* sp.	MZ960783	MZ960832	MZ961480	MZ960880	MZ961525	MZ961390	MZ961435
IT 3449	Italy	*Tanacetum* sp.	MZ960784	MZ960833	MZ961481	MZ960881	MZ961526	MZ961391	MZ961436
IT 3504	Italy	*Orobanche sp.*	MZ960785	MZ960834	MZ961482	MZ960882	MZ961527	MZ961392	MZ961437
IT 3556	Italy	*Sorbus aria*	MA960786	MZ960835	MZ961483	MZ960883	MZ961528	MZ961393	MZ961438
IT 3598	Italy	*Quercus pubescens*	MZ960787	MZ960836	MZ961484	MZ960884	MZ961529	MZ961394	MZ961439
IT 3651	Italy	*Sambucus nigra*	MZ960788	MZ960837	MZ961485	MZ960885	MZ961530	MZ961395	MZ961440
CBS 102596 (=A. citrimacularisT)	USA	*Citrus jambhiri*	KP124328	KP124480	KP124950	KP125104	KP124796	KP124183	KP123877
CBS 102599 (=A. turkisafriaT)	Turkey	*Minneola tangelo*	KP124330	KP124482	KP124952	KP125106	KP124798	KP124185	KP123879
CBS 102600 (=A. toxicogenicaT)	USA	*Citrus reticulate*	KP124331	KP124483	KP124953	KP125107	KP124799	KP124186	KP123880
CBS 102602 (=A. perangustaT)	Turkey	*Minneola tangelo*	KP124332	KP124484	KP124954	KP125108	KP124800	KP124187	KP123881
CBS 102603 (=A. interruptaT)	Israel	*Minneola tangelo*	KP124333	KP124485	KP124955	KP125109	KP124801	KP124188	KP123882
CBS 102604 (=A. dumosaT)	Israel	*Minneola tangelo*	KP124334	KP124486	KP124956	KP125110	KP124802	AY562410	AY563305
CBS 106.24 (=A. maliT)	USA	*Malus sylvestris*	KP124298	KP124449	KP124919	KP125073	KP124766	KP124155	KP123847
CBS 106.34 (=A. liniT)	Unknown	*Linum usitatissimum*	Y17071	KP124454	KP124924	KP125078	KP124771	JQ646308	KP123853
CBS 112252 (=A. tenuissima)	Unknown	*Unknown*	KP124340	KP124492	KP124962	KP125116	KP124808	KP124194	KP123888
CBS 113013 (=A. tenuissima)	South Africa	*Malus domestica*	KP124341	KP124493	KP124963	KP125117	KP124809	KP124195	KP123889
CBS 113014 (=A. tenuissima)	South Africa	*Malus domestica*	KP124342	KP124494	KP124964	KP125118	KP124810	KP124196	KP123890
CBS 113015 (=A. tenuissima)	South Africa	*Malus domestica*	KP124343	KP124495	KP124965	KP125119	KP124811	KP124197	KP123891
CBS 113024	South Africa	*Minneola tangelo*	KP124344	KP124496	KP124966	KP125120	KP124812	KP124198	KP123892
CBS 113025	South Africa	*Citrus clementina*	KP124345	KP124497	KP124967	KP125121	KP124813	KP124199	KP123893
CBS 113054 (=A. tenuissima)	South Africa	*Malus domestica*	KP124346	KP124498	KP124968	KP125122	KP124814	KP124200	KP123894

Table 4.9 (continued)

Strain number (Taxa name)	Origin	Host	GenBank accession numbers						
			ITS	LSU	SSU	TEF1-α	RPB2	GAPDH	Alt-al
CBS 115069 (=A. tenuissima)	South Africa	*Malus domestica*	KP124347	KP124499	KP124969	KP125123	KP124815	KP124201	KP123895
CBS 115199	South Africa	*Minneola tangelo*	KP124351	KP124503	KP124973	KP125127	KP124819	KP124205	KP123899
CBS 115200	South Africa	*Minneola tangelo*	KP124352	KP124504	KP124974	KP125128	KP124820	KP124206	KP123900
CBS 116749	Netherlands	*Unknown*	KP124353	KP124505	KP124975	KP125129	KP124821	KP124207	KP123901
CBS 117.44 (=A. godetiaeT)	Denmark	*Godetia sp.*	KP124303	KP124455	KP124925	KP125079	KP124772	KP124160	KP123854
CBS 117130	Italy	*Arbutus unedo*	KP124354	KP124506	KP124976	KP125130	KP124822	KP124208	KP123902
CBS 117143	Italy	*Capsicum annuum*	KP124355	KP124507	KP124977	KP125131	KP124823	KP124209	KP123903
CBS 119115	Greece	*Prunus sp.*	KP124360	KP124512	KP124982	KP125136	KP124828	KP124214	KP123909
CBS 119399 (=A. postmessiaT)	USA	*Minneola tangelo*	KP124361	KP124513	KP124983	KP125137	KP124829	JQ646328	KP123910
CBS 119543 (=A. citricancriT)	USA	*Citrus paradisi*	KP124363	KP124515	KP124985	KP125139	KP124831	KP124215	KP123911
CBS 119778 (=A. eichhorniae)	India	*Eichhornia crassipes*	KC146356	KP124579	KP125049	KP125204	KP124895	KP124276	KP123973
CBS 120829	Greece	*Punica granatum*	KP124364	KP124516	KP124986	KP125140	KP124832	KP124216	KP123912
CBS 121344 (=A. turkisafriaR)	Israel	*Minneola tangelo*	KP124365	KP124518	KP124988	KP125142	KP124834	KP124217	KP123913
CBS 121346 (=A. turkisafriaR)	South Africa	*Minneola tangelo*	KP124366	KP124519	KP124989	KP125143	KP124835	KP124218	KP123914
CBS 121455 (=A. broussonetiaeT)	China	*Broussonetia papyrifera*	KP124368	KP124522	KP124992	KP125146	KP124838	KP124220	KP123916
CBS 121492 (=*Ulocladium cucumisis*)	China	*Cucumis melo*	KP124370	KP124524	KP124995	KP125149	KP124841	KP124223	KP123919
CBS 121492 (=*Ulocladium cucumisis*)	China	*Cucumis melo*	KP124370	KP124524	KP124994	KP125148	KP124840	KP124222	KP123918
CBS 121547 (=*A. yali-inficiens*^{T})	China	*Pyrus bretschneideri*	KP124372	KP124526	KP124996	KP125150	KP124842	KP124224	KP123920
CBS 124277 (=*A. tenuissima*)	Denmark	*Prunus* sp.	KP124373	KP124527	KP124997	KP125151	KP124843	KP124225	KP123921
CBS 124278 (=*A. tenuissima*)	Denmark	*Prunus* sp.	KP124374	KP124528	KP124998	KP125152	KP124844	KP124226	KP123922
CBS 125606	India	Human	KP124375	KP124529	KP124999	KP125153	KP124845	KP124227	KP123923
CBS 126071 (=*A. tenuissima*)	Namibia	Soil	KP124376	KP124530	KP125000	KP125154	KP124846	KP124228	KP123924
CBS 126910 (=*A. tenuis*)	USA	Soil	KP124379	KP124533	KP125003	KP125157	KP124849	KP124231	KP123927
CBS 127334	USA	Soil	KP124380	KP124534	KP125004	KP125158	KP124850	KP124232	KP123928

Table 4.9 (continued)

Strain number (Taxa name)	Origin	Host	GenBank accession numbers						
			ITS	LSU	SSU	TEF1-α	RPB2	GAPDH	Alt-al
CBS 127671 (=*A. seleniiphila*^{T})	USA	*Stanleya pinnata*	KP124381	KP124535	KP125005	KP125159	KP124851	KP124233	KP123929
CBS 127672 (=*A. astragali*^{T})	USA	*Astragalus bisulcatus*	KP124382	KP124536	KP125006	KP125160	KP124852	KP124234	KP123930
CBS 130254	India	Human sputum	KP124383	KP124537	KP125007	KP125161	KP124853	KP124235	KP123931
CBS 130255	India	Human sputum	KP124384	KP124538	KP125008	KP125162	KP124854	KP124236	KP123932
CBS 130258	India	Human sputum	KP124385	KP124539	KP125009	KP125163	KP124855	KP124237	KP123933
CBS 130259	India	Human sputum	KP124386	KP124540	KP125010	KP125164	KP124856	KP124238	KP123934
CBS 130260	India	Human sputum	KP124387	KP124541	KP125011	KP125165	KP124857	KP124239	KP123935
CBS 130261	India	Human sputum	KP124388	KP124542	KP125012	KP125166	KP124858	KP124240	KP123936
CBS 130262	India	Human sputum	KP124389	KP124543	KP125013	KP125167	KP124859	KP124241	KP123937
CBS 130263	India	Human sputum	KP124390	KP124544	KP125014	KP125168	KP124860	KP124242	KP123938
CBS 130265	India	Human sputum	KP124391	KP124545	KP125015	KP125169	KP124861	KP124243	KP123939
CBS 195.86 (=*A. angustiovoidea*^{T})	Canada	*Euphorbia esula*	KP124317	KP124469	KP124939	KP125093	KP124785	KP124173	JQ646398
CBS 267.77 (=*A. citri*)	USA	*Citrus paradisi*	KP124311	KP124463	KP124933	KP125087	KP124779	KP124168	KP123864
CBS 447.86 (=*A. malvae*)	Marocco	*Malva* sp.	KP124318	KP124470	KP124940	KP125094	KP124786	JQ646314	JQ646397
CBS 479.90 (=*A. pellucida*^{T})	Japan	*Citrus unshiu*	KP124319	KP124471	KP124941	KP125095	KP124787	KP124174	KP123870
CBS 489.92 (=*A. eichhorniae*^{T})	Indonesia	*Eichhornia crassipes*	KP124426	KP124580	KP125050	KP125205	KP124896	KP124277	-
CBS 603.78	USA	Air	KP124312	KP124464	KP124934	KP125088	KP124780	KP124169	KP123865
CBS 686.68 (=*A. tenuissima*)	Sahara	Desert sand	KP124306	KP124458	KP124928	KP125082	KP124775	KP124163	KP123859
CBS 795.72	USA	*Plantago aristida*	KP124309	KP124461	KP124931	KP125085	KP124778	KP124166	KP123862
CBS 806.96	Papua New Guinea	Cyperaceae	KP124325	KP124477	KP124947	KP125101	KP124793	KP124180	KP123874
CBS 877.95 (=*A. tenuissima*)	India	Human, sinusitis	KP124321	KP124473	KP124943	KP125097	KP124789	KP124176	KP123871
CBS 880.95 (=*A. tenuissima*)	Belgium	*Fragaria vesca*	KP124322	KP124474	KP124944	KP125098	KP124790	KP124177	-
CBS 911.97 (=*A. tenuissima*)	India	*Artemisia brevifolia*	KP124326	KP124478	KP124948	KP125102	KP124794	KP124181	KP123875
CBS 916.96^{T}	India	*Arachis hypogaea*	AF347031	DQ678082	KC584507	KC584634	KC584375	AY278808	AY563301

Table 4.9 (continued)

Strain number (Taxa name)	Origin	Host	GenBank accession numbers						
			ITS	LSU	SSU	TEF1-α	RPB2	GAPDH	Alt-al
CBS 965.95 (=*A. tenuissima*)	India	*Triticum* sp.	KP124323	KP124475	KP124945	KP125099	KP124791	KP124178	KP123872
CBS 966.95 (=*A. tenuissima*)	India	*Solanum lycopersicum*	KP124324	KP124476	KP124946	KP125100	KP124792	KP124179	KP123873
CBS104.26	Unknown	Unknown	KP124299	KP124450	KP124920	KP125074	KP124767	KP124156	KP123848
DMS15	Thailand	Grass	MZ960799	MZ960848	MZ961496	MZ960896	MZ961541	MZ961405	MZ961451
H-49	China	*Capsicum annuum*	MZ960789	MZ960838	MZ961486	MZ960886	MZ961531	MZ961396	MZ961441
H-71	Thailand	*Coffea arabica*	MZ960801	MZ960850	MZ961498	MZ960898	MZ961543	MZ961408	MZ961453
HAM-02	China	Grass	MZ960795	MZ960844	MZ961492	MZ960892	MZ961537	MZ961402	MZ961447
HAM-03	China	*Capsicum annuum*	MZ960796	MZ960845	MZ961493	MZ960893	MZ961538	MZ961403	MZ961448
HKM-1	China	*Raphanus sativus*	MZ960792	MZ960841	MZ961489	MZ960889	MZ961534	MZ961399	MZ961444
HMRC-51	Thailand	Dead leaves	MZ960798	MZ960847	MZ961495	MZ960895	MZ961540	MZ961405	MZ961450
HSH-01	Thailand	Bamboo	MZ960797	MZ960846	MZ961494	MZ960894	MZ961539	MZ961404	MZ961449
HTWD-01	Thailand	Palm	MZ960800	MZ960849	MZ961497	MZ960897	MZ961542	MZ961407	MZ961452
HWP 01	Thailand	*Bidens pilosa*	MZ960802	MZ960851	MZ961499	MZ960899	MZ961544	MZ961409	MZ961454
HXB-08	China	*Dimocarpus longan*	MZ960794	MZ960843	MZ961491	MZ960891	MZ961536	MZ961401	MZ961446
KIB-H2	China	Bamboo	MZ960791	MZ960840	MZ961488	MZ960888	MZ961533	MZ961398	MZ961443
PB 12	China	*Zea mays*	MZ960793	MZ960842	MZ981490	MZ960890	MZ961535	MZ961400	MZ961445

Note The type strains are indicated by superscript "T" and thereference strains are indicated by superscript "R". The newly generated sequences are indicated in blue. Abbreviations: CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; KUMCC: Kunming Institute of Botany Culture Collection, Chinese Science Academy, Kunming, China; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

CHAPTER 5

ESTABLISHMENT OF ZYGOSPORIACEAE *FAM. NOV.* (XYLARIALES, SORDARIOMYCETES) BASED ON RDNA SEQUENCE DATA TO ACCOMMODATE *ZYGOSPORIUM*[4]

Abstract

Zygosporium species are widespread on hosts and usually associated with monocotyledonous plants including Arecaceae (palms) and Pandanaceae. *Zygosporium* forms colonies as darkened spots on the host surface. This study recovers two *Zygosporium* species from China and Thailand and makes an attempt to clarify the systematic placement of *Zygosporium* based on morphological characteristics and phylogenetic analyses based on rDNA sequence data. Phylogenetic analyses of a combined LSU and ITS sequence data generated under Maximum Likelihood and Bayesian criteria indicate that *Zygosporium* species form a strongly supported monophyletic lineage basal to the Coniocessiaceae and not to the Microdochiaceae as previously assumed. Given the distinct morphological features of *Zygosporium* and its strongly supported monophyletic nature, a new family, Zygosporiaceae is introduced within the Xylariales.

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5.1 Introduction

Zygosporium (Xylariales, Sordariomycetes) species are usually found as saprobes occurring on various angiosperms and some gymnosperms with a cosmopolitan distribution from temperate to tropical regions (Photita et al., 2001; Whitton et al., 2002; Manoharachary et al., 2006; McKenzie et al., 2007; Abbas et al., 2011; Pratibha et al., 2012; Taheriyan et al., 2014; Farr & Rossman, 2017). Currently there are 16 described species (Wijayawardene et al., 2017). Sequence data of *Zygosporium gibbum* (Sacc., M. Rousseau & E. Bommer) S. Hughes is available in GenBank. *Zygosporium majus* Piroz. has been reported as a parasite on leaves of *Brillantaisia patula* while *Z. gibbum* was found as a hyperparasite on *Coleosporium plumeriae*, the latter causing *Plumeria* (frangipani) rust (Ellis, 1976; Abbas et al., 2011; Manimohan & Mannethody, 2011). *Zygosporium masonii* has been reported as a fungal antagonist, which produces some unidentified secondary metabolites that are effective against plant pathogens and multi-drug resistant bacteria (Kanoh et al., 2008; Ajith & Lakshmidevi, 2012).

Zygosporium was first described by Montagne (1842) with the type species *Zygosporium oscheoides* Mont. The genus is known only from its hyphomycetous asexual morph and is characterized by setiform conidiophores, dark pigmented, incurved vesicular cells that give rise to 2–4 ampulliform conidiogenous cells and single-celled conidia that are typically ellipsoid or globose and smooth or variously ornamented. Only one conidium is produced per conidiogenous cell (Mason, 1941; Hughes, 1951; Meredith, 1962; Photita et al., 2001; Whitton et al., 2002; Manoharachary et al., 2006; McKenzie et al., 2007; Abbas et al., 2011).

Mason (1941) reviewed the genus and referred to the vesicular portion of the conidiophore as a ‘falx’, and the setiform conidiophores that gives rise to the vesicular cells as the ‘falciphore’. Some authors have used these terms with respect to *Zygosporium* taxonomy (Hughes, 1951; Barron, 1968; Pirozynski, 1972; Thakur & Udipi, 1976; Subramanian & Bhat, 1987), but there is really little need for such terms whose definitions appear to be rather vague (Photita et al., 2001; Whitton et al., 2002; Manoharachary et al., 2006). *Zygosporium* has usually been described as lacking true setae, with the setiform structures being described as conidiophores or setiform conidiophores (Montagne, 1842;

Mason, 1941; Hughes, 1951; Ellis, 1971, 1976; Photita et al., 2001; Whitton et al., 2002; Manoharachary et al., 2006; McKenzie et al., 2007; Abbas et al., 2011). However, it has been very confusing to use these morphological features to segregate species. For example, *Z. deightonii* M.B. Ellis (Ellis, 1976) and *Z. echinosporum* Bunting & E.W. Mason (Hughes, 1951) produced setiform structures without vesicles and these have been referred to the true setae group (Whitton et al., 2002). In contrast, *Zygosporium* species which produce vesicles from the side of the setiform structures referred as setiform conidiophores include *Z. geminatum* S. Hughes, *Z. majus*, *Z. minus* S. Hughes and *Z. oscheoides* (Manoharachary et al., 2006; McKenzie et al., 2007; Abbas et al., 2011). The differences of setiform conidiophores and vesicular conidiophores was discussed following a terminology more or less utilized by Ellis (1971, 1976) and discussed by Whitton et al. (2002), Manoharachary et al. (2006), McKenzie et al. (2007) and Abbas et al. (2011).

Zygosporium species have mostly been identified using only morphological characteristics given the paucity of DNA sequence data and few species are being encountered and described in the field. To date, there are 16 described species which include 27 epithets listed in Index Fungorum (2017) but the genus is poorly represented with sequence data in GenBank. The placement of the genus is uncertain and is listed in Ascomycota genera *incertae sedis* (Wijayawardene et al., 2012, 2017; Index Fungorum, 2017). However Hernández-Restrepo et al. (2017) showed that the genus belongs to the Xylariales based on analysis of LSU sequence data.

During this investigations in China and Thailand, we recovered two *Zygosporium* species, which are described and illustrated herein. Phylogeny of these taxa, together with *Z. gibbum* was analysed based on a combined LSU and ITS sequence data. A new family Zygosporiaceae, is introduced herein to accommodate *Zygosporium*.

5.2 Materials and Methods

5.2.1 Isolation and Identification

The fungal species were collected from dead leaves of *Ptychosperma macarthurii* (H. Wendl. ex H. J. Veitch) H. Wendl. ex Hook. f. (Arecaceae) in Thailand and from *Pandanus* sp. (Pandanaceae) in China, during the years 2014–2017. Samples were returned to the laboratory for examination and description of morphological characters. The specimens were observed under a Motic SMZ 168 series dissecting stereo-microscope. Macro-morphological structures were photographed using a Discovery V.8 stereo-microscope fitted with a CARL ZEISS Axio Cam ERc5S microscope camera. The conidial structures were picked up with a surgical needle and transferred into 10% lacto-glycerol on a clean slide and examined using a Nikon Eclipse 80*i* compound microscope and photographs taken with a Canon 600D digital camera using DIC microscopy. Tarosoft® Image Frame Work version 0.9.0.7 and Adobe Photoshop CS5 Extended version 10.0 software (Adobe Systems, USA) were used for measurements and compiling the photographic plates respectively. The species were described following the terminologies of subsequent authors to avoid taxonomic confusion (Whitton et al., 2002; Manoharachary et al., 2006; McKenzie et al., 2007; Abbas et al., 2011).

Single spore isolation was carried out to obtain pure cultures after a few conidiophores with conidia were picked from the substrate using a sterilized needle and placed in few drops of sterilized distilled water mounted on a sterilized cavity slide. Conidiophores with conidia were stirred to separate the conidia and obtain the conidia suspension. The suspension was inoculated on to fresh malt extract agar (MEA) or potato dextrose agar (PDA) plates and incubated at room temperature. Germinating conidia were transferred aseptically to PDA and MEA plates and grown at 25–30°C in alternating day and night light conditions. The characters of fungal colonies were observed and recorded after one week and at weekly intervals. The specimens are deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and the Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (HKAS), Yunnan, China. Living cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Institute of Botany Culture Collection (KUMCC). Faces of Fungi numbers

and Index Fungorum numbers are registered (Jayasiri et al., 2015; Index Fungorum, 2017). Establishment of the new family followed guidelines as described in Liu et al. (2016).

5.2.2 DNA Extraction, PCR Amplification and Sequencing

Fresh fungal mycelia and individual fungal conidia were scraped off and placed in a 1.5 ml the sterilized micro-centrifuge tube for DNA extraction as outlined by Jeewon et al. (2002). The Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China) was used to extract fungal genomic DNA from mycelium and the Omega DNA Extraction Kit (OMEGA® E.Z.N.A.) was used to extract fungal genomic DNA from conidia, following the protocols in the manufacturer's instructions. DNA amplification was performed by polymerase chain reaction (PCR) using the nuclear large subunit ribosomal RNA gene (28S, LSU) and the internal transcribed spacer (ITS). Respective primers LROR and LR5 (Vilgalys & Hester, 1990) were used to amplify the partial ribosomal RNA for the LSU gene; ITS5 and ITS4 primer pairs were used to amplify the ITS and 5.8S regions of the rDNA gene (White et al., 1990) following the protocols as described in Li et al. (2017). Purification and sequencing of PCR products were carried out at Shanghai Majorbio Biopharm Technology Co., China.

5.2.3 Sequence Alignment and Phylogenetic Analyses

Sequences generated from this study were analyzed together with related taxa in the order Xylariales, which were obtained from GenBank and derived from Maharachchikumbura et al. (2015, 2016) and Senanayake et al. (2014, 2015) (Table 5.1). Consensus sequences were obtained from both forward and reverse directions using Geneious Pro.v4.8.5. Sequence alignments were automatically aligned with MAFFT v.6.864b (Katoh & Standley, 2013; http://mafft.cbrc.jp/alignment/server/) and manually aligned wherever necessary in MEGA version 6.0 (Tamura et al., 2013). The sequence datasets were combined using BioEdit v.7.2.3 (Hall, 1999). The individual gene alignments were initially analysed for comparing the tree topologies and the combined LSU and ITS sequence data was analysed and conducted the phylogenetic tree based upon the Randomized Accelerated Maximum Likelihood (RAxML) and Bayesian Inference (BI) analyses.

The evolutionary model of nucleotide substitution for BI and RAxML were selected independently for each locus using MrModeltest 2.2 (Nylander, 2004), implemented in PAUP v. 4.0b10 under the Akaike Information Criterion (AIC). RAxML was performed by using RAxMLGUI v. 0.9b2 (Stamatakis, 2006, 2014; Stamatakis et al., 2008; Silvestro & Michalak, 2010) with 1,000 rapid bootstrap replicates using the GTR + GAMMAI model of nucleotide substitution.

Bayesian Inference analysis (BI) (Huelsenbeck et al., 2001) was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001) to evaluate posterior probabilities (PP) (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 and 10,000 trees were obtained. The first 2,000 trees, representing the burn-in phase of the analyses, were discarded, the remaining 8,000 trees were used for calculating posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic is 0.01) (Cai et al., 2006).

The phylograms were represented in Treeview X (Page, 1996), drawn in Microsoft Power Point and converted to jpeg file in Adobe Photoshop version CS5 (Adobe Systems, USA). The newly generated sequences were submitted to GenBank (Table 5.1). The alignment was deposited in TreeBASE (2017) under the accession number 21406.

Table 5.1 Taxa used in the phylogenetic analysis and their corresponding GenBank numbers

Species	Culture collection	GenBank accession numbers	
		LSU	ITS
Apiosordaria verruculosa	F-152365	AY346258	–
Apiospora bambusae	ICMP 6889	DQ368630	–
Apiospora setosa	ICMP 4207	DQ368631	–
Arecophila bambusae	HKUCC 4794	AF452038	–
Arthrinium hyphopodii	MFLUCC 15-0003	KY356093	KY356088
Arthrinium phaeospermum	HKUCC 3395	AY083832	–
Arthrinium sp.	MFLUCC 15-0002	KU863135	KU940148
Arthrinium subglobosa	MFLUCC 11-0397	KR069113	KR069112
Atrotorquata spartii	MFLUCC 13-0444	KP325443	–
Barrmaelia moravica	CBS 142769	MF488987	MF488987

Table 5.1 (continued)

Species	Culture collection	GenBank accession numbers	
		LSU	ITS
Barrmaelia rappazii	CBS 142771	MF488989	MF488989
Barrmaelia rhamnicola	CBS 142772	MF488990	MF488990
Beltrania rhombica	CPC 27482	KX306778	KX306749
Beltraniella carolinensis	DQ810262	DQ810233	–
Cainia anthoxanthis	MFLUCC 15-0539	KR092777	NR138407
Cainia graminis	CBS 136.62	AF431949	–
Cainia graminis	MFLUCC 15-0540	KR092781	KR092793
Camillea obularia	ATCC 28093	KY610429	KY610384
Ciferriascosea uniseptata	MFLUCC 15-0542	KR092776	KR092786
Ciferriascosea wavyseptata	MFLUCC 15-0541	KR092778	KR092789
Coniocessia anandra	Co108	GU553349	GU553338
Coniocessia cruciformis	Co113	GU553348	GU553337
Coniocessia cruciformis	Co115	GU553346	GU553335
Coniocessia cruciformis	Co116	GU553347	GU553336
Coniocessia maxima	Co117	GU553344	GU553332
Coniocessia minima	Co111	GU553345	GU553334
Coniocessia nodulisporioides	CBS 281.77	AJ875224	GU553342
Coniocessia nodulisporioides	Co106	GU553351	GU553343
Coniocessia nodulisporioides	Co107	GU553350	GU553339
Coniocessia nodulisporioides	Co126	GU553352	GU553333
Creosphaeria sassafras	CBS119001	DQ840056	–
Daldinia concentric	CBS 113277	KT281895	AY616683
Diatrype disciformis	MFLUCC 15-0538	KR092784	KR092795
Diatrype palmicola	MFLUCC 11-0018	KP744481	KP744439
Diatrype whitmanensis	ATCC MYA-4417	FJ430587	FJ430595
Eutypa flavovirens	MFLUCC 13-0625	KR092774	KR092798
Eutypa lata	CBS 208.87	DQ836903	DQ006927
Graphostroma platystoma	CBS 270.87	DQ836906	JX658535
Hyponectria buxi	UME 31430	AY083834	–
Hypoxylon fragiforme	MUCL 51264	KM186295	KC477229
Idriella lunata	CBS 204.56	KP858981	KP859044
Idriella lunata	CBS 177.57	KP858980	KP859043
Immersidiscosia eucalypti	KT 2091	AB593722	AB594790

Table 5.1 (continued)

Species	Culture collection	GenBank accession numbers	
		LSU	ITS
Immersidiscosia eucalypti	KT 2115	AB593723	AB594791
Iodosphaeria ongrenensis	MFLU 15-0393	KR095283	KR095282
Jackrogersella multiforme	CBS 119016	KT281893	KC477234
Kretzschmaria deusta	CBS 163.93	KT281896	KT281901
Lopadostoma americanum	CBS 133211	KC774568	NR 132027
Lopadostoma dryophilum	CBS 107.39	KC774573	KC774573
Lopadostoma fagi	MFLUCC 15-0008	KU820973	KU820972
Lopadostoma quercicola	CBS 134633	KC774610	NR 132035
Lopadostoma turgidum	CBS 133207	KC774618	KC774618
Melogramma campylosporum	MBU	JF440978	JF440978
Microdochium fisheri	CBS 242.91	KY777594	KY777595
Microdochium phragmitis	CBS 285.71	KP858949	KP859013
Microdochium tainanense	CBS 269.76	KP858945	KP859009
Oxydothis garethjonesii	MFLUCC 15-0287	KY206762	KY206773
Oxydothis metroxylonicola	MFLUCC 15-0281	KY206763	KY206774
Oxydothis metroxylonis	MFLUCC 15-0283	KY206764	KY206775
Oxydothis palmicola	MFLUCC 15-0806	KY206765	KY206776
Phlogicylindrium eucalyptorum	CBS 111689	KF251708	KF251205
Phlogicylindrium uniforme	CBS 131312	JQ044445	JQ044426
Podosordaria tulasnei	CBS 128.80	KT281897	KT281902
Poronia punctata	CBS 656.78	KT281900	KT281904
Pseudomassaria carolinensis	PC1	DQ810233	–
Pseudomassaria chondrospora	MFLUCC 15-0545	KR092779	KR092790
Pseudomassaria sepincoliformis	CBS129022	JF440984	JF440984
Sarcoxylon compunctum	CBS 359.61	KT281898	KT281903
Seynesia erumpens	SMH 1291	AF279410	–
Sordaria fimicola	HKUCC 3714	AF132330	AY681188
Vialaea mangifia	MFLUCC 12-0808	KF724975	KF724974
Vialaea minutella	BRIP 56959	KC181924	KC181926
Xylaria hypoxylon	CBS 122620	KY610495	KY610407
Xylaria polymorpha	MUCL 49884	KT281899	KY610408
Zygosporium gibbum	CBS 137306	KY853546	KY853482
Zygosporium minus	HKAS99625	MF621586	MF621590
Zygosporium oscheoides	MFLUCC 14-0402	MF621585	MF621589

Table 5.1 The newly generated sequences are indicated in red bold, while the type strains are in black bold. Abbreviations: ATCC: American Type Culture Collection, Virginia, U.S.A.; BRIP: Plant Pathology Herbarium, Department of Agriculture, Fisheries and Forestry, GPO Box 267, Brisbane, Queensland 4001, Australia; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; Co: Centre de Ressources Biologiques de l'Institut Pasteur (CRBIP), 25-28 rue du Docteur Roux 75724 Paris Cedex 15 France; CPC: Culture collection of Pedro Crous, housed at CBS, Utrecht, The Netherlands; F: Fernandez, F.A., The Field Museum of Natural History, 1400 S. Lake Shore Dr., Chicago, IL 60605-2496, USA; GKM: G.K. Mugambi; HKAS: The Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica, Kunming, China. HKUCC: Ecology & Biodiversity, University of Hong Kong, Pokfulam Road, Hong Kong SAR, People's Republic of China; ICMP: International Collection of Micro-organisms from Plants, Landcare Research, Private Bag 92170, Auckland, New Zealand; IFO: Institute for Fermentation, Osaka, Yodogawa-ku, Osaka, Japan; KT: K. Tanaka; MAFF: Ministry of Agriculture, Forestry and Fisheries, Japan; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUCL: (Agro) industrial fungi & yeasts collection, Mycotheque de l'Universite catholique de Louvain Place Croix du Sud 3,B-1348 Louvain-la-Neuve, Belgium; PC: P.W. Crous; SMH: S.M. Huhndorf; UME: The herbarium of the Department of Ecology and Environmental Science of Umeå University, Umeå,

5.3 Taxonomy

The family Zygosporiaceae is introduced to accommodate a single genus *Zygosporium.* The family is justified based on morphologically differences from other taxa in Xylariales and is phylogenetically supported. Detailed descriptions, illustrations of macro-and micro-morphological characteristics of two representative *Zygosporium* species are provided.

5.3.1 Zygosporiaceae J.F. Li, Phookamsak & K.D. Hyde, fam. nov.

Synonym: Zygosporaceae Locq., Mycol. gén. struct. (Paris): 202. 1984. (nom. inval., Art 39.1, Melbourne Code).

Index Fungorum number: IF10473; Facesoffungi number: FoF03760

Saprobic on plant litter, especially on monocotyledons. *Colonies* effuse, white to light pink. *Mycelium* immersed or partly superficial, composed of smooth, thin-walled, white or light pink hyphae. *Setae* or setiform conidiophores with sphaerical apex. *Conidiophores* macronematous, mononematous, solitary or in small groups, pale brown, thin-walled, unbranched, septate, smooth, bearing swollen dark brown, thick-walled vesicles. *Conidiogenous cells* holoblastic, discrete, hyaline or light brown, smooth, sphaerical to ellipsoid, borne in groups of 2–4 conidiogenous cells on the vesicular cell. *Conidia* solitary, aseptate, hyaline or pale brown, globose or ellipsoid, thin- or thick-walled.

Notes: Zygosporiaceae was invalidly published as 'Zygosporaceae' (Nom. inval., Art. 39.1, Melbourne) by Locquin (1984) to accommodate the genus *Zygosporium* (Index Fungorum, 2017; MycoBank, 2017). However, the taxonomic placement of *Zygosporium* is still ambiguous due to its unique morphological characters of setae/setiform or vesicular conidiophores, with a sphaerical apex and distinct vesicles which are different from other hyphomycetous taxa. Lack of molecular data to clarify its phylogenetic position is also a problem. In this study, two representative species, *Z. oscheoides* and *Z. minus* were collected and molecular data was obtained. Phylogenetically, *Zygosporium* forms a distinct lineage separated from other taxa in Xylariales. Therefore, the new family Zygosporiaceae is validly introduced herein based on the morphological and phylogenetic support.

Type genus: *Zygosporium* Mont.

5.3.2 *Zygosporium* Mont., in Sagra, *Annls Sci. Nat.*, Bot., sér. 2 17: 120 (1842)

Facesoffungi number: FoF03761

Saprobic on plant litter. *Colonies* effuse, white to light pink. *Mycelium* immersed or partly superficial, composed of smooth, thin-walled, white or light pink hyphae. *Setae*/*setiform conidiophores* with a sphaerical apex. *Conidiophores* macronematous, mononematous, pale brown, thin-walled, unbranched, septate, smooth, bearing swollen dark brown, thick-walled vesicles on the base, solitary or in small groups. *Conidiogenous cells* usually on the base of conidiophores, born on the apex of thick-walled dark vesicles, holoblastic, thin-walled, smooth, discrete, sphaerical to ellipsoid, borne in groups of 2–4

conidiogenous cells on the vesicular cell. *Conidia* solitary, aseptate, hyaline or pale brown, globose or ellipsoid, smooth to minutely verruculose or verruculose, thin- or thick-walled.

Notes – Montagne (1842) established *Zygosporium* and designated *Z. oscheoides* as the type species. Morphologically, the genus has a set of unique characters consisting of darkly pigmented, incurved vesicular cells usually born from the side of setiform conidiophores and the vesicles give rise to ampulliform conidiogenous cells that produce aseptate, globose or ellipsoid, smooth or variously ornamented conidia (Mason, 1941; Hughes, 1951).

Type species: *Zygosporium oscheoides* Mont., Annls Sci. Nat., Bot., sér. 2, 17: 121 (1842)

5.3.3 *Zygosporium oscheoides* Mont., Annls Sci. Nat., Bot., sér. 2, 17: 121 (1842)

Facesoffungi number: FoF03285

Saprobic on dead leaves of *Ptychosperma macarthurii* (Arecaceae). Sexual morph: Undetermined. Asexual morph: *Colonies* effuse, white to light pink. *Mycelium* immersed or partly superficial, composed of smooth, thin-walled, white or light pink hyphae. *Conidiophores* setiform portion 24–45.5 µm long × 2–4 µm diam. ($\overline{x}$ = 35.4 × 2.7 µm, n = 10), macronematous, mononematous, consisting of a setiform portion with a vesicle borne on a short cell near its base, light brown to pale brown, thin-walled, smooth, solitary or in small groups; *Conidiogenous cells* (6.6–)8.2–15.5 µm long × 4.7–10 µm diam. ($\overline{x}$ = 11.5 × 6.8 µm, n = 20), on the apex of vesicular conidiophores, holoblastic, thick-walled in the dark vesicular cell, light brown to dark brown, smooth, solitary. *Conidia* (8.6–)9.9–10.6 × 9.2–10 µm diam. ($\overline{x}$ = 10.5× 9.6 µm, n = 10), smooth to minutely verruculose, solitary, aseptate, hyaline to pale brown, globose to ellipsoid, thin-walled.

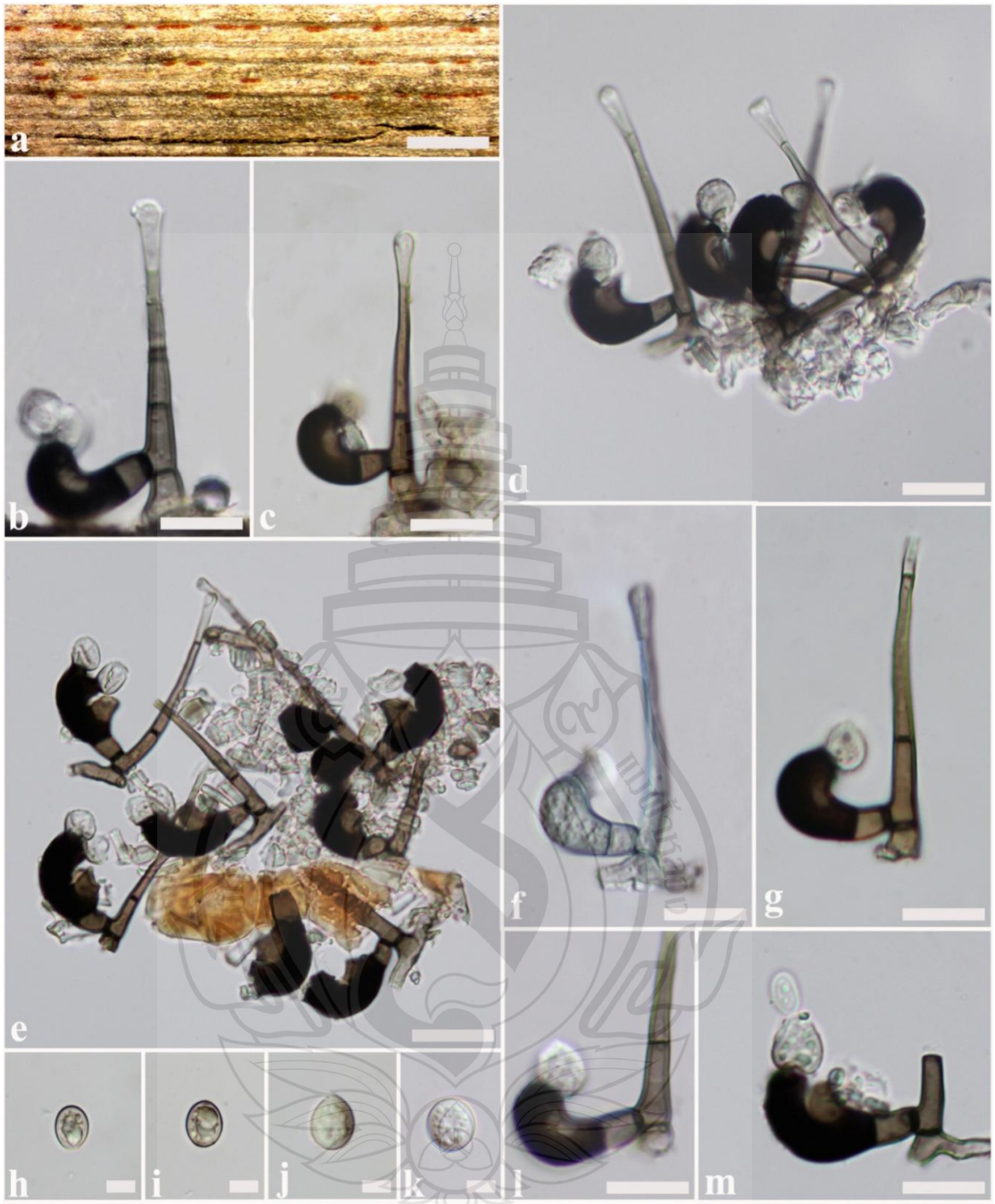
Cultural characteristics**:** Conidia germinating on MEA within 10 hours, producing a single germ tube, colonies reaching 5 mm diam. within 20 days at 30ºC, cottony, white to pale pink. Mycelium partly superficial, partly immersed, slightly effuse, with regular edge, white to light pink hyphae; sexual or asexual spores not formed within 60 days.

Material examined: THAILAND, Chiang Rai Province, Mae Fah Luang University, on dead leaves of *Ptychosperma macarthurii* H. Wendl. & H.J. Veitch (Arecaceae), 9 January 2014, J.F. Li, H-17 (MFLU 16-0273, reference specimens designated here), living culture MFLUCC 14-0402, KUMCC 17-0172.

Known hosts (not molecular based): *Agave fourcroydes* Lem., *Areca* sp., Arecaceae, *Carica papaya* Linn., *Chamaedorea* sp., *Cocos nucifera* Linn., *Ficus carica* Linn., *Ficus* sp., *Jasminum sambac* (L.) Aiton, *Musa sapientum* (L.) O. Ktze., *Pinus elliottii* Engelm., *Podocarpus* sp., *Pucciniopsis caricae* Earle, *Rhapis flabelliformis* L'Hert, *Sabal palmetto* (Walt.) Lodd., *Saccharum officinarum* Linn., *Thuja* sp., *Tillandsia* sp., *Ptychosperma macarthurii* Linn. (Montagne, 1842; Mason, 1941; Hughes, 1951; Ellis, 1971, 1976; Whitton et al., 2002; Farr & Rossman, 2017).

Distribution: distributed in tropical and temperate regions.

Notes: *Zygosporium oscheoides* is unique in having a vesicular cell produced from the side of the setiform conidiophores. Apical cell sub-hyaline of the setiform conidiophores is smooth, acute or narrowly clavate. The conidia are sphaerical, globose to ellipsoid, hyaline to pale brown, smooth to minutely verruculose. Our isolate (MFLU 16-0273) is morphologically typical *Z. oscheoides* and was collected from different host and location with holotype. Therefore, MFLU 16-0273 is assigned here as the reference specimen following the procedures described in Ariyawansa et al. (2014).



Note a Appearance of the fungus on dead leaves of *Ptychosperma macarthurii*. b–g Conidiophores with vesicles. h–k Conidia. l–m Vesicles and conidiogenous cells. *Scale bars* a = 200 µm, b–g = 10 µm, h–m = 5 µm.

Figure 5.1 Morpholoy of *Zygosporium oscheoides* (MFLU 16-0273)

5.3.4 *Zygosporium minus* S. Hughes, Mycological Papers 44: 6 (1951)

Facesoffungi number: FoF03762

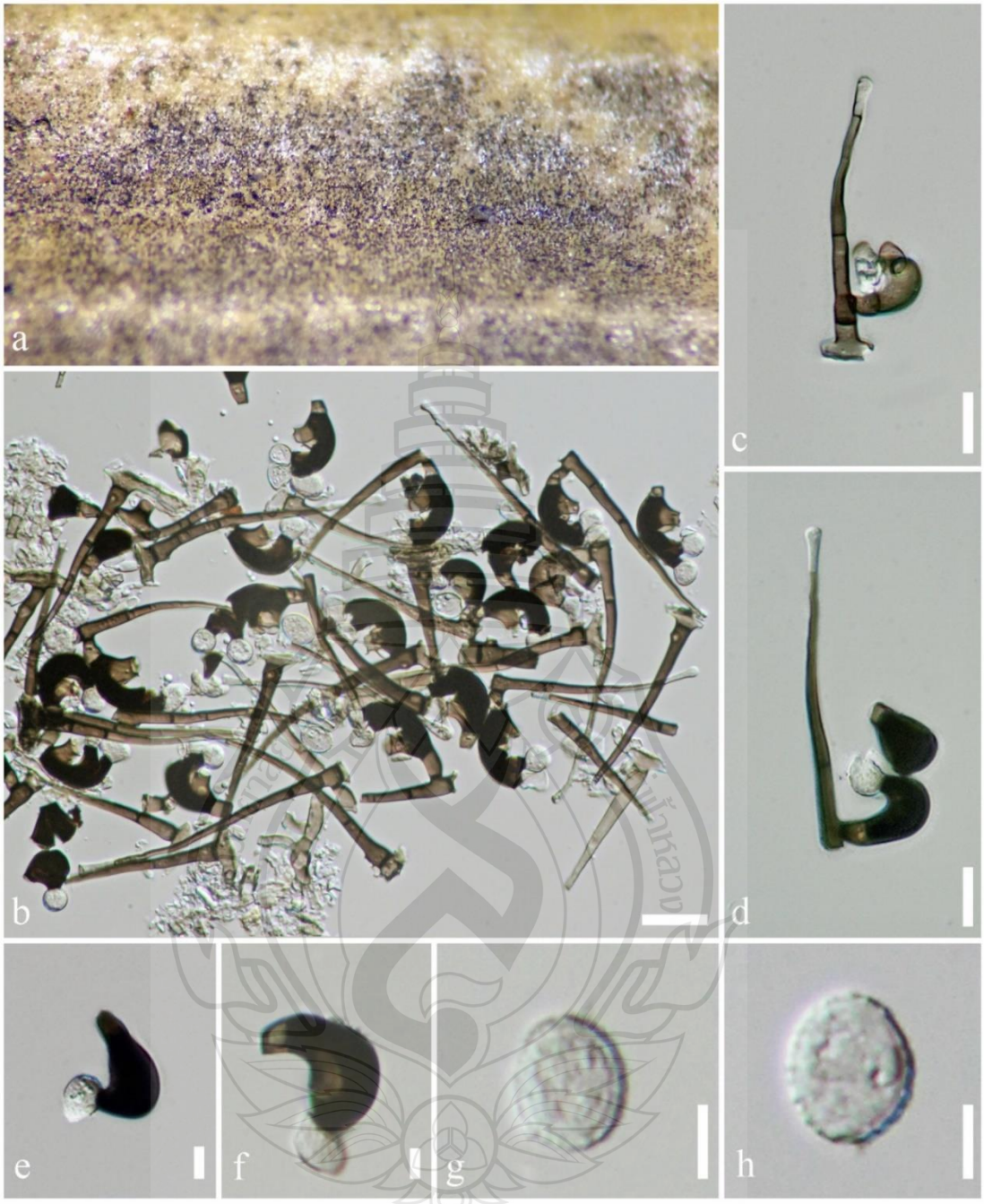
Saprobic on fallen dead and decaying leaves of *Pandanus* sp. Sexual morph: Undetermined. Asexual morph: *Colonies* effuse to compact, forming a thin layer, spread on the substrate surface, numerous. *Mycelium* mostly superficial, consisting of cylindrical, dark brown to black, smooth, septate, branched hyphae with slightly thickened walls. *Conidiophores* setiform portion 43–78.6 µm long × 2.9–6.4 µm diam. ($\overline{x}$ = 56.6 × 4.5 µm, n = 20), macronematous, mononematous, scattered, wide towards the base, 3–4-septate, erect, straight or slightly flexuous, smooth, unbranched, dark brown at the base with connecting the conidiophores with each other, sub-hyaline narrowing towards the apex, swollen vesicles on short stalk arising from the side of the first cell of the conidiophores, vesicular cylindrical, brown, smooth. *Conidiogenous cells* 13.1–27.7 × 6.4–10.4 µm ($\overline{x}$ = 20 × 8.3 µm, n = 20), holoblastic, discrete, terminal, ellipsoidal to ampulliform, upwardly curved, smooth, pale brown, apex obtuse, thin-walled, borne in pairs, arising directly from the vesicular cell. *Conidia* 5.3–10 × 5.3–10.7 µm ($\overline{x}$ = 7.4 × 6.9 µm, n = 40), solitary, aseptate, verruculose, hyaline, globose to broadly ellipsoid, thick-walled.

Material examined: CHINA, Yunnan, Xishuangbanna Tropical Botanical Garden, on fallen dead and decaying leaves of *Pandanus* sp. (Pandanaceae), 28 April 2017, R. Phookamsak and N. I. de Silva, XTBG17 (HKAS99625, reference specimens designated here).

Hosts (not based on molecular data): *Polyalthia longifolia* Sonn., *Pandanus* sp. (Hughes, 1951; Whitton et al., 2002).

Distribution: China, Cuba, Ghana, Great Britain, Myanmar, Sierra Leone, Tamil Nadu (India), Tanzania, Venezuela, West Bengal (India), Zambia.

Notes: Hughes (1951) introduced *Zygosporium minus* based on its morphology having subulate conidiophores, with thickened at the base, bearing a single vesicle laterally on a short stalk and sphaerical, hyaline to very pale brown, verruculose conidia. This species can be found on *Pandanus* spp. worldwide (Whitton et al., 2002). This is first record of *Z. minus* from China. DNA sequence analyses indicate that *Z. oscheoides* and *Z. minus* cluster together with very high support (100% ML/0.99 PP; Fig 3). Due to same species found on different host and location with holotype, HKAS99625 is assigned here as the reference specimen following the procedures described in Ariyawansa et al. (2014).



Note a Appearance of the fungus on dead leaves of *Pandanus* sp. b–d Conidiophores, vesicles and conidiogenous cells. e–f Vesicles and conidiogenous cells. g–h Conidia. Scale bars: b = 20 µm, c–d = 10 µm, e–h = 5 µm.

Figure 5.2 Morphology of *Zygosporium minus* (HKAS99625)

5.4 Phylogenetic Analyses

The combined LSU and ITS sequence data comprised 78 taxa with *Apiosordaria verruculosa* (F-152365) and *Sordaria fimicola* (HKUCC 3714) selected as the outgroup taxa. The best scoring RAxML tree (Final ML Optimization Likelihood: -21750.480571) is chosen to represent the phylogenetic relationships of *Zygosporium* spp. with other genera in Xylariales (Sordariomycetes). The phylogenetic trees obtained from maximum likelihood and Bayesian Inference analyses gave essentially similar topologies with regards to the position of *Zygosporium* within the Xylariales and did not differ significantly. *Zygosporium oscheoides* (MFLUCC 14-0402), *Z. minus* (HKAS99625) and *Z. gibbum* (CBS 137306) isolates sequenced and analysed constitute a strongly supported distinct clade basal to the Coniocessiaceae (76% ML, 0.98 PP).



Figure 5.3 Phylogenetic tree constructed from RAxML analysis based on a combined multigene

Figure 5.3 Phylogenetic tree constructed from RAxML analysis based on a combined dataset of LSU and ITS DNA sequences. RAxML bootstrap support values equal or greater than 50% and Bayesian posterior probabilities equal or higher than 0.90 PP (red) are provided below or above the node. The tree is rooted to *Apiosordaria verruculosa* (F-152365) and *Sordaria fimicola* (HKUCC 3714). Newly generated strains are indicated in red. Type strains are in bold.

5.5 Discussion

The taxonomy of *Zygosporium* has always been a contentious issue especially with regards to its taxonomic position. Only a few species have been described so far with limited DNA sequence data, which make it almost impossible to refer them to an appropriate familial position. In addition, its prevalence only in its asexual morphs (with no known sexual morphs to date) makes it also difficult to ascertain to which group of fungi it might be closely related to and hence DNA sequence data is essential to clarify taxonomic relationships. It is noteworthy to mention that *Zygosporium* species are characterized by unique and peculiar morphological characters, in particular vesicular cells usually born from the side of setiform conidiophores and the vesicles give ampulliform conidiogenous cells that set it apart from other known asexual morphs of the Xylariales.

Whitton et al. (2002) reviewed the taxonomy of the genus *Zygosporium* while describing two new species and provided key information on species differences which was later updated by Manoharachary et al. (2006), McKenzie et al. (2007) and Abbas et al. (2011). There was no DNA sequence data for any species until Hernández-Restrepo et al. (2017) recovered *Z. gibbum* from unidentified dead leaves in Spain. The latter analysed rDNA sequence data and reported a close affinity of *Z. gibbum* to the family Microdochiaceae, but this relationship received no support at all. The authors also reported that *Zygosporium* constitutes a separate and independent lineage within the Xylariales and referred it to an uncertain position due to sparse taxon sampling.

In this study, two additional species, viz *Zygosporium oscheoides* and *Z. minus* collected from Thailand and China, are analysed phylogenetically to provide a better taxonomic interpretation. All three species of *Zygosporium* analysed herein constitute a

strong monophyletic group with high support and basal to the family Coniocessiaceae, as compared to a sister relationship to the family Microdochiaceae as proposed by Hernández-Restrepo et al. (2017). It should be pointed out that *Zygosporium* is characterized by asexual morphs that are quite distinct from both families. Microdochiaceae species are characterized by globose, erumpent stromata of minute, hyaline cells, small papillate conoid conidiogenous cells and solitary, fusiform to subfalcate, hyaline conidia (Hernández-Restrepo et al., 2016). *Zygosporium* species are morphologically distinguished from species in Coniocessiaceae as *Zygosporium* species have dark setae and setiform conidiophores with a sphaerical head and distinct vesicles. On the other hand, Coniocessiaceae species lack setae, have hyaline, macronematous or micronematous conidiophores with integrated or discrete conidiogenous cells that bear conidia on conspicuous denticles (Asgari & Zare, 2010). No sexual morph is known for *Zygosporium*, but Coniocessiaceae species have distinct ascomata, asci and ascospores (Asgari & Zare, 2010).

Taking into account the robustness of our phylogeny herein and more taxon sampling, a new family, Zygosporiaceae is being proposed to accommodate *Zygosporium* species. At the species level, our phylogeny herein also depicts a close relationship between *Z. oscheoides* and *Z. minus* with high support (Figure 5.3). However these two taxa are recognized as morphologically distinct species as *Z. oscheoides* is characterized by setiform conidiophores, apical cell subhyaline, smooth, acute or narrowly clavate and conidia not sphaerical, smooth or minutely verruculose, ellipsoid, hyaline to pale-brown, smooth to minutely verruculose whereas *Z. minus* possesses setiform conidiophores bearing a single vesicle laterally on a short stalk and sphaerical and conidia hyaline to very pale brown, verruculose (Figure 5.1, 5.2; Montagne, 1842; Hughes, 1951; Whitton et al., 2002). *Zygosporium gibbum* on the other hand is morphologically distinct from the above two with darkly pigmented, incurved vesicular cells usually born from the side of setiform conidiophores; the vesicles may be stalked or sessile, and give rise to 2–4 ampulliform conidiogenous cells that produce aseptate, ellipsoid or globose, smooth or variously ornamented conidia (Mason, 1941; Hughes, 1951). In addition, all these species have been described from different hosts. Even though Zygosporiaceae was originally invalidly published (Art. 39.1, Melbourne) by Locquin (1984), the Zygosporiaceae is being introduced herein as a new family to accommodate *Zygosporium* with morphologically distinct characters coupled with phylogenetic support.

CHAPTER 6

MARINOPHIALOPHORA GARETHJONESII GEN. ET SP. NOV.: A NEW HYPHOMYCETE ASSOCIATED WITH *HALOCYPHINA* FROM MARINE HABITATS IN THAILAND [5]

Abstract

Marinophialophora garethjonesii sp. nov., representing a novel genus *Marinophialophora* gen. nov., associated with the basidiomycete fungus, *Halocyphina* on mangrove wood from Phetchaburi, Thailand, is described and illustrated. *Marinophialophora* morphologically resembles *Phialophora* and *Junctospora* (Herpotrichiellaceae, Chaetothyriales) in having macronematous, unbranched or branched conidiophores, globose to subglobose, solitary, acrogenous, pale to subhyaline, aseptate, conidia in chains and phialidic conidiogenous cells. *Marinophialophora* mainly differs from other related genera due to its conidia borne in basipetally developing branched chains and septate conidiophores. Phylogenetic analyses of a combined ITS, LSU and SSU sequence data show that *Marinophialophora garethjonesii* constitutes an independent lineage with high statistical support basal to the genus *Exophiala* within the family Herpotrichiellaceae (Chaetothyriales). The new marine taxon is described herein with illustrations and relationships inferred from DNA sequence data.

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[5] This paper has been published in Phytotaxa, 2018, 345(1), 001–012. doi:10.11646/phytotaxa.345.1.1

6.1 Introduction

Asexual morph of ascomycetes within the order Chaetothyriales comprise morphologically diverse hyphomycetes (Shenoy et al., 2006; Liu et al., 2015), which are ubiquitous with different ecological lifestyles such as epilithic on rocks, lichenized and lignicolous fungi, saprobic on dead materials and pathogenic on plants, humans and animals (Gueidan et al., 2008; Chomnunti et al., 2012a, 2012b; Réblová et al., 2013; Selbmann et al., 2013; Hubka et al., 2014; Liu et al., 2015). Herpotrichellaceae is the best known family in the order Chaetothyriales (Nascimento et al., 2016). About one-third of the species of this family were described from pathogenic strains isolated from human diseases, and occasionally infections can be fatal (Li et al., 2011). Nevertheless, there are poorly known marine fungi within this family. Marine habitats, especially mangroves are well-known to harbour many novel species of fungi (Jones et al., 2009, 2015). Species in genus *Halocyphina* are well-known to occur on core mangrove species (Pang & Jheng, 2012). Recent collections of *Halocyphina* species on decayed mangrove wood from the Southern parts of Thailand recovered an unusual species. The present study investigates the morphological characterisation and phylogenetic position of this new taxon, *Marinophialophora garethjonesii*, collected from a tropical marine habitat.

6.2 Material and Methods

6.2.1 Sample Collection, Morphological Study and Isolation

The specimen was collected from *Halocyphina* sp. on decaying mangrove wood in Phetchaburi Province, southern Thailand and brought to the laboratory for examination and description of fungal morphology. The specimens were observed using a Motic SMZ 168 series dissecting stereo-microscope. The conidial structures were picked up using a surgical needle and a squash mount was prepared in distilled water for examination under a Nikon Eclipse 80i compound microscope. Photographs were made with a Canon 600D digital camera using DIC microscopy. The macro-morphological structures were photographed using a Discovery V.8 stereo-microscope fitted with a CARL ZEISS Axio Cam ERc5S microscope camera. Tarosoft (R) Image Frame Work program and Adobe Photoshop

version CS5 (Adobe Systems, USA) were used for measurements and to prepare photographic plates. Single spore isolation was carried out to obtain a pure culture as described in Chomnunti et al. (2014). The type specimen is deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and duplicated in the Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (HKAS), Yunnan, China. Ex-type living cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Institute of Botany Culture Collection (KUMCC). Faces of Fungi and Index Fungorum numbers were provided as in Jayasiri et al. (2015) and Index Fungorum (2016). New taxa are defined based on recommendations of Jeewon and Hyde (2016).

6.2.2 DNA Extraction, Amplification and Sequencing

The Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China) was used to extract fungal genomic DNA, following the protocols in the manufacturer's instructions.

The DNA amplification was performed by polymerase chain reaction (PCR) for the following ITS, LSU and SSU gene regions using the primer pairs: ITS4 and ITS5 (White et al., 1990) to amplify the internal transcribed spacer (ITS); LROR and LR5 (Vilgalys & Hester, 1990) to amplify the partial ribosomal RNA for the nuclear large subunit (28s, LSU); NS1 and NS4 (White et al., 1990) to amplify the partial ribosomal RNA for the nuclear small subunit (18s, SSU). The final volume of the PCR reaction was 25 µl, containing 1 µl of DNA template, 1 µl of each forward and reward primer, 12.5 µl of 2×Power Taq PCR MasterMix and 9.5 µl of ddH_2O. The PCR thermal cycling conditions of ITS, LSU and SSU amplification, as outlined by Jeewon et al. (2002, 2003, 2013) is as follows: initialization at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 50 sec, elongation at 72°C for 1 min, and a final extension at 72°C for 10 min. Purification and sequencing of PCR products with the primers mentioned above were carried out at Shanghai Majorbio Biopharm Technology Co., China.

6.2.3 Sequence Alignment and Phylogenetic Analysis

Phylogenetic analyses were performed based on a combined ITS, LSU and SSU sequence data. Sequences generated from this study were analysed with other similar sequences obtained from GenBank and those derived from Liu et al. (2015) (Table 6.1). Consensus sequences were assembled with Sequencher 4.9 for Windows (Gene Codes Corp., Ann Arbor, Michigan) and aligned using MAFFT v.7.110 online program (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley, 2013) and manually adjusted via MEGA version 6.0 (Tamura et al. 2013), follow the method described by Phookamsak et al. (2015).

The phylogenetic analyses were obtained from Randomized Accelerated Maximum Likelihood (RAxML) and Bayesian analyses. The evolutionary model of nucleotide substitution for Bayesian analysis and maximum-likelihood were selected independently for each locus using MrModeltest 2.2 (Nylander, 2004). Randomized Accelerated Maximum Likelihood (RAxML) was performed by using RAxMLGUI v. 0.9b2 (Stamatakis, 2006, 2014; Stamatakis et al., 2008, Silvestro & Michalak, 2010) with 1,000 rapid bootstrap replicates using the GAMMAI model of nucleotide substitution. Best scoring is the Final ML Optimization Likelihood: -11550.999264.

Bayesian analysis (BI) (Huelsenbeck et al., 2001) was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001) to evaluate posterior probabilities (PP) (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 and 10,000 trees were obtained. The first 2000 trees, representing the burnin phase of the analyses, were discarded, the remaining 8000 trees were used for calculating posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic is 0.01) (Cai et al., 2006).

Bayesian Posterior Probabilities (BYPP) equal or greater than 0.90 is given below or above each node (Figure 6.3). The phylograms were represented in Treeview X (Page, 1996) and drawn in Microsoft power point and converted to jpeg file in Adobe Photoshop version CS5 (Adobe Systems Inc., The United States). The new sequences were submitted in GenBank (Table 6.1).

The phylograms were represented in Treeview (Page, 1996) and drawn in Adobe Microsoft Power Point and converted to jpeg file in Adobe Photoshop version CS5 (Adobe

Systems, USA). The alignment was deposited in TreeBASE (2017) under the accession number 21365.

Table 6.1 Culture collection codes and GenBank accession numbers of fungi used in phylogenetic analysis of *Marinophialophora*

Species	Culture collection	GenBank accession numbers		
		ITS	LSU	SSU
Capronia pilosella	AFTOL-ID 657	DQ826737	DQ823099	DQ823106
Cladophialophora arxii	CBS 306.94	EU103986	-	-
Cladophialophora australiensis	CBS 112793	EU035402	EU035402	-
Cladophialophora bantiana	CBS 173.52	EU103989	-	-
Cladophialophora boppii	CBS 126.86	EU103997	FJ358233	FJ358301
Cladophialophora carrionii	CBS 160.54	EU137266	FJ358234	FJ358302
Cladophialophora carrionii	CBS 260.83	EU137292	KF928518	AY554285
Cladophialophora chaetospira	CBS 491.70	EU035405	EU035405	-
*Cladophialophora chaetosp*ira	CBS 114747	EU035403	KF928514	-
Cladophialophora devriesii	CBS 147.84	EU103985	KC809989	KF155192
Cladophialophora emmonsii	CBS 640.96	EU103995	KC809995	KF155193
Cladophialophora immunda	CBS 834.96	EU137318	KC809990	KF155194
Cladophialophora minourae	CBS 556.83	AY251087	FJ358235	FJ225734
Cladophialophora minourae	CBS 987.96	EU103988	-	-
Cladophialophora parmeliae	CBS 129337	JQ342180	JQ342182	-
Cladophialophora potulenturum	CBS 115144	-	DQ008141	-
Cladophialophora potulenturum	CBS 112222	EU035409	EU035409	-
*Cladophialophora samoens*is	CBS 259.83	EU137291	KC809992	-
Cladophialophora saturnica	CBS 118724	EU103984	-	-
Cladophialophora subtilis	CBS 122642	FJ385273	-	-
Cladophialophora yegresii	CBS 114405	EU137322	KC809994	KF155195
Cyphellophora laciniata	CBS 190.61	EU035416	FJ358239	FJ358307
Exophiala jeanselmei	CBS 507.90	AY156963	FJ358242	FJ358310
*Exophiala pisciphil*a	AFTOL-ID 669	DQ826739	DQ823101	DQ823108
Fonsecaea pedrosoi	CBS 271.37	AY366914	-	-
Fonsecaea monophora	CBS 102243	EU938579	FJ358247	FJ358315
Marinophialophora garethjonesii	MFLUCC 16-1449	KY305174	KY305176	KY305178
Marinophialophora garethjonesii	KUMCC 16-0066	KY305175	KY305177	KY305179
Melanoctona tectonae	MFLUCC 12-0389	KX258778	KX258779	KX258780
Minimelanolocus aquaticus	MFLUCC 15-0414	KR215607	KR215612	KR215617
Minimelanolocus asiaticus	MFLUCC 15-0237	KR215604	KR215610	KR215615

Table 6.1 (continued)

Species	Culture collection	GenBank accession numbers		
		ITS	LSU	SSU
Minimelanolocus melanicus	MFLUCC 15-0415	KR215608	KR215613	KR215618
Minimelanolocus obscurus	MFLUCC 15-0416	KR215606	KR215611	KR215616
Minimelanolocus yunnanensis	MFlucc 16-0764	KX258781	KX258782	KX258783
Phialophora verrucosa	MUCL 9760	AF050281	EF413615	EF413614

Notes Ex-type strains are given in bold, the new genus in this study is in red. Abbreviations: AFTOL-ID: Assembling the Tree of Life consortium, Europe; CBS Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; M: Mitochondrion DNA. Botanical Museum, Finnish Museum of Natural History, PO Box 7, Helsinki 00014, Finland; KUMCC: Kunming Institute of Botany Culture Collection, Chinese Academy of Sciences, Kunming, China; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUCL: Culture Collection, Catholic University of Louvain (UCL), Place Croix du Sud 3, Louvain-la-Neuve B-1348, Belgium; MUV: Wendy Untereiner; SJ: San Juan Islands, Washington State Ferries, U.S.A.; TRN: The thalamic reticular nucleus projects.

6.3 Taxonomy

6.3.1 *Marinophialophora* J.F. Li, Phookamsak & K.D. Hyde, gen. nov.

Index Fungorum number: IF552733; Facesoffungi number: FoF 02832.

Etymology: Named after its marine habitat and morphological similarity to *Phialophora*.

Hyphomycetous associated with *Halocyphina* species on decaying mangrove wood. Sexual morph: Undetermined. Asexual morph: *Mycelium* partly superficial, partly immersed on the substrate, composed of septate, branched, smooth, thin-walled, hyaline to subhyaline hyphae. *Conidiophores* macronematous, mononematous, straight or curved, aseptate, smooth, hyaline to subhyaline, unbranched, erect, single or caespitose, smooth, thin-walled, cylindrical, arising from hyphae. *Conidiogenous cells* phialidic, integrated, terminal or intercalary, determinate, hyaline to subhyaline, globose to ellipsoidal, smooth, without a conspicuous collarette. *Conidia* phialoconidia, hyaline or subhyaline, globose to subglobose, aseptate, borne in basipetally developing unbranched or branched chains, clavate, smooth, thin-walled.

Type species: *Marinophialophora garethjonesii* J.F. Li, Phookamsak, Dayarathne & K.D. Hyde, *sp. nov*.

Notes: *Marinophialophora* is described herein as a monotypic genus and typified by *M. garethjonesii*. The species was collected from decaying wood of mangrove in southern Thailand. This genus is characterized by its mononematous, macronematous conidiophores, aseptate subhyaline conidia and phialidic conidiogenesis without conspicuous collarette and colourless.

Marinophialophora morphologically resembles *Phialophora* Meldar, *Phialomyces* Misra & Talbot, *Junctospora* Minter & Hol.-Jech and *Cladophiaphora* Borelli. However, *Marinophialophora* differs from *Phialophora* in having phialides without a conspicuous collarette, which in *Phialophora* are very large and conspicuous. The genus differs from *Junctospora* in having intercalary phialides, as well as terminal phialides, and from *Phialomyces* due to its integrated conidiogenous cells, which in *Phialomyces* are discrete. *Cladophialophora* has holoblastic conidiogenesis.

6.3.2 *Marinophialophora garethjonesii* J.F. Li, Phookamsak, Dayarathne & K.D. Hyde, sp. nov.

Index Fungorum number: F552734; Facesoffungi number: FOF 02833

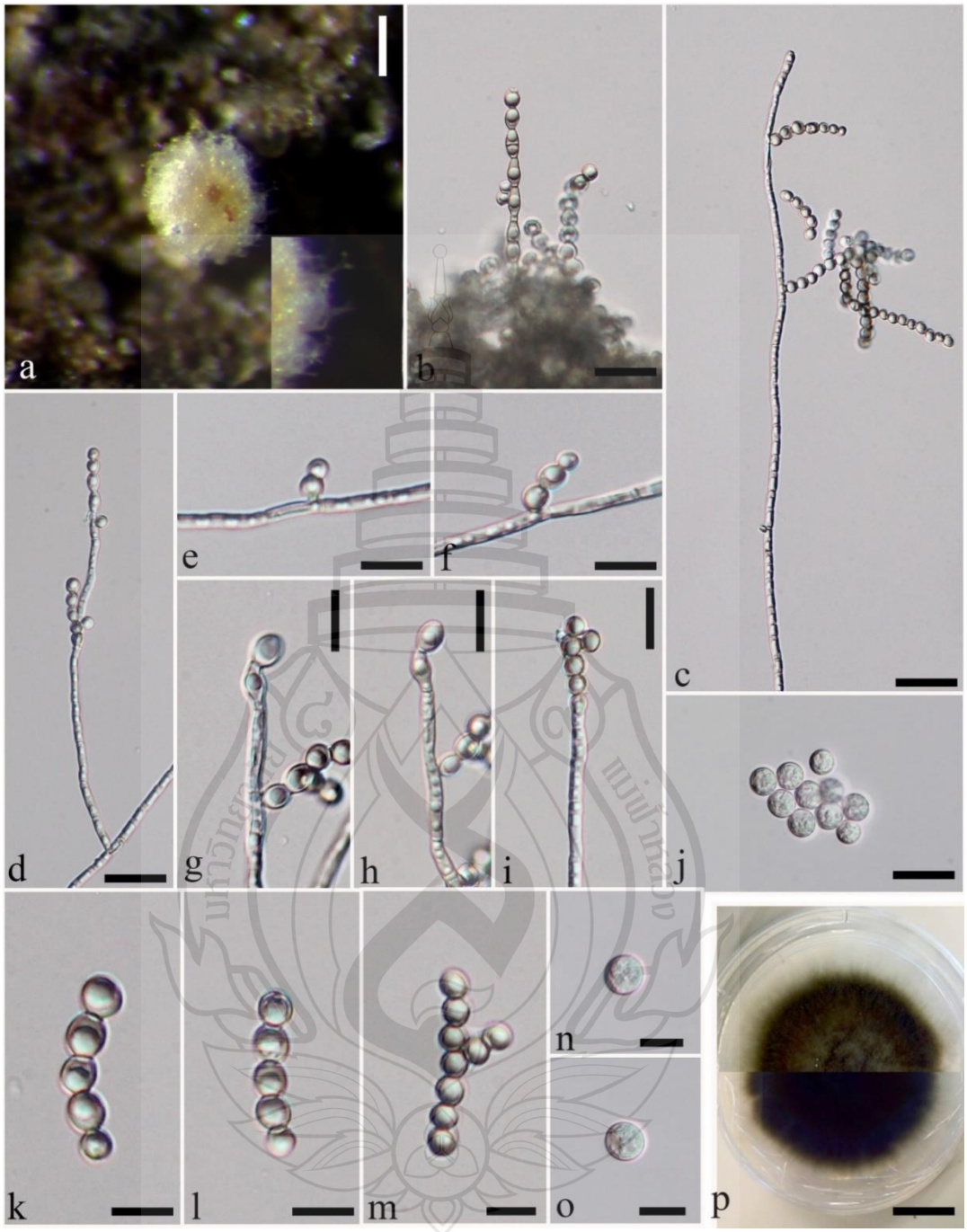
Etymology: Named in honour of the mycologist Professor E.B. Gareth Jones, in recognition of his contribution to the study of marine fungi.

Holotype: MFLU 16-2821

Hyphomycetous associated with *Halocyphina* species on decaying mangrove wood. Sexual morph: Undetermined. Asexual morph: *Mycelium* partly superficial, partly immersed on the substrate, composed of septate, branched, smooth, thin-walled, hyaline to subhyaline hyphae. *Conidiophores* 75–135 × 1.1–2 µm ($\bar{x}$ = 105 × 1.6 µm, n = 20), macronematous, mononematous, arising from hyphae, hyaline to subhyaline, thin-walled, smooth, aseptate, unbranched to branched, straight, cylindrical. *Conidiogenous cells* 1.7–2.5 × 1.3–1.9 µm ($\bar{x}$ = 2.1 × 1.7 µm, n = 20), phialidic, integrated, terminal or intercalary, determinate, hyaline to subhyaline, globose to ellipsoidal, smooth, without a conspicuous collarette. *Conidia* 1.3–1.5 × 1.4–1.7 µm ($\bar{x}$ = 1.4 × 1.6 µm, n = 20), phialoconidia, hyaline to subhyaline, globose to subglobose, aseptate, borne in basipetally developing unbranched or branched chains, clavate, smooth, thin-walled.

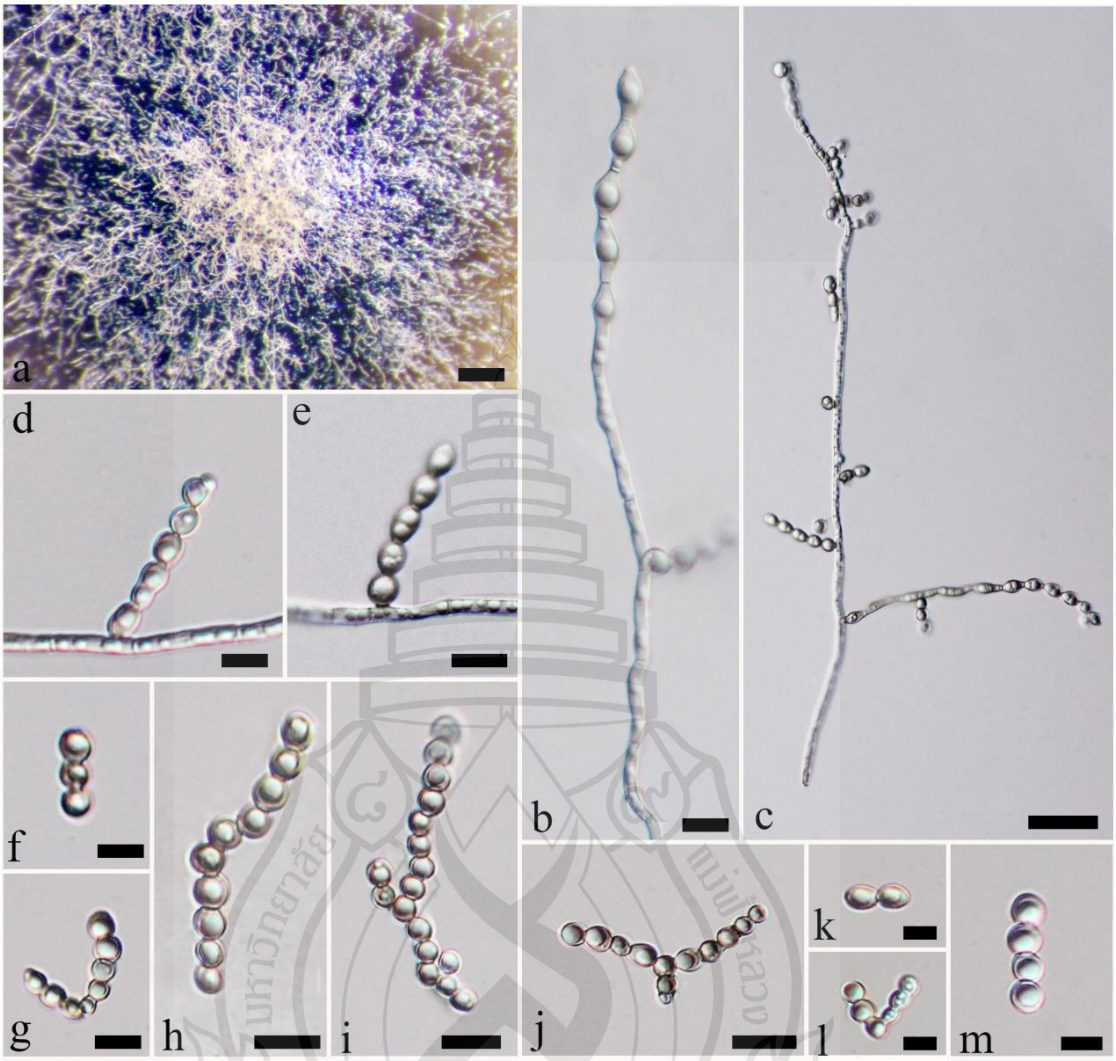
Cultural characteristics: Conidia germinating on PDA within 12 hrs at 25ºC, germ tubes produced from apex. Colonies growing on PDA reaching 5 mm diam. after 2 weeks at 25ºC, mycelium semi-immersed to superficial, irregular in shape, flat, slightly raised, with trim edge, slightly rough on surface, hairy to powdery, cottony from above, initially dark brown, becoming black after 2 weeks; from below, always black.

Material examined: THAILAND, Phetchaburi Province, Hat Chao Samran, associated with *Halocyphina* sp. (*Niaceae*, Agaricamycetes), on intertidal decaying wood of mangrove stand, 28 August 2015, Monika C. Dayarathne, H-42 (MFLU 16-2821, holotype; HKAS96301, isotype), ex-type living culture, KUMCC 16-0066, MFLUCC 16-1449.



Note a. Appearance of mycelium growing on *Halocyphina* on mangrove wood. b Hyphae on *Halocyphina*. c–d. Conidiophores bearing conidia. e–i. Conidiophores with conidiogenous cells. k–m. Conidia in chain. j, n–o. Conidia. p. Culture characteristic on PDA. Scale bars: p = 1 cm, a = 200 μm, b–d, i = 10 μm, e–h, j–m = 5 μm, n–o = 2 μm.

Figure 6.1 Morphology of *Marinophialophora garethjonesii* (MFLU 16-2821)



Note a. Sporulating culture on PDA. b–c. Hyphae with conidiophores and conidiogenous cells. d–e. Conidiogenous cells producing conidia. f–m. Conidia. Scale bars: a = 100 µm, c = 10 µm, h–j = 5 µm, b, d–g, m = 2 µm, k–l = 1 µm.

Figure 6.2 Morphology of sporulation of *Marinophialophora garethjonesii* (KUMCC 16-0066, from ex-type culture)

6.4 Phylogenetic Relationships of *Marinophialophora garethjonesii*

The combined ITS, LSU and SSU dataset comprises 39 taxa, (with *Cyphellophra laciniata* (CBS 190.61) as the outgroup taxon) analysed either under ML or Bayesian criteria resulted in trees which were topologically congruent with respect to the position of the new taxa investigated herein. Figure 3 represents the phylogram generated under the ML analysis (loglikelihood: -11550.999264). Bootstrap support values for maximum likelihood (black) and Bayesian posterior probabilities (red) greater than 70% and 0.90 are given above the nodes. Species collected in this study are supported in the multigene phylogenetic analyses as members of Herpotrichellaceae in Chaetothyriales (Liu et al., 2015).

In our phylogenetic reconstructions, the new species *Marinophialophora garethjonesii* forms a distinct clade with reasonable statistical support. Our 2 new strains cluster together with very good support but its affinities to other genera, such as *Exophiala* received weak support. This provides further evidence to support the new taxon as a new genus in family Herpotrichellaceae (Chaetothyriales, Eurotiomycetes).

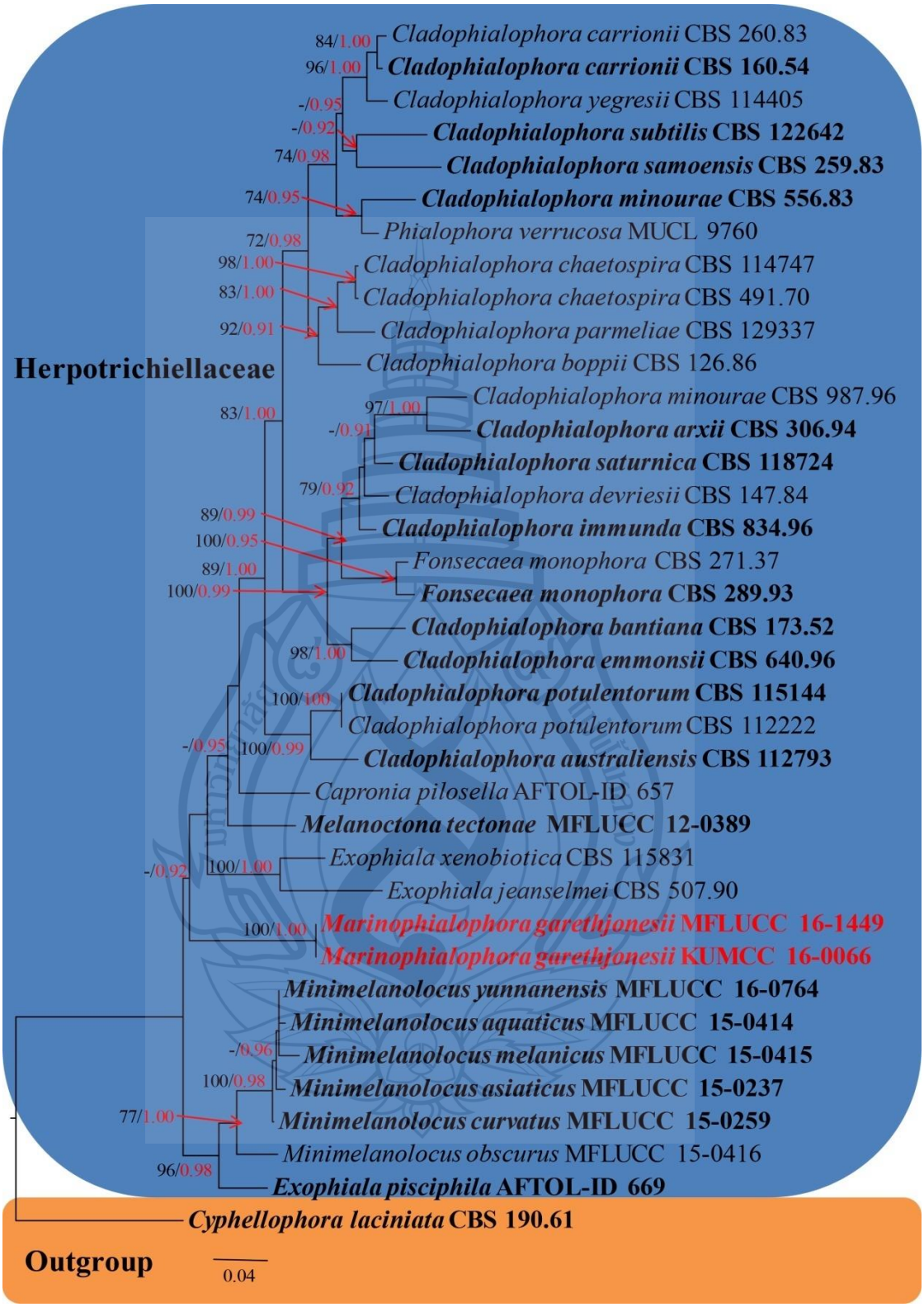


Figure 6.3 Phylogenetic construction based on RAxML analysis of a combined multigene

Figure 6.3 Phylogenetic construction based on RAxML analysis of a combined ITS, LSU and SSU dataset. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 70 % and Bayesian posterior probabilities (PP, red) equal to or greater than 0.90 are shown above the nodes. The tree is rooted to *Cyphellophra laciniata*. The type strains are in black bold and the newly generated sequences are indicated in red bold.

6.5 Discussion

Since Johnson and Sparrow (1961) classified marine fungi, a number of taxonomic studies targeting marine fungi have revealed that the marine environment and marine plants harbour a huge diversity of new fungal species (Kohlmeyer & Kohlmeyer, 1979; Swe et al., 2008; Huang et al., 2017). There has also been an impressive increase in molecular phylogenetic data for marine fungi and this has largely contributed to provide further taxonomic insights into their classification (Jones et al., 2009; Suetrong et al., 2009; Hyde et al., 2013, 2016). Jones et al. (2009) were the first to properly document the taxonomy of marine fungi with full details on phylogenetic relationships. Sakayaroj et al. (2011) and Pang and Jheng (2012) provided keys to genera of the Halosphaeriaceae, where DNA sequence data and phylogenetic data are available for about 75% of species. Another major group of fungi has recently been well investigated based on morphology and phylogeny are marine Dothideomycetes (Suetrong et al., 2009; Hyde et al., 2013, 2016). Further phylogenetic studies on the taxonomy of marine fungi include those by Wijayawardene et al. (2014), Ariyawansa et al. (2015), Li et al. (2016); Maharachchikumbura et al. (2015) and Senanayake et al. (2015) on the Sordariomycetes and Jones et al. (2015) on Ascomycota, Basidiomycota, Blastocladiomycota and Chytridiomycota. A recent revision of marine fungi has led to an estimate of more than 10,000 species (Jones, 2011). This would obviously means that there are a number of species there awaiting to be discovered.

During our study on fungal diversity in Thailand, we collected a new species with unique morphology from the surface of *Halocyphina* sp. on intertidal decayed wood of mangrove stand. Close microscopic examinations revealed that our species is interesting due to its particular characters that could not be ascribed to any known taxa. In addition,

we also noted morphological differences among extant phialophorioid and cladophialophorioid genera and hence a new monotypic genus, *Marinophialophora* is introduced. Our molecular phylogeny based on a concatenated dataset also provides further evidence to support the new genus within the family Herpotrichellaceae (Chaetothyriales, Eurotiomycetes). In particular, both strains of *M. garethjonesii* cluster with each other with high support and constitute an independent lineage basal to the genus *Exophiala* (Figure 6.3). The latter exhibit asexual morphs as well but its host and morphological characters are very distinct from *Marinophialophora* and hence not comparable. Furthermore, the ITS and LSU sequences of our species were also compared with closely related taxa and sufficient differences are noted to warrant generic rank. The new taxon was isolated from the surface of *Halocyphina* sp., but at the moment there is insufficient evidence to demonstrate that the new species could be a hyperparasite on *Halocyphina* sp. It is interesting to note that the new species was incubated in sterilized marine water and it was observed that the mycelium grew well and formed a hairy floc in the marine water.

CHAPTER 7

FIFTEEN NEW SPECIES AND THREE NEW RECORDS OF HYPHOMYCETES ON PLANT LITTERS[6]

Abstract

This study reported the discovery of fifteen new species, (*Acremonium chiangraiensis*, *Alternaria doiliconidium*, *A. italica*, *Curvularia palmicola*, *Corynespora doipuiensis*, *Pseudohelicomyces menglunicus*, *Lophium zalerioides*, *Myrothecium septentrionale*, *Periconia palmicola*, *Pseudocercospora maetaengensis*, *Pse. rosae*, *Pseudopithomyces palmicola*, *Pse. chartarum*, *Pse. maydicus*, *Vamsapriya breviconidiophora*), three new records *Hermatomyces sphaericus* (*H. chromolaenae*), *H. krabiensis* (*H. chiangmaiensis*), and *Nigrospora oryzae* were reported respectively in Dothediomycetes and Sodariomycetes, from China, Russia and Thailand were reported.

[6] This paper has been published in Fungal diversity, 2015, 75(1), 27–274. doi:10.1007/s13225-015-0346-5; in Fungal diversity, 2017, 83(1), 1–261. doi:10.1007/s13225-017-0378-0; in Fungal Diversity, 2017, 87(1), 1–235. doi:10.1007/s13225-017-0391-3; in Fungal diversity, 2018, 89(1), 1–236. doi:10.1007/s13225-018-0395-7; in Fungal Diversity, 2019, 95, 1–273. doi:10.1007/s13225-019-00421-w; in Fungal Diversity, 2020, 100, 5–277. doi:10.1007/s13225-020-00439-5; in Asian Journal of Mycology, 2020, 3(1), 22–294. doi:10.5943/ajom/3/1/3

7.1 Introduction

Pleosporales is the largest order in the Dothideomycetes, comprising a quarter of all species in Dothideomycetes (Kirk et al., 2008). Members in class Dothideomycets occur in various habitats, both in Terrestrial and aquatic, while can be endophytes or parasites, epiphytes and saprophytes of living plants and animals, hyperparasites on fungi or insects, lichenized. (Kruys et al., 2006; Zhang et al., 2012). Asexual morphs of Pleosporales are consists of coelomycetes and hyphomycetes. Phoma or Phoma-like anamorphic stages are indicated as most common anamorphs of species in the order (Aveskamp et al., 2010; de Gruyter et al., 2009, 2010; Hyde et al., 2011; Zhang et al., 2012). Some species in order Pleosproles are reported connection of their sexual and asexual morphs, based on the phylogeny and anamorphic stages on single spore isolated cultures (Hyde et al., 2011; Zhang et al., 2012). The phylogenetic studies of Pleosporales, the genes applied mostly including LSU, SSU, SSU and ITS gene, as well as the protein genes, such as RPB2 and TEF-1α (Hyde et al., 2011, 2016, 2017, 2019, 2020; Phookamsak et al., 2015, 2019; Zhang et al., 2012). In this study, eight new species and two new geographical records within Pleosporales were reported on different plant hosts from China, Italy and Thailand.

The order Tubeufiales was introduced by Boonmee et al. (2014) to accommodate the type family Tubeufiaceae based on phylogenetic analyses, which is a large family comprising over 20 genera of freshwater ascomycetes (Doilom et al., 2017; Lu et al., 2018). The majority of tubeufiaceous species is generally have superficial, globose to subglobose, light to dark pigmented ascomata, often with aerial mycelium and/or setae, cylindrical, clavate asci, multi-septate hyaline ascospores; asexual morph as hyphomycetous, often helicosporous conidia (Kodsueb et al., 2006; Boonmee et al., 2011, 2014; Lu et al., 2018). Members in this order are widely occurs on woody substrates in terrestrial and freshwater habitats (Hyde & Goh, 1998; Ho et al., 2001; Cai et al., 2002). In this work, a new species *Pseudohelicomyces menglunensis* on seed coats from China is decribed.

Species in order Mycosphaerellales are one of the largest and most diverse groups of hyphomycetes and cause a wide range of diseases, on various of hosts such as vegetables, fruits, cereals and other wild plants. Mycosphaerella-like hyphomycetes are generally referred to as cercosporoid fungi (Crous & Braun, 2003; Braun et al., 2013; Meswaet et al.,

2021). Cercosporoid fungi are morphological as dematiaceous hyphomycetes is having conidiophores formed arranged in sporodochia or in synnemata, singly or in groups, with integrated, terminal or intercalary conidiogenous cells (Crous & Braun, 2003). In this work, two new taxa in genus *Pseudocercospora* (Mycosphaerellaceae, Mycosphaerellales) were isolated from plant issue in China and Thailand.

Mytilinidioid fungi was historically classified within the family Hysteriaceae, based on morphologically having ascocarp morphology, specifically its means of longitudinal dehiscence (Boehm et al., 2009). Fungi in the Mytilinidiaceae morphologically in hving laterally compressed, connivent, thin-walled conchate ascomata, reminiscent of miniature bivalve molluscs. These mytilinidioid ascomata typically dehisce by an evaginated slit, in some species forming a longitudinal keel or cristate apex (Barr, 1990). This family was placed in the Melanommatales by Barr (1987) based on characterized a thin-walled peridium of scleroparenchymatous cells enclosing a hamathecium of narrow trabeculate pseudoparaphyses, asci borne in a peripheral layer and with ascospores typically showing bipolar symmetry. Boehm et al. (2009) refined the genera *Mytilinidion* and *Lophium* based on phylogeny analyses, which these genera formed a strongly supported clade within the monophyletic Mytilinidiaceae, of which the Mytilinidiales was established. In this work, a new species *Lophium zalerioides* in family Mytilinidiaceae (Mytilinidiales) from Italy are reported.

Sordariomycetes is one of the largest classes of Ascomycota. Members in this order are widely distributed and occur in a variety of habitats, including plant and/or animal pathogenic, endophytic, saprobic, epiphytic, coprophilous, fungicolous and lichenicolous taxa (Kruys et al., 2014; Hongsanan et al., 2016; Jayawardena et al., 2019; Luo et al., 2019). Asexual sordariomycetous fungi are generally reported as ceolomycetes and hyphomycetes (Maharachchikumbura et al., 2016). In this work, tree new species *Acremonium chiangraiensis* (Bionectriaceae, Hypocreales), *Myrothecium septentrionale* (Stachybotryaceae, Hypocreales), *Vamsapriya breviconidiophora* (Xylariaceae, Xylariales) and a new record *Nigrospora oryzae* (Apiosporaceae, Xylariales) are collected on different plant hosts from Thailand.

7.2 Materials and Methods

Materials and methods follow the previous study (Hyde et al., 2016; Tibpromma et al., 2017; Phookamsak et al., 2019). Taxa described in this study were collected from China, Italy and China. Taxa were described and illustrated based on morphological features coupled based on phylogenetic analyses performed by maximum likelihood and Bayesian inference criteria. The new taxa are justified based on guidelines of Jeewon and Hyde (2016).

7.3 Phylogeny and Taxonomy

7.3.1 Pleosporales

7.3.1.1 *Alternaria doliconidium* J.F. Li, Camporesi & K.D. Hyde, sp. nov.

Index Fungorum number: IF554202; Facesoffunginumber: FoF 04041.

Etymology: Named after its doliiform conidia

Holotype: HKAS100840.

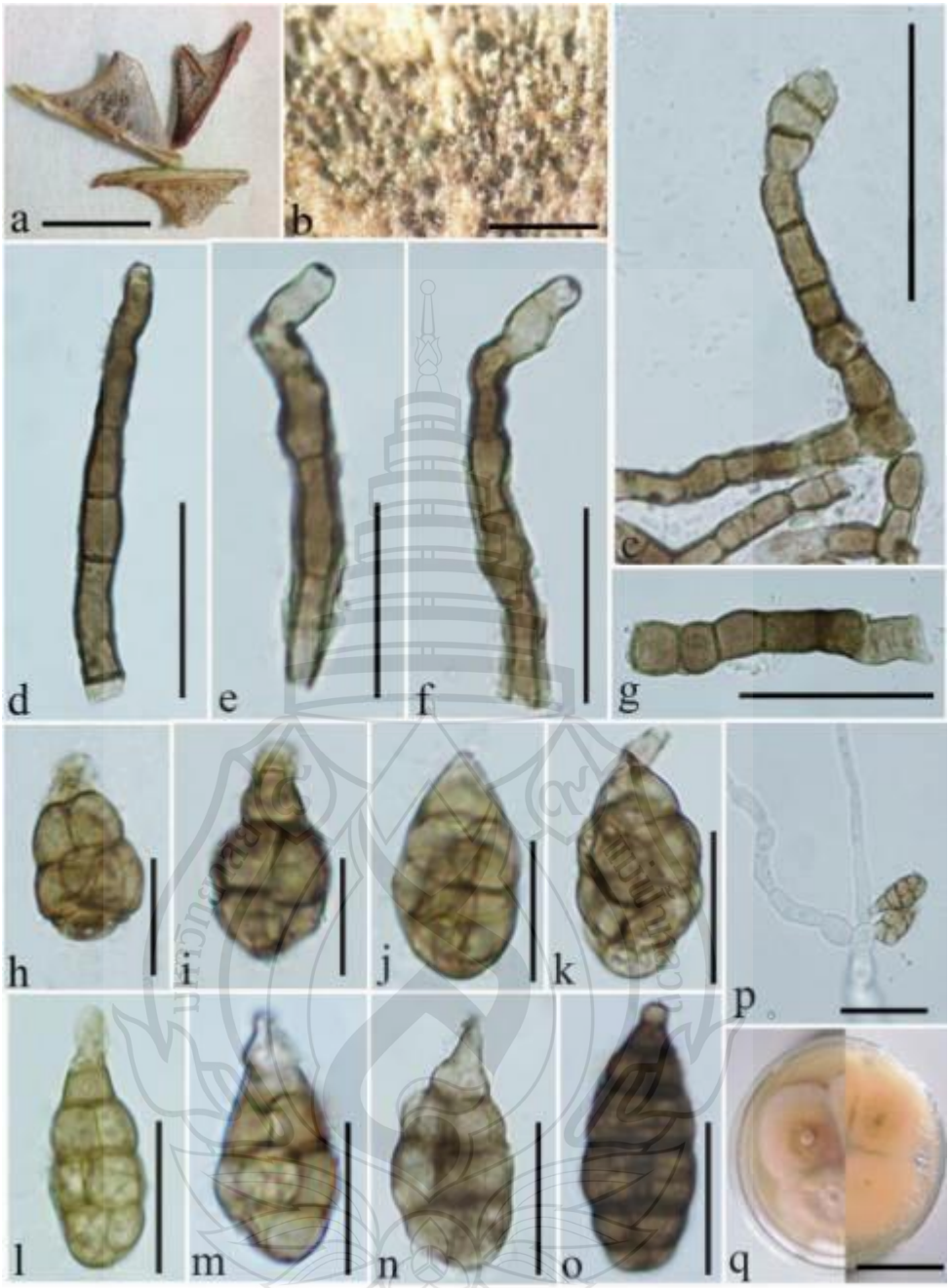
Saprobic on spines of *Rosa canina*. Sexual morph: Undetermined. Asexual morph: Mycelium superficial on the substrate, composed of septate, branched, smooth, thin-walled, white to light pink coloured hyphae Conidiophores (105–)114.2–126(–129.5) µm long × (8.7–)8.9–9.6(–11.4) µm diam ($\bar{x}$ =121.4µm× 9.2 µm, n = 100), macronematous, mononematous, light brown to brown, thick-walled, smooth, septate, branched on base, straight or flexuous, cylindrical. Conidiogenous cells (6.2–)6.8–9.5(–11.5) µm long × (12.5–)14.8–15.4(–17) µm diam ($\bar{x}$ = 8.5µm × 15.2 µm, n = 100), monoblastic, integrated, terminal, determinate or percurrent, cylindrical, doliiform, subhyaline, smooth, colourless, thin-walled. Conidia (58.2–)62.2–79(–81.2) µm long× (22.5–)27–31.2(–32.5) µm diam ($\bar{x}$ =68.6 µm× 31.7µm, n = 100) acrogenous, holoblastic, solitary, brown to dark brown, multiseptate, dry, simple, straight, curved, fusiform, obpyriform or obturbinate, subglobose, borne in chain, sometimes rostrate, unsmooth, thin-walled.

Cultural characteristics: Conidia germinating on PDA within 14 h and germ tubes produced from upper cells. Colonies growing on PDA, hairy or cottony, white to light brown, reaching 5 mm in 15 days at 25ºC, mycelium superficial, effuse, radially striate, with regular edge, white to light pink coloured hyphae; Asexual spores produced within 15 days; Sexual spores not formed within 60 days.

Material examined: ITALY, Province of Forli-Cesena [FC], Raggio di Santa Sofia, on dead aerial spines of *Rosa canina* L. (Rosaceae), 16 October 2014, Erio Camporesi 2165 (HKAS100840), living culture = KUMCC 17-0263.

GenBank numbers: ITS: MG828864, LSU: MG828980, SSU: MG829094.

Notes: *Alternaria doliconidium* is described here as new species and is unique based on its dark coloured, doliiform to subglobose, multi-septate conidia and conidiophores with swollen knots. In the phylogenetic analyses, *A. doliconidium* clusters with *A. aborescens* with high support. This is also the first record *Alternaria* sp. found on *Rosa canina*.



Note a. Plant tissue. b. Colonies on dead stem. c. Conidiophores with Conidiogenous cells. d-g.Conidiophores. h-o. Conidia. p. Germinated spore. q. Cultures on PDA media. Scale bars: a=0.5cm, b =500µm, c=100µm, d-p=50µm

Figure 7.1 Morphology of *Alternaria doliconidium* (HKAS100840)

7.3.1.2 *Alternaria italica* J.F. Li, Camporesi & K.D. Hyde sp. nov.

Index Fungorum numbers: IF553987, Facesoffungi number: FoF 03880

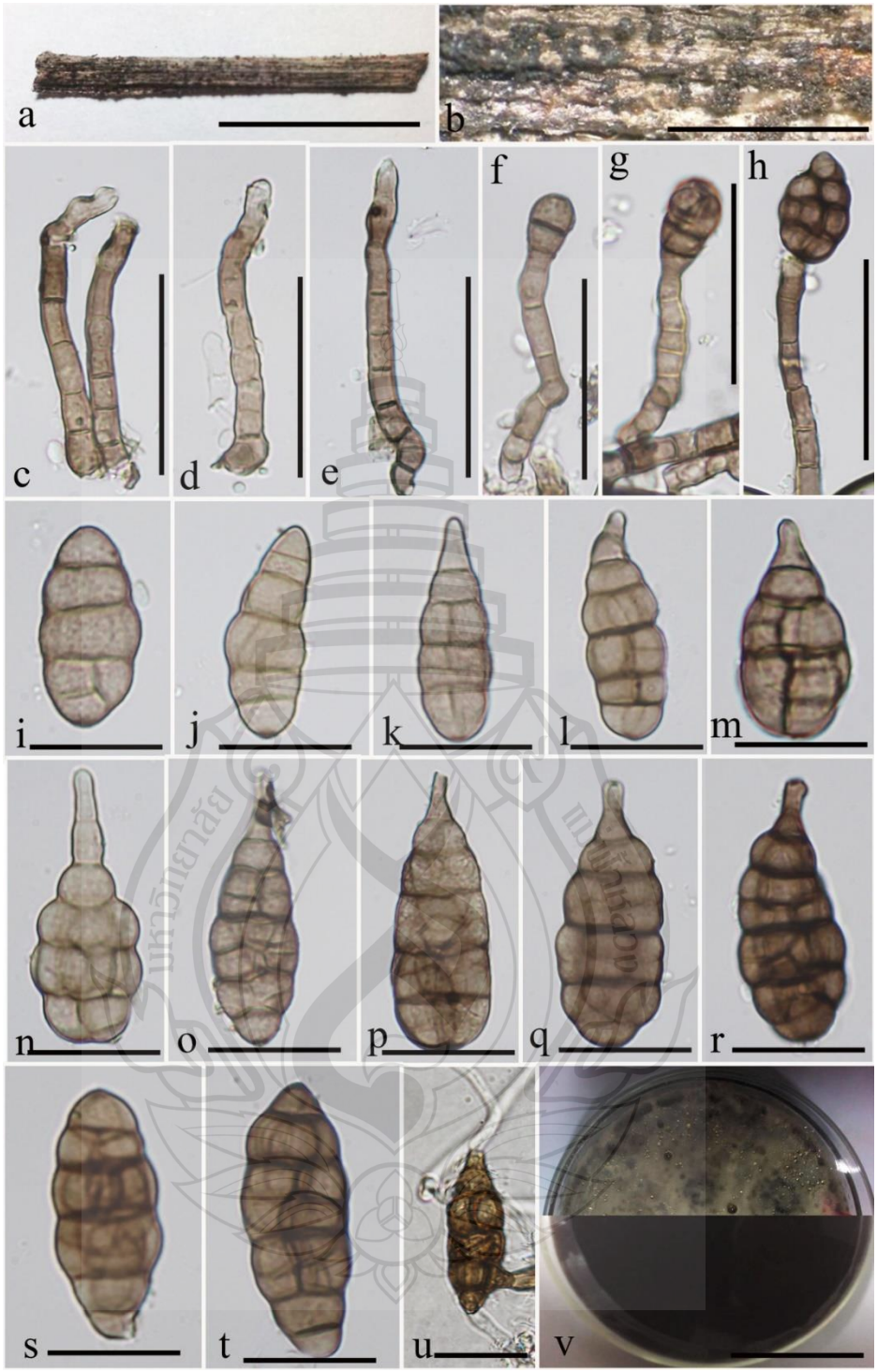
Holotype: HKAS 100717

Saprotrophic on dead and aerial branch of *Vitis vinifera*. Sexual morph: Not observed. Asexual morph: Mycelium superficial on substrate, composed of septate, branched, smooth, thin-walled, brown to dark brown hyphae. Conidiophores 250–300×17–21 µm (x =278×18 µm, n=100), macronematous, mononematous, light brown to brown, thick-walled, smooth, septate, branched at the base, straight or flexuous, cylindrical. Conidiogenous cells 8–13×9–15 µm (x =10×11 µm, n=100), cicatrized, monoblastic, integrated, terminal, determinate or percurrent, cylindrical, doliiform, subhyaline, smooth, thin-walled. Conidia 76–98×23–39 µm (x =86×32 µm, n=100), acrogenous, holoblastic, solitary, light grey to brownish grey, 8–11-septate or more, dry, simple, straight, curved, fusiform, obpyriform, obturbinate or subglobose, catenate, sometimes rostrate, smooth, thin-walled.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies growing on PDA, hairy or cottony, brown to dark brown, reaching 5 mm in 20 days at 25ºC, mycelium superficial, effuse, radially striate, with irregular edge, brown to dark brown hyphae; asexual spores and sexual spores not formed within 60 days.

Material examined: ITALY, Province of Forlì-Cesena, Via Pietro Nenni – Forlì, on dead and aerial branch of *Vitis vinifera*, 12 November 2013, Erio Camporesi, IT 1513 (HKAS 100717 -holotype), culture MFLUCC 14–0421= KUMCC 17-0123.

Notes: Based on morphology and multigene phylogentic support we introduce *A. italica* to accommodate the new taxon.



Note a. Host tissue. b. Colonies on dead branch. c-e. Conidiophores. f-h. Conidiophores with Conidiogenous cells. i-t. Conidia. u. Germinated conidia. v. Culture on PDA media. Scale bars: a=1cm, b=1000µm, c-h=100µm, i-u=20µm, v=5cm.

Figure 7.2 Morphology of *Alternaria italica* (HKAS 100717)



Figure 7.3 Phylogenetic tree of the genus *Curvularia*

Figure 7.3 Phylogram generated for *Curvularia* from a maximum likelihood analysis based on the combined ITS, GPDH and TEF alignment. Maximum likelihood bootstrap values are shown above the branches. Newly sequence strain is in red bold. All ex-type cultures are printed in balck bold.

7.3.1.3 *Curvularia palmicola* J.F. Li, Phookamsak & K.D. Hyde sp. nov.

Index Fungorum number: IF553198, Facesoffungi number: FoF: FoF 03284

Etymology: The name palmicola, refers to the common host name and Latin cola meaning loving.

Holotype: MFLU 15-0913

Saprobic on branches of *Acoelorrhaphe wrightii* Griseb. & H. Wendl. (Arecaceae). Sexual morph: Undetermined. Asexual morph: *Colonies* effuse, dark brown to black, superficial, dry. *Mycelium* superficial or partly immersed on the substrate, composed of septate, branched, smooth, thin-walled, hyaline hyphae. *Conidiophores* 94–157 µm long × 4.7–7.1 µm diam ($\bar{x}$ = 124.6 × 6.3 µm, n = 10), micronematous, mononemous, partly packed together, light brown, thick-walled, septate, smooth, unbranched. *Conidiogenous cells* 5.5–8.7 µm long × 2.12–4.7 µm diam ($\bar{x}$ = 6.8 × 3.2 µm, n = 10), monoblastic, integrated, terminal, determinate, light brown, wide in the middle. *Conidia* 23.9–34.7 µm long × 9.3–15.7 µm diam ($\bar{x}$ = 29.9 × 13.7 µm, n = 10), acrogenous, solitary, simple, obconical, curyed, subhyaline to moderately brown, acrogenous, pseudoseptate, guttulate, thick-walled, smooth, with a dark basal scar, wide at below half, narrow and elongated at above half.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from top end. Colonies growing on PDA, reaching 5 mm in 10 days at 30ºC, mycelium partly superficial, partly immersed, slightly effuse, radially striate, with irregular edge, dark brown to black; sexual or asexual spores not formed within 60 days.

Material examined*:* THAILAND, Chiang Rai Provice, Khorn Khun water fall, on dead branches of *Acoelorrhaphe wrightii* (Arecaceae), 9 Janurary 2014, Junfu Li, H-19 (MFLU 15-0913, holotype), ex-type living culture MFLUCC 14-0404, KUMCC (isotype in HKAS).

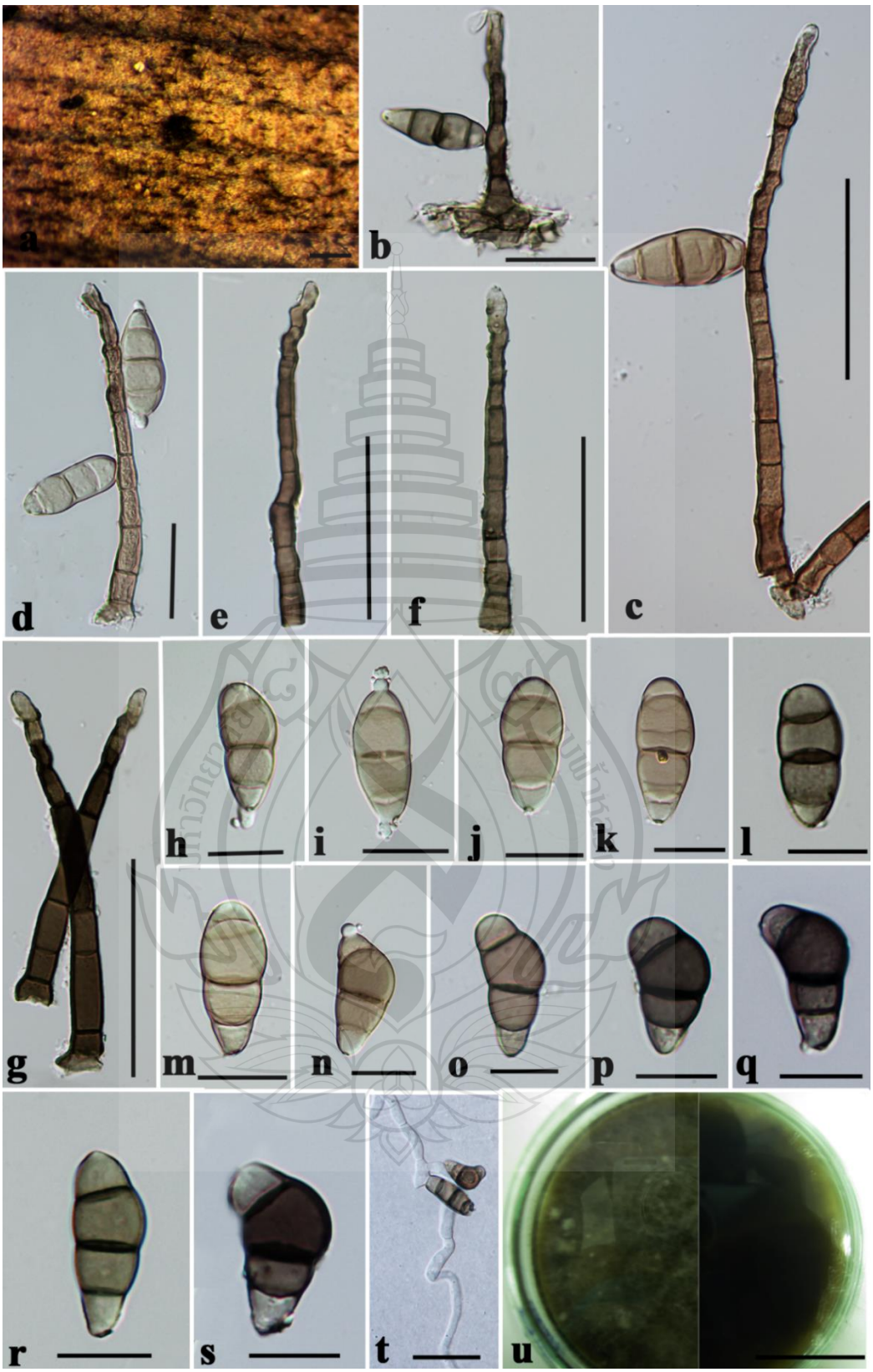


Figure 7.4 Morphology of *Curvularia palmicola* (MFLU 15-0913)

Figure 7.4 a. Colonies on branch. b. Conidiogenous cells and Conidiophores bearing conidia. c–d. Conidiophores bearing conidia. e–g. Conidiophores. h–s. Conidia. t. Germinated spore. u. Culture. *Scale bars* a = 200µm, b = 20µm, c, e–g = 50µm, d–f = 20µm, h–s = 10µm, t = 20µm, u = 3cm.

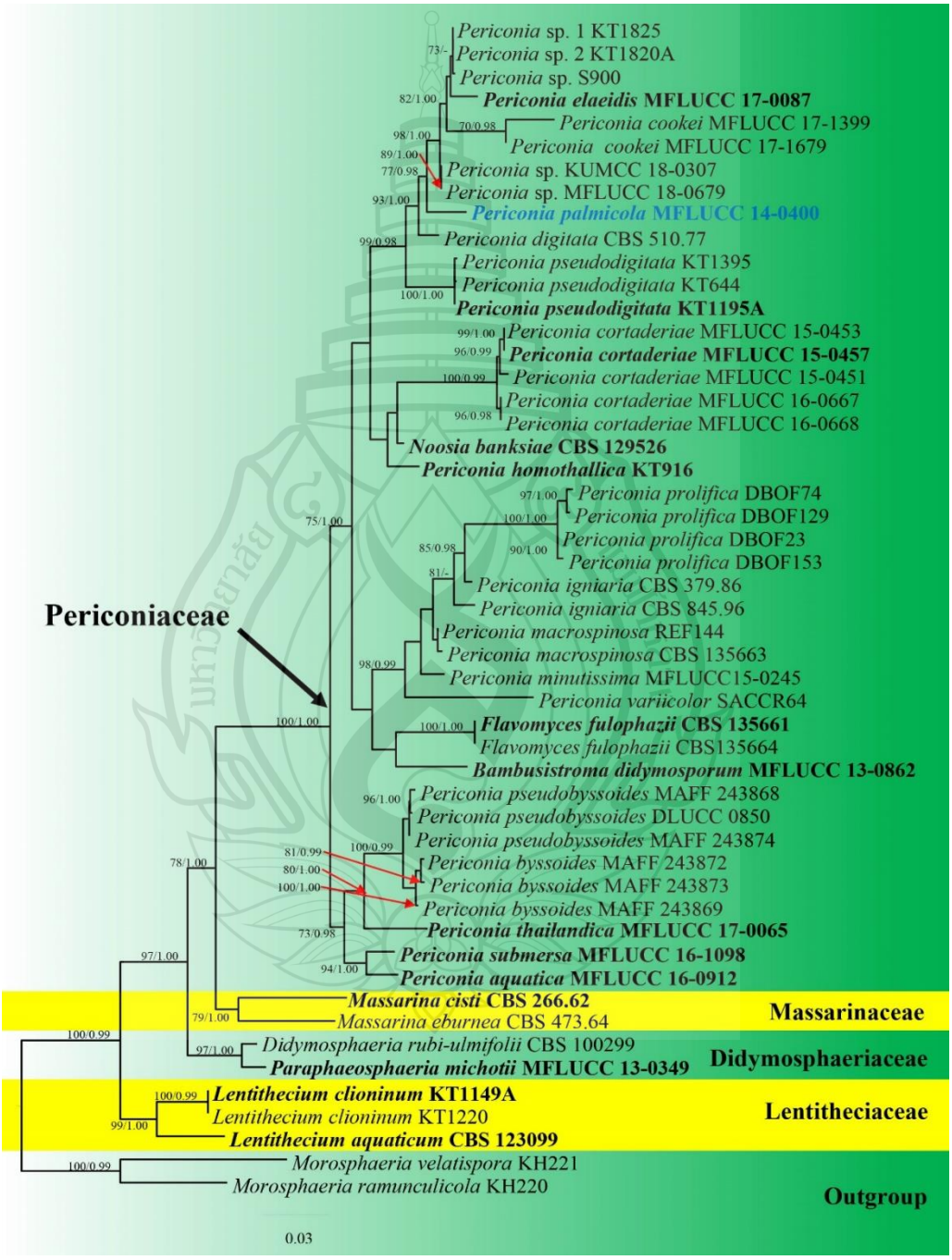


Figure 7.5 Phylogeny of the family Periconiaceae

Figure 7.5 Phylogram generated from the best scoring of the RAxML tree based on combined ITS, LSU and TEF1-α sequenced data of taxa in Periconiaceae and other related families (Massarinaceae, Didymellaceae and Lentitheciaceae). Taxa in Morosphaeriaceae, *Morosphaeria ramunculicola* (KH 220) and *M. velatispora* (KH 221) were selected as the outgroup taxa. The best RAxML tree with a final likelihood value of -13065.728774 is presented. RAxML analysis yielded 982 distinct alignment patterns and 28.84% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.245718, C = 0.219687, G = 0.310546, T = 0.224049, with substitution rates AC = 1.522214, AG = 2.988589, AT = 1.846846, CG = 0.504980, CT = 12.777992, GT = 1.000000. The gamma distribution shape parameter alpha = 0.317094. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with final average standard deviation of split frequencies = 0.008294. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 70%; BYPP (red) equal to or greater than 0.95 are given above or below the nodes. Type sequences are in black bold and newly generated sequences are indicated in blud bold.

7.3.1.4 *Periconia palmicola* J.F. Li & R. Phookamsak, sp. *nov*.

Indexfungorum number: IF556889, Facesoffungi number: FoF07053

Etymolgy: The specific epithet "palmicola" refers to the host palm (unidentified), of which the fungus was living.

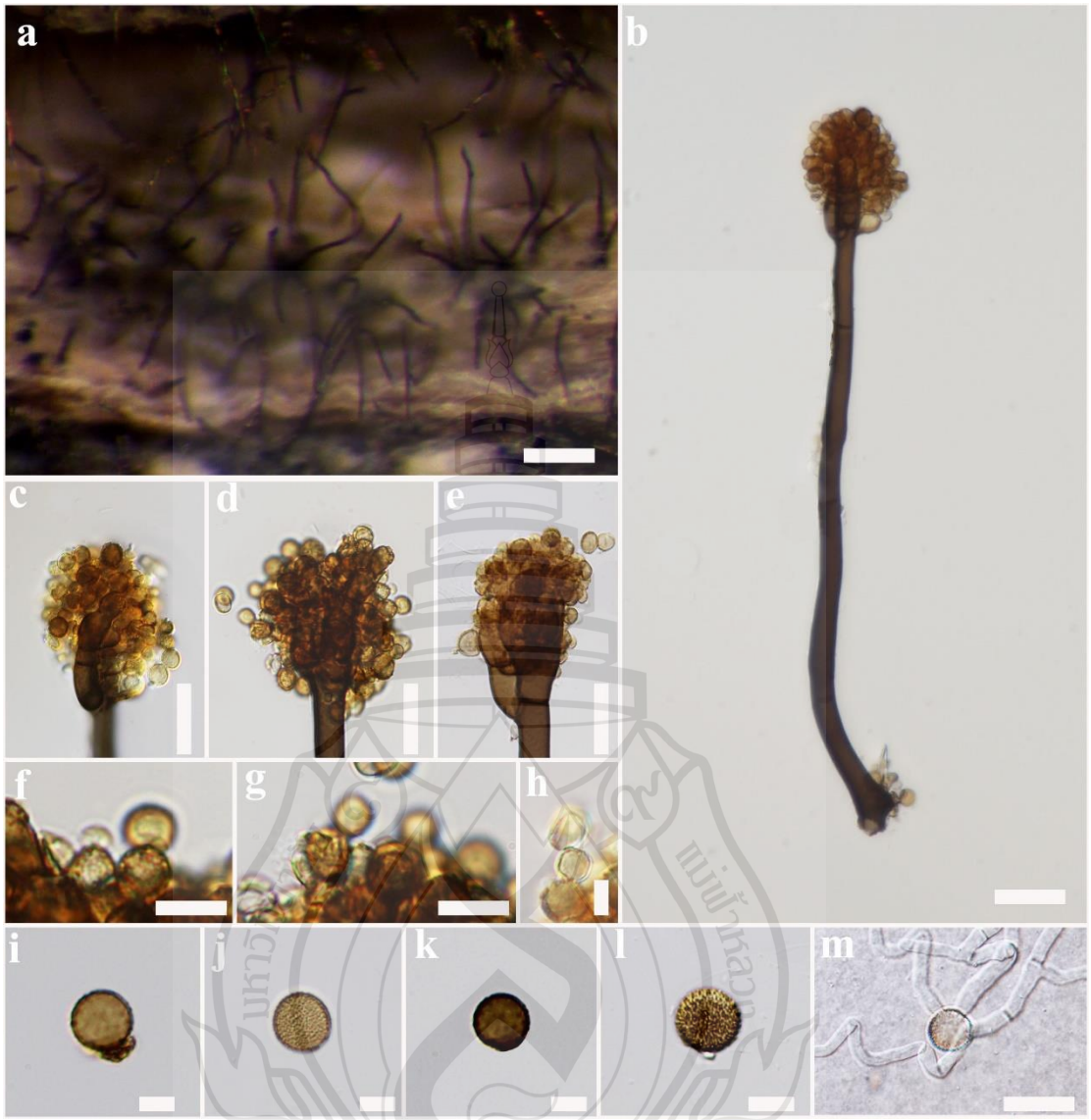
Holotype: MFLU 14-0198

Saprobic on dead, fallen leaves of unidentified palm. Sexual morph Undetermined. Asexual morph Colonies on the substratum superficial, numerous, effuse, black, floccose. Mycelium immersed on the substrate, composed of septate, branched, smooth, dark-colored hyphae. Conidiophores 151–188 µm long × 5.6–8 µm wide ($\bar{x}$ = 175.4 × 6.8 µm, n = 10), macronematous, mononematous, septate, partly forming sphaerical heads and branched at apex, erect, mostly slightly flexuous to curved, dark brown to black. Conidiogenous cells 3–3.5 µm long × 3–4.8 µm wide ($\bar{x}$ = 3.2 × 4 µm, n = 10), mono- to polyblastic, discrete, terminal, globose, hyaline to subhyaline. Conidia 5.1–7.4 µm long × 4.8–6.1 µm wide ($\bar{x}$ = 6.2 × 5.5 µm, n = 20), solitary to catenate, in acropetal chains, subglobose to globose, light brown to brown, verruculose.

Culture characteristic: Conidia germinating on PDA within 14 h and germ tubes produced from top cells. Colonies growing on PDA, hairy or cottony, white to grey, reaching 5 mm in 20 days at 25°C, mycelium superficial, effuse, radially striate, with regular edge, subhyaline to pale white coloured hyphae; Asexual spores and sexual spores were not formed within 60 days.

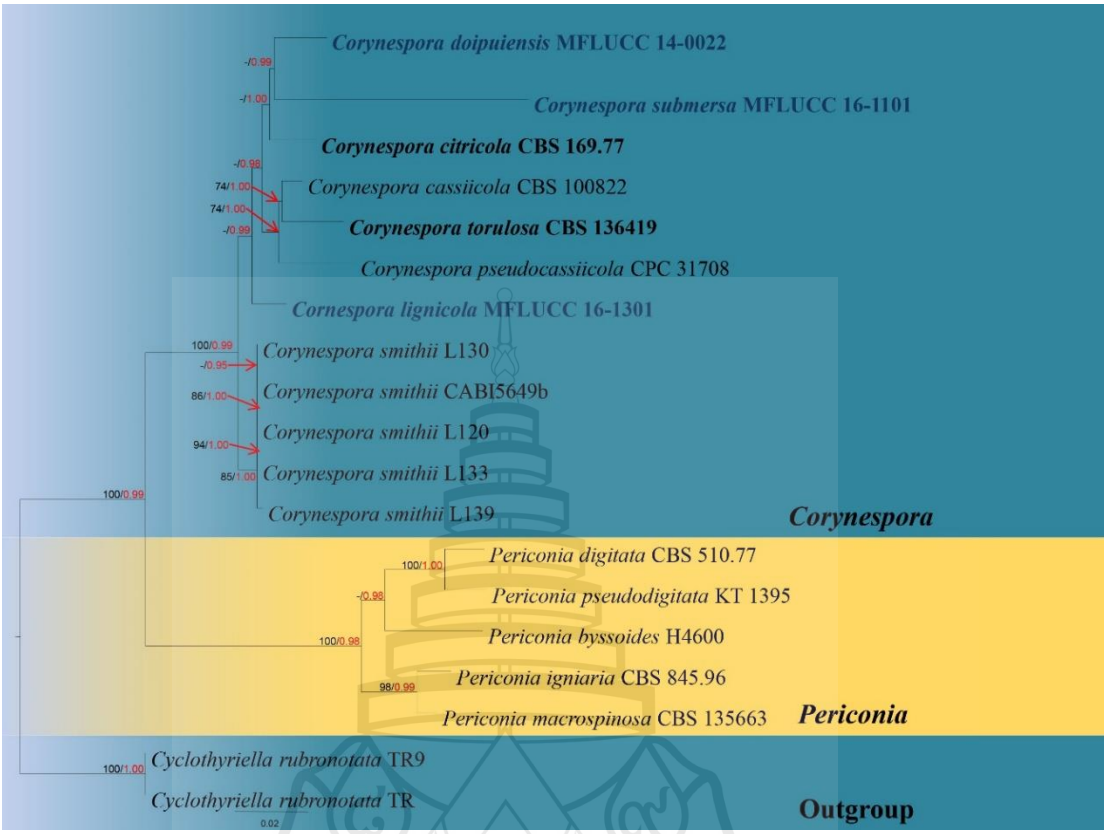
Material examined: Thailand, Chiang Rai Province, Mae Fah Luang University, Herb Garden, on dead, fallen leaves of palm, 28 December 2013, Junfu Li, H-13 (MFLU 14-0198, holotype), ex-type living culture MFLUCC14-0400, KUMCC.
GenBank numbers: LSU: MN648327, SSU: MN648319, TEF1-α: MN821070.

Notes: *Periconia palmicola* described herein as a new species. *Periconia palmicola* is unique in its colorless conidiogenous cells and dark colored conidiophres. The phylogenetic of combined ITS, LSU and TEF1-α, Periconia palmicola formed a independent clade to *P. digitata* (77% ML, 0.98 BYPP). Therefore, a new species, *Periconia palmicola* is established.



Note a Appearance of fungal colonies on host substrate. b Conidiophores. c–e Closed up conidiophores attached with conidiogenous cells. f–h Conidiogenous cells. i–l Conidia. m Germinated conidia. Scale bars a = 100 µm, c–e = 20 µm, b, m = 10 µm, f–h = 5 µm, i–l = 2 µm.

Figure 7.6 Morphology of *Periconia palmicola* (MFLU 14-0198)



Note Phylogram generated from the best scoring of the RAxML tree based on combined ITS, LSU, SSU and TEF1-α sequenced data of taxa in *Corynespora* and other related families (*Periconiaceae*). Taxa in *Cyclothyriella*, *Cyclothyriella rubronotata* (TR9) and *C. rubronotata* (TR) were selected as the outgroup taxa. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 70%. BYPP (red) equal to or greater than 0.95 are given above or below the nodes. Newly generated sequences are indicated in blue.

Figure 7.7 Phylogeny of the genus *Corynespora*

7.3.1.5 *Corynespora doipuiensis* J.F. Li & R. Phookamsak, sp. nov.

Indexfungorum number: IF557020; Facesoffungi number: FoF07056.

Etymology: Name reflects the location from which it was collected, Doi Pui, Chiang Rai, Thailand.

Holotype: MFLU 14–0388

Saprobic on branches of handing dead branch of unidentified herb. Sexual morph undetermined. Asexual morph hyphomycetous, colony on natural substrate effuse, dark brown or black. *Colonies* effuse, thin, cottony, pale to dark grey. *Mycelium* superficial, consisting of branched, septate, smooth, thin-walled, pale hyphae. *Conidiophores* 211.87–425.79× 9.98–15.2 µm ($\bar{x}$ = 313.45 × 13.89 µm, n = 40), macronematous, mononematous, setiferous, erect, straight or flexuous, often arising in groups, septate, unbranched, thick-walled, dark brown, sometimes light brown at the tip, percurrently proliferating from cut ends. *Conidiogenous cells* 27.42–35.27× 9.95–16.45 µm ($\bar{x}$ = 33.71 × 13.34 µm, n = 100) holoblastic, monoblastic, integrated, terminal, light to moderately dark brown, often percurrently proliferating. *Conidia* 135.9–165.27× 4.86–25.5 µm ($\bar{x}$ = 143.7 × 21.31 µm, n = 100), solitary, simple, obconical, curved, subhyaline to moderately brown, acrogenous, pseudoseptate, guttulate, thick-walled, smooth, with a dark basal scar, wide at below half, narrow and elongated at above half.

Cultural characteristics: Conidia germinating on PDA within 12 hours and germ tubes produced from both tops. Colonies growing on PDA, cottony, white to pale grey, reaching 5 mm in 7 days at 30ºC, mycelium partly superficial, partly immersed, slightly effuse, radially striate, with irregular edge, white; Asexual and sexual spores are not formed within 60 days.

Material examined: THAILAND, Chiang Rai Provice, Doi Pui, on dead herbaceous branch, 9 January 2014, Junfu Li, H-15 (MFLU 14-0388), living culture at MFLUCC 14-0022.

Notes: In this study, our strain (MFLUCC 14–0022) shares a size range of the conidial characters with the type and other respective specimens (Voglmayr & Jaklitsch, 2017). However, *Corynespora doipuiensis* is unique in having lanky, curve, sunhyaline conidia with long and brown conidiophores. Furthermore, phylogenetic analysis shows that our strain (MFLUCC 14–0022) forms a well-supportedsubclade (0.99 BYPP) with *Corynespora* sp. (MFLUCC 16-1101) with available support. Our specimen is identified

as a novel species *Corynespora doipuiensis* collected from herbaceous aerial dead branches from Chiang Rai, Thailand.



Note a. Colonies on handing dead herbaceous branch. b-c. Conidiophores. d. Conidiophores bearing conidia. e-d. Conidiogenous cells. g-f. Conidia. n. Germinated conidia. Scale bars: a=200 µm, b=100 µm, c-d= 50µm, e,i=100 µm, f-h=50µm.

Figure 7.8 Morphology of *Corynespora doipuienesis* (MFLU 14-0388)

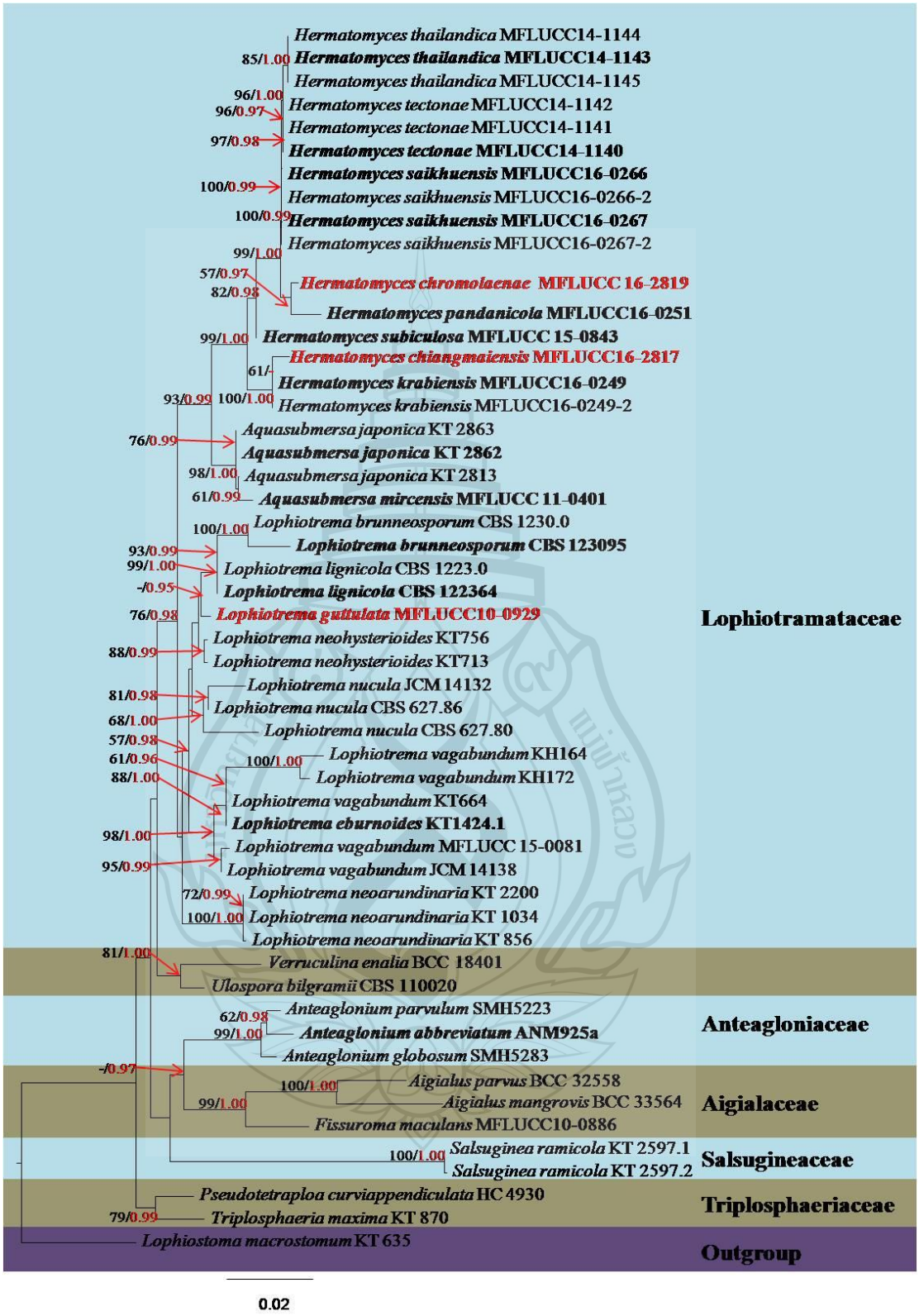


Figure 7.9 Phylogeny of the family Lophiotramataceae

Figure 7.9 Phylogenetic construction using RAxML-based analysis of a combined LSU and SSU, dataset. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 50 % and Bayesian posterior probabilities (PP, red) equal to or greater than 0.95 are shown above the nodes. The tree is rooted to *Lophiostoma macrostomum*. The type strains are in black bold and the newly generated sequences are indicated in red bold.

7.3.1.6 *Hermatomyces krabiensis* Tibpromma et al., Phytotaxa 275: 132 (2016).

Synonym: *Hermatomyces chiangmaiensis* J.F. Li et al., Index Fungorum 342: 45 (2017).

Indexfungorum number: IF552735, Facesoffungi number: FoF02480

Holotype: MFLU16-2888

Saprobic on dead (not land) leaves of Pandanus. Sexual morph: Undetermined. Asexual morph: *Colonies* discrete on host, black, powdery. *Mycelium* immersed on the substrate, composed of septate, branched, smooth, light brown-coloured hyphae. *Conidiophores* 3.8–5.3 µm long × 4.5–5 µm diam ($\bar{x}$ = 4.8 × 4.7 µm, n = 10), micronematous, mononematous, erect, hyaline, smooth, thin-walled, consist of 1–2 globose cell, subcylindrical to subglobose, arising from prostrate hyphae. *Conidiogenous cells* 3.8–4.5 µm long × 4.8–6.7 µm diam ($\bar{x}$ = 4.4 × 5.8 µm, n = 10), monotretic, integrated, terminal, ampulliform to doliiform, sub-hyaline to hyaline, smooth, thin-walled, dark on loci. *Conidia* (10–)13.5–18 µm long × (7.3–)8.6–10.4 µm diam ($\bar{x}$ = 13.5 × 9.6 µm, n = 20) solitary, acrogenous, simple, dark brown to black, with a layer of hyaline outer cells, multi-septate, globose to subglobose. *Conidial secession* schizolytic.

Cultural characteristics: Conidia germinating on PDA within 10 h and germ tubes produced from the apex. Colonies growing on PDA, reaching 5 cm in 14 days at 30ºC, mycelium partly superficial, partly immersed, slightly effuse, cottony, with regular edge, Olivierish-green to grayish-green; sexual or asexual spores not formed within 60 days.

Material examined: THAILAND, Chiang Mai, Mae Taeng (M.R.C.), on a dead (not land) leave of Pandanus, 25 March 2016, Junfu Li, H-MRC40B (MFLU 16-2888, holotype). ex-type living culture MFLUCC 16-2817, KUMCC (isotype in HKAS).

Notes: *H. chiangmaiensis* is reduced to synonymy based on morphological and multigene phylogenetic analysis by Koukol et al. (2018).

7.3.1.7 *Hermatomyces sphaericus* (Sacc.) S. Hughes, Mycol. Pap. 50: 100 (1953).

Synonyms: *Hermatomyces chromolaenae* Jun F. Li et al., Index Fungorum 342: 1 (2017).

Indexfungorum number: IF552736, Facesoffungi number: FoF02481

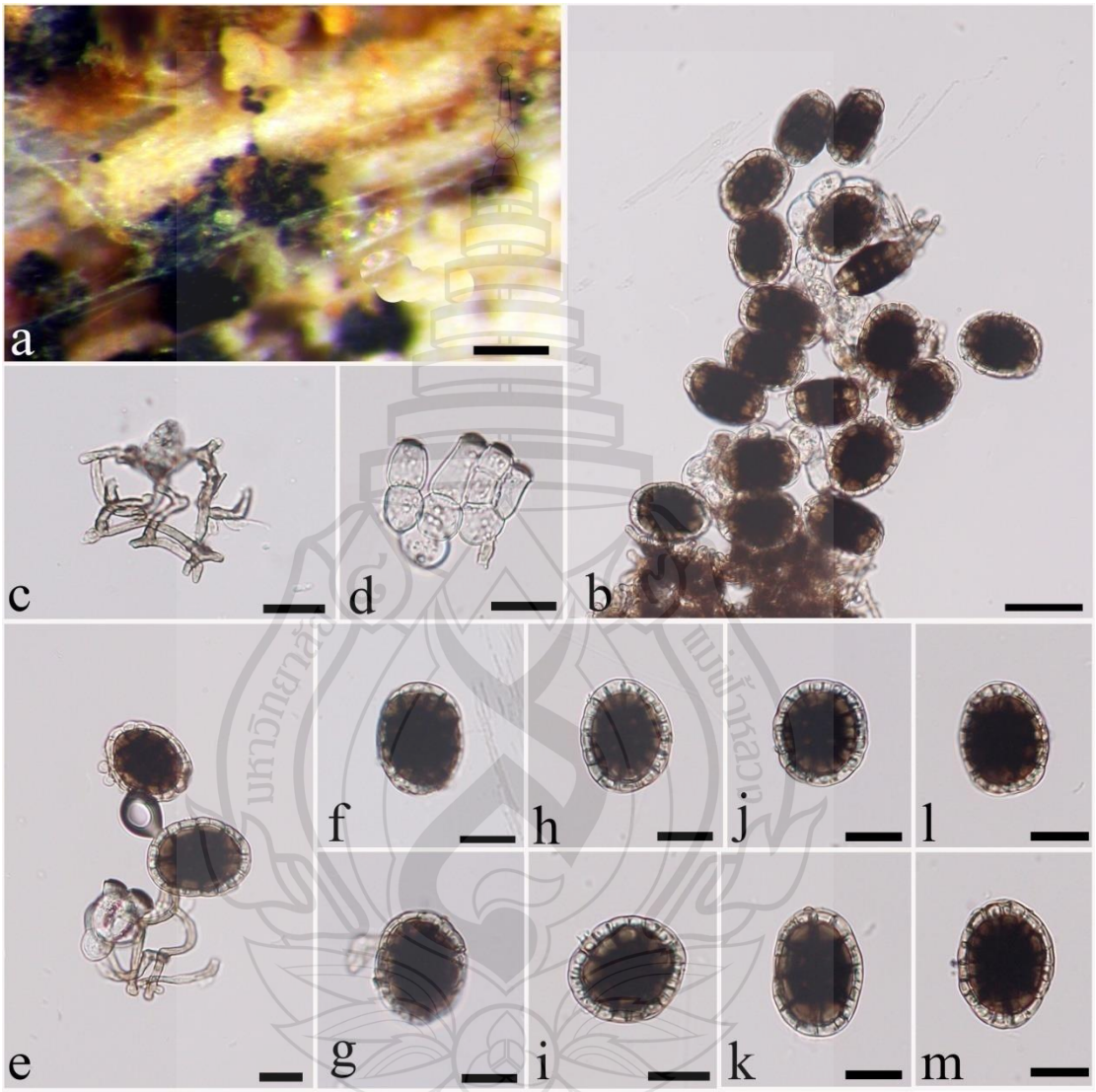
Holotype: MFLU16-2890

Saprobic on dead stem of *Chromolaena odorata* Linn. (Asteraceae). Sexual morph: Undetermined. Asexual morph: Colonies on natural substrate dry, blackish brown, velvety, circular, doughnut-shaped, dull, consisting of a sterile mycelial outer zone and a round, glistening, abundantly sporulating centre, conidia readily liberated when agitated. Mycelium 1.3–2.1 µm wide, superficial, composed of a network of branched, septate, brown, thick-walled, smooth hyphae. Conidiophores 3–4.3 µm long × 1.8–1.9 µm wide (x = 3.6 × 1.8 µm, n = 10), micronematous, straight or flexuous, pale brown, smooth, unbranched, arising from prostrate hyphae at the centre of circular colony. Conidiogenous cells holoblastic, monoblastic, integrated, terminal, cylindrical, hyaline to subhyaline. Conidia 9.2–10.4 µm high × 10.2–11.5 µm wide (x = 9.7 × 10.7 µm, n = 20) monomorphic, thick-walled, smooth, disk-shaped, with central cells dark brown to black, with peripheral cells pale brown. Two halves of the disk-shaped conidia symmetrically adpressed, forming a deep constriction at lower and upper end in lateral view, each half with 6–7 cells, hyaline to light brown at lower and upper cells, dark brown in middle cells. *Cylindrical conidia* 10–16.4 µm high, × 16–19.5 µm wide in broadest part of lower cells, (x= 13.2 × 17.5 µm, n = 20), with 3–4 columns of 2–4 cells arising from a common basal cell, each column, with rectangular to globose cells, constricted at septa, pale to subhyaline, granulate, smooth, terminating with a brown upper cell.

Cultural characteristics: Conidia germinating on PDA within 10 h and germ tubes produced from the apex. Colonies growing on PDA, reaching 5 cm in 14 days at 30ºC, mycelium partly superficial, partly immersed, slightly effuse, cottony, with regular edge, Olivierish-green to grayish-green; sexual or asexual spores not formed within 60 days.

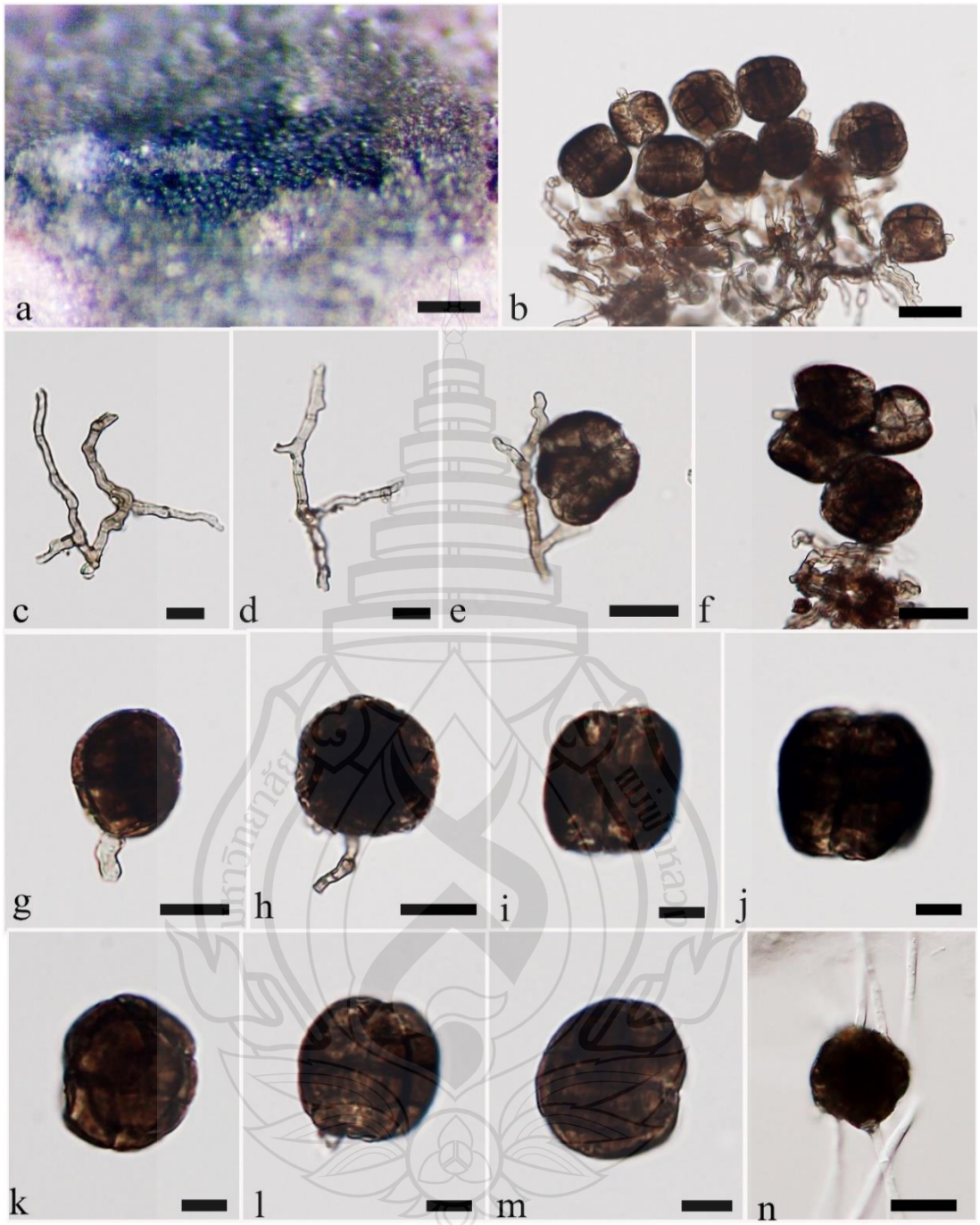
Material examined: THAILAND, Chiang Rai, Doi Pui, on a dead (not land) stem of *Chromolaena odorata*, 5 October 2016, Junfu Li, DPK4 (MFLU 16-2890, holotype). ex-type living culture MFLUCC 16-2819, KUMCC (isotype in HKAS).

Notes: Koukol et al. (2018) synonymized *Hermatomyces chromolaenae*, *H. saikhuensis*, *H. tectonae* into *H. sphaericus*.



Note a Colonies on dead leave of Pandanus. b Conidia in mass. c Hyphea. d Conidiophores with conidiogenous cells. e Hyphea with conidiogenous cell and conidia. f–m Conidia. *Scale bars* (a) 100 µm, (b) 10 µm, (c–m) 5 µm

Figure 7.10 Morphology of *Hermatomyces krabiensis* (MFLU 16-2890, holotype of *H. chiangmaiensis*)



Note a Colonies on dead stem. b Conidia in mass. c–d Hyphea. e–h Conidiophores with conidia. i–m Conidia. *Scale bars* (a) 100 µm, (b) 10 µm, (c–d, e–h, n) 5 µm, (i–m) 2 µm

Figure 7.11 Morphology of *Hermatomyces sphaericus* (MFLU 16-2890, holotype of *H. chromolaenae*)

7.3.1.8 *Pseudopithomyces chartarum* (Berk. & M.A. Curtis) J.F. Li, Ariyawansa & K. D. Hyde, *comb. nov*.

Basionym: *Sporidesmium chartarum* Berk. & M.A. Curtis, in Berkeley, Grevillea 3(no. 26): 50 (1874).

≡ *Pithomyces chartarum* (Berk. & M.A. Curtis) M.B. Ellis, Mycol. Pap. 76: 13 (1960).

Index Fungorum number: IF551393, Facesoffunginumber: FoF 00938

Pathogenic on human or sheep skin. *Colonies* black, separate, later becoming confluent, up to 0.5 mm diam. *Conidiophores*2–5×1.5–2.5 µm. micronematous or semi-macronematous, mononematous, branched and anastomosing, pale olive, smooth oroccasionally verruculose. *Conidiogenous cells* integrated. *Conidia* 18–29×10–17 µm, acropleurogenous, mainly pleurogenous, broadly ellipsoid, 3-septateto multi-septate, mainly with 3–4 transverse septa and usually divided by longitudinal septum, often constricted at the septa, mid brown to dark brown, finely echinulate to verruculose, rhexolytic, smooth or occasionally verruculose, a small piece of the denticle invariably remains attached to the base of the conidium (Ellis, 1971; Dingley, 1963).

Notes: *Pesudopithomyces chartarum* was introduced as *Sporidesmium chartarum* by Berkeley and Curtis (1874). In this study we used the reference strain of *Pse. chartarum* for our phylogenetic analysis, which was used by da Cunha et al. (2014). Our results clearly show that the strains of *Pse.chartarum* (MUCL 15905 and UBC F15184) forms a separate clade sister to the *Pse. palmicola* clade, including some other putative strains of *Pse.chartarum* (MUCL 4329, UTHSC 04 2495 and UTHSC 06 214), which we rename.

7.3.1.9 *Pseudopithomyces palmicola* J.F. Li, Ariyawansa & K. D. Hyde, sp.nov.

Index Fungorum number: IF551394, Facesoffunginumber: FoF 00939.

Etymology: The name *palmicola*, refers to thecommon host name and Latin *cola* meaning loving.

Holotype: MFLU 15-1474

Saprobic on leaves of *Acoelorrhaphe wrightii* (Griseb. & H. Wendl.) H. Wendl. ex Becc. Sexual morph: Undetermined. Asexual morph: *Colonies* effuse, dark brown to black. *Mycelium* superficial or partly immersed on the substrate, composed of septate,

branched, smooth, thin-walled, pale to grey hyphae. *Conidiophores* 3.6–6 × 3.2–3.5µm ($\bar{x}$ = 5.5 × 3.3µm, n = 100), micronematous, mononemous, closely packed, hyaline, thin-walled, aseptate, smooth, branched, flexuous. *Conidiogenous cells* 3.5–5.5 × 2.5–3.5µm ($\bar{x}$ = 4.5× 3.3 µm, n = 100), monoblastic, phialidic, integrated, terminal, determinate, hyaline, globose or subglobose. *Conidia* 21.5–30.5 × 10–16.5µm ($\bar{x}$ = 27.9× 14.2µm, n = 100), acrogenous, muriform, subglobose, 6–8-septate, verruculose to echinulate, amygdaliform or ovoid, thin-walled, light brown to brown.

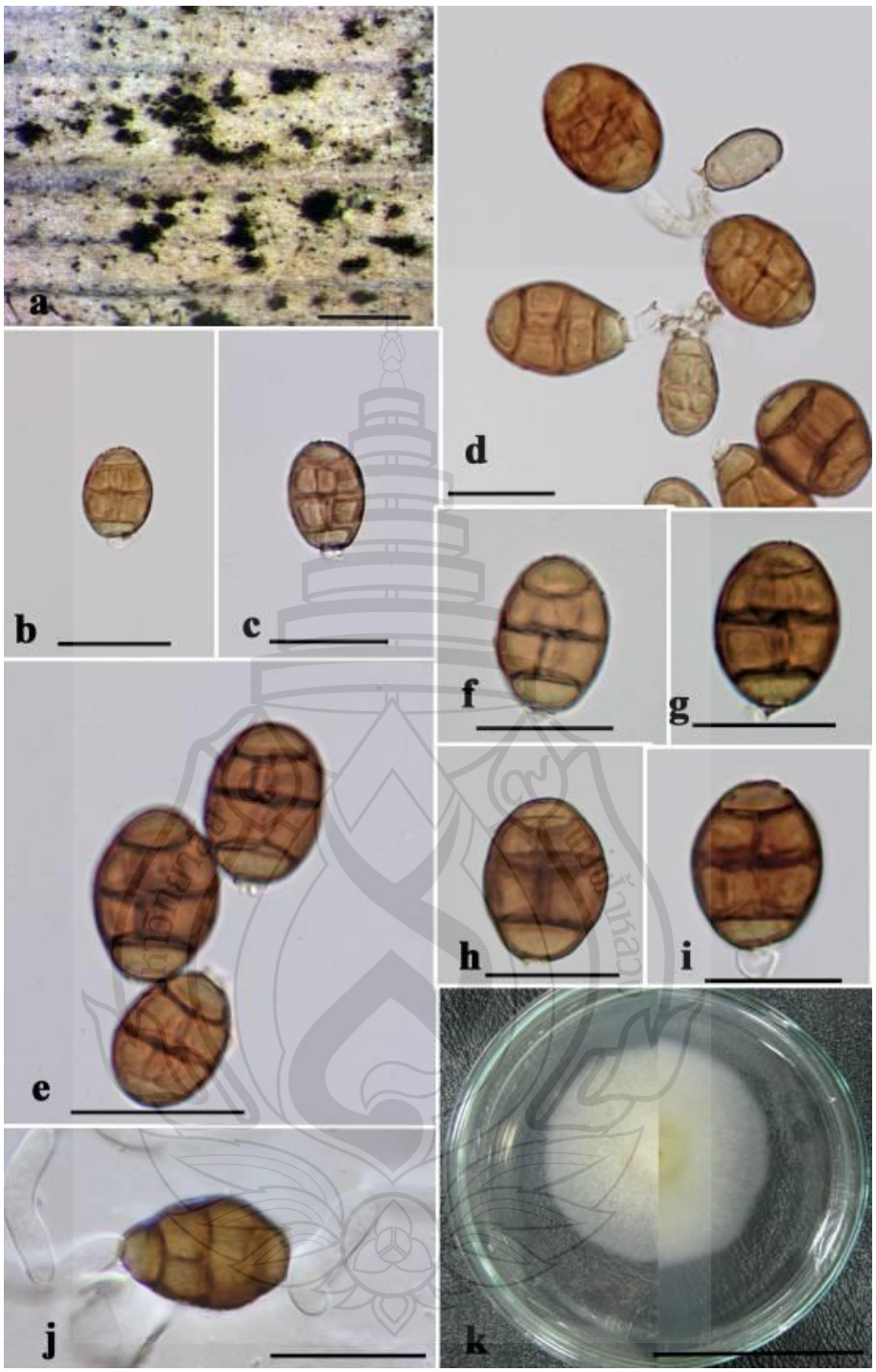
Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from end cells. Colonies growing on PDA, cottony, grey to brown, reaching 5 mm in 20 days at 25ºC, mycelium superficial, effuse, radially striate, with regular edge, hyphae pale to yellow white; asexual spores and sexual spores were not formed within 60 days.

Material examined: THAILAND, Chiang Rai Provice, Mae Fah Luang, on leaves of *Acoelorrhaphe wrightii* (Griseb. & H.Wendl.) H.Wendl. ex Becc. (*Arecaceae*), 26 January 2014, J.F. Li, H-4l (MFLU 15-1474, holotype), ex-type living culture, MFLUCC 14-0392, KUMCC 15-0100.

Notes: Phylogenetic analysis based on ITS and LSU sequence data showed that *Pseudopithomyces palmicola* is closely related to, but forms a distinct lineage from the *Pse. Chartarum* clade (Figure 7.21). *Pseudopithomyces palmicola* is similar to *P. chartarum*in having three constricted transverse septa and 1–2 longitudinal septa in the central cells. *Pseudopithomyces palmicola* differs from *Pse. chartarum* in having thick and hyaline conidiophores and mostly globose, pale brown conidia with slightly constricted septa, while *Pse. palmicola* has thin and heavily pigmented conidiophores with dark brown conidia which are comparatively more constricted at the septa (da Cunha et al., 2014). Furthermore *P. palmicola* forms pale to yellow white colonies on PDA, while *Pse. Chartarum* forms white to dark grey colonies on PDA (Table 7.1).

Table 7.1 Synopsis of *Pseudopithomyces* species with similar morphological features discussed in this study

Taxon	Hosts	Conidiophores	Conidia	Culture characters	Reference
Pseudopithomyces chartarum	Humans, sheep	Micromanetous, branch and anastomosing, pale olive, smooth or occasionally verruculose, 2–5 µm	With 3 or more septa, broadly ellipsoidal, mid to dark brown, 12–33 × 9–19 µm	Hairy or cottony, with white to dark gray colonies	Ellis (1960), Ellis (1971), da Cunha et al. (2014)
Pseudopithomyces palmicola	*Acoelorrhaphewrightii*	Micromanetous, branched and closely packed on base, hyaline, smooth, 3.5–5.5 µm	With 6–8 septa, verruculose to echinulate, light brown to brown, 21.5–30.5 × 10–16.5 µm	Cottony, pale to yellow white colonies	This study
Pseudopithomyces maydicus	*Acoelorrhaphe wrightii*	Semi-micronematous, unbranched and closely packed together, hyaline to subhyaline, smooth, 27 × 4.5µm	With 3–4 septa, cylindrical or slightly clavate, brown to dark brown,13–24.5 × 5.5–8.5µm	Hairy or cottony, white to dark grey colonies	Dingley (1963), da Cunha et al. (2014), this study
Pseudopithomyces sacchari	Soil, rotten pericarp	--	With 2–5 septa, ellipsoidal or slightly clavate, brown. 13.2–24.5 × 5.4–8.3 µm	Hairy or cottony, beige and gray or olivaceous, colonies brown	Ellis (1960), Ellis (1971), da Cunha et al. (2014)



Note a Colonies on palm leaves b,c Immature conidia d Conidiophores bearing conidia e-i Conidia j Germinating conidium k Upper and lower view of colony. Scale bars: a=500 µm, b-j=20 µm, k=5 cm.

Figure 7.12 Morphology of *Pseudopithomyces palmicola* (MFLU 15-1474)

7.3.1.10 *Pseudopithomyces maydicus* (Sacc.) J.F. Li, Ariyawansa & K.D. Hyde, *comb. nov.*

Basionym: *Clasterosporium maydicum* Sacc., Nuovo G. bot. ital.23(2): 213 (1916)

≡ *Pithomyces maydicus*(Sacc.) M.B. Ellis, *Mycol. Pap.*76: 15 (1960)

For other synonyms see Index Fungorum (2015)

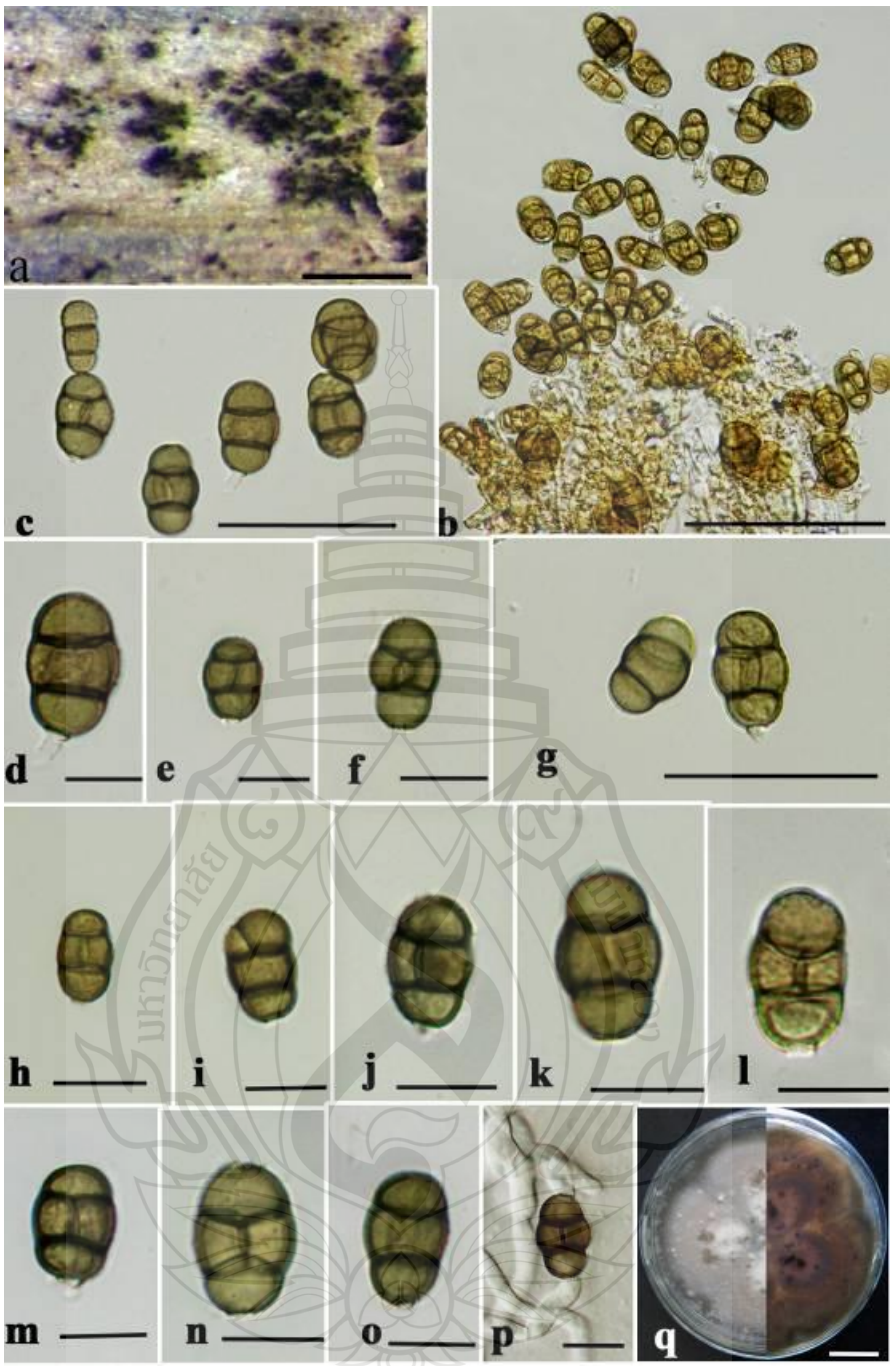
Index Fungorum number: IF 551395, Facesoffunginumber: FoF 00940.

Saprobic on leaves of *Acoelorrhaphe wrightii*. Sexual morph: Undetermined. Asexual morph: *Colonies* effuse, dark brown to black. *Mycelium* superficial or partly immersed in the substrate, composed of septate, branched, smooth, thin-walled, white hyphae. *Conidiophores* 4–6 × 2.5–3 µm ($\bar{x}$ = 4.4 × 2.7µm, n = 100), semi-micronematous, mononemous, closely packed, hyaline to subhyaline, thin-walled, aseptate, smooth, unbranched, flexuous. *Conidiogenous cells* 3.5–5 × 1.4–4µm ($\bar{x}$ = 3.6× 2.2µm, n = 100), monoblastic, integrated, terminal, determinate, hyaline, globose or subglobose. *Conidia* 13.5–24.5 × 5.5–8.5 µm ($\bar{x}$ = 21.8 × 5.6 µm, n = 100), wide in the middle, acrogenous, muriform,3–4-septate, thin-walled, brown to dark brown, fusiform to pyriform, clavate.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from apical cells. Colonies growing on PDA, hairy or cottony, white to dark grey, reaching 5 mm in 20 days at 25ºC, mycelium superficial, effuse, radially striate, with regular edge, hypha white to grey. Asexual spores and sexual spores not formed within 60 days.

Material examined: THAILAND, Chiang Rai Provice, Mae Fah Luang, on dead leaves of *Acoelorrhaphe wrightii* (*Arecaceae*), 26 Janurary 2014, J.F. Li, H-4e (MFLU 15-1473, reference specimen designated here), living culture, MFLUCC 14-0391, KUMCC 15-0101.

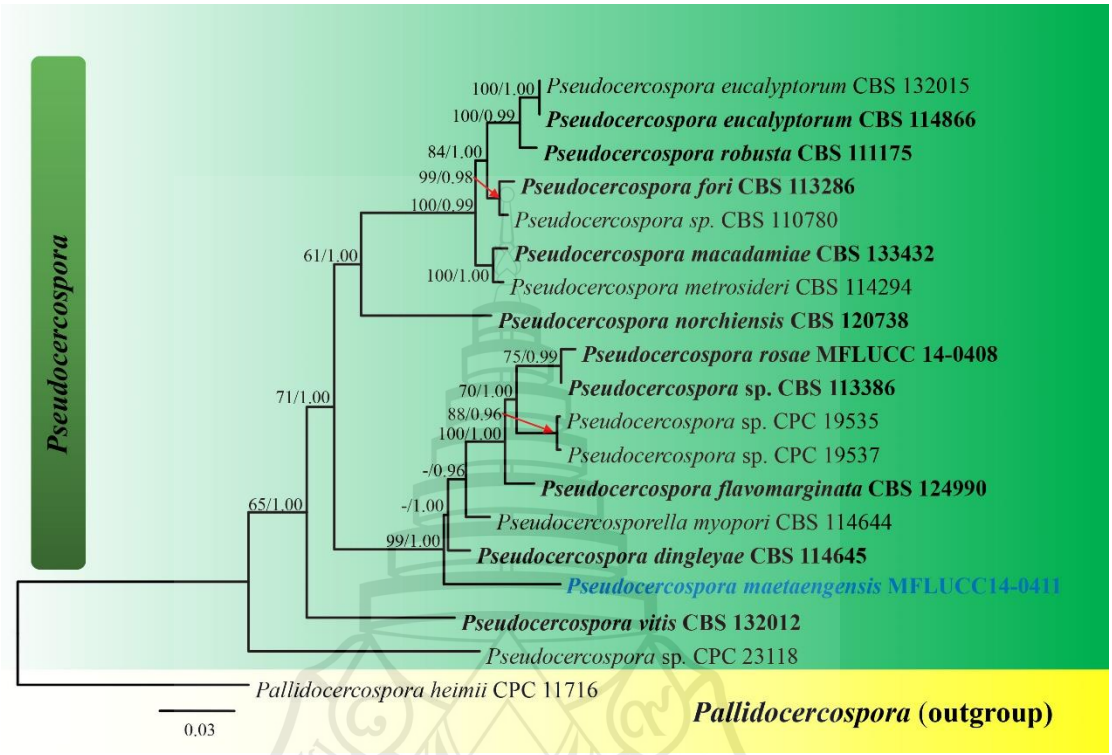
Notes: *Pseudopithomyces maydicus*is characterised by cylindricalor slightly clavate, pale brown to dark brown, verrucose conidia with two to three transverse septa, rarely with one oblique or longitudinal septum and in its white to grayish yellowcolonies on PDA. The characters of our collection are typical with that of *Clasterosporium maydicum* (Ellis, 1960). Furthermore our phylogeny also concluded that the putative strains of *Pse. maydicus* (UTHSC 06 3954 and UTHSC 06 1549) clustered with our strain.



Note a Colonies on palm stem b Conidiogenous cells and conidiophores bearing conidia c-o Conidia p Germinated conidium q Upper and lower view of colony. Scale bars: a=500 µm, b=100 µm, c,g=50µm, d-n= 20µm, q=3cm.

Figure 7.13 Morphology of *Pseudopithomyces maydicus* (MFLU 15-1473)

7.3.2 Mycosphaerellales



Note Phylogram generated from the best scoring of the RAxML tree based on combined ITS and LSU sequenced data of taxa in *Pseudocercospora*. Taxa in *Pallidocercospora heimii* (CPC 11716) is selected as the outgroup taxa. The best RAxML tree with a final likelihood value of -10821.117889 is presented. RAxML analysis yielded 609 distinct alignment patterns and 19.20% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.248806, C = 0.241445, G = 0.281063, T = 0.228686, with substitution rates AC = 1.648825, AG = 3.373595, AT = 1.202983, CG = 0.838400, CT = 6.421832, GT = 1.000000. The gamma distribution shape parameter alpha = 0.165747. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with final average standard deviation of split frequencies = 0.008723. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 70%; BYPP (red) equal to or greater than 0.95 are given above or below the nodes. Type sequences are in black bold and newly generated sequences are indicated in blud bold.

Figure 7.14 Phylogeny of the genus *Pseudocercospora*

7.3.2.1 *Pseudocercospora maetaengensis* J.F. Li & R. Phookamsak, sp. nov.

Index Fungorum number: IF556890*; Facesoffungi number*: FoF 07054.

Etymology: Name reflects the location from which it was collected, Mae Taeng, Chiang Mai, Thailand.

Holotype: MFLU 14-0206.

Saprobic on fallen dead leaves. Sexual morph: Unknown. Asexual morph: Synnemata 100–130 μm high, 5.9–8 μm wide, erect, simple, unbranched, dark brown to black, conidiophres twisted together in synnemata, with bubble-like, tightly interwoven, branched hyphae, compacted into an elongate bundle. *Conidiophores* 100–130 μm long × 1.8–3.5 μm diam ($\overline{x}$ = 124 μm × 2.6 μm, n = 10). *Conidiogenous cells* 2.5–3 μm long × 1.6–2 μm diam ($\overline{x}$ = 2.8 μm × 1.8 μm, n = 20), monoblastic, integrated, terminal, determinate or percurrent, cylindrical, doliiform, hyaline, smooth, colourless, thin-walled. *Conidia* 13–28 μm long × (2.5–)2.7–3.6(–4) μm diam. ($\overline{x}$ = 21 μm × 3.1 μm, n = 20), acrogenous, solitary, hyaline, ellipsoidal, clavate, 2–4 septate, dry, simple, straight, curved, fusiform, smooth-walled, thin-walled.

Cultural characteristics: Conidia germinating on PDA within 14 h and germ tubes produced from top cells. Colonies growing on PDA, hairy or cottony, grey to dark grey, reaching 5 mm in 20 days at 25°C, mycelium superficial, effuse, grey to dark grey coloured hyphae; Asexual spores and sexual spores were not formed within 60 days.

GenBank numbers: ITS: MN648323, LSU: MN648328, SSU: MN648320, TEF1-α: MN821071.

Material examined: THAILAND, Chiang Mai Province, Mae Teang (M.R.C.) on unidentified fallen dead leaves, 25 November 2013, Xiangyu Zeng (MFLU 14-0206, holotype), ex-type living culture MFLUCC 14-0411, KUMCC.

Notes: *Pseudocercospora maetaengensis* described herein as a new species. Morphologically, *P. maetaengensis* resemble *P. vitis* in having septated, straight or slightly curved, with a rounded apex conidia and with conidiophores grouped in synnema (Ellis, 1971). However, *P. maetaengensis* unique in its short, dark color synnemata and smaller, less septa, sub-hyaline, fusiform conidia. The phylogenetic of combined ITS and LSU, *P. maetaengensis* formed a independent clade distinct with *P. vitis* as well differ with other species in *Pseudocercospora* with high support (77% ML, 0.98 BYPP). Therefore, a new species, *Pseudocercospora maetaengensis* is established.



Note a, b Appearance of fungal colonies on host substrate. c, e–g Closed up conidiophores attached with conidiogenous cells. d Conidiophores. h–o Conidia. p Germination of conidia. q–r Culture. *Scale bars* a = 1 cm, b = 50 µm, c–d, e–g = 10 µm, h–p = 5 µm, q–r = 0.5 cm.

Figure 7.15 Morphology of *Pseudocercospora maetaengensis* (MFLU 14-0206)

7.3.2.2 *Pseudocercospora rosae* J.F. Li, A. Mapook & K.D. Hyde, *sp. nov*.

Index Fungorum number: IF554125; Facesoffungi number: FoF 03961

Etymology: Name reflects its host in Rosaceae

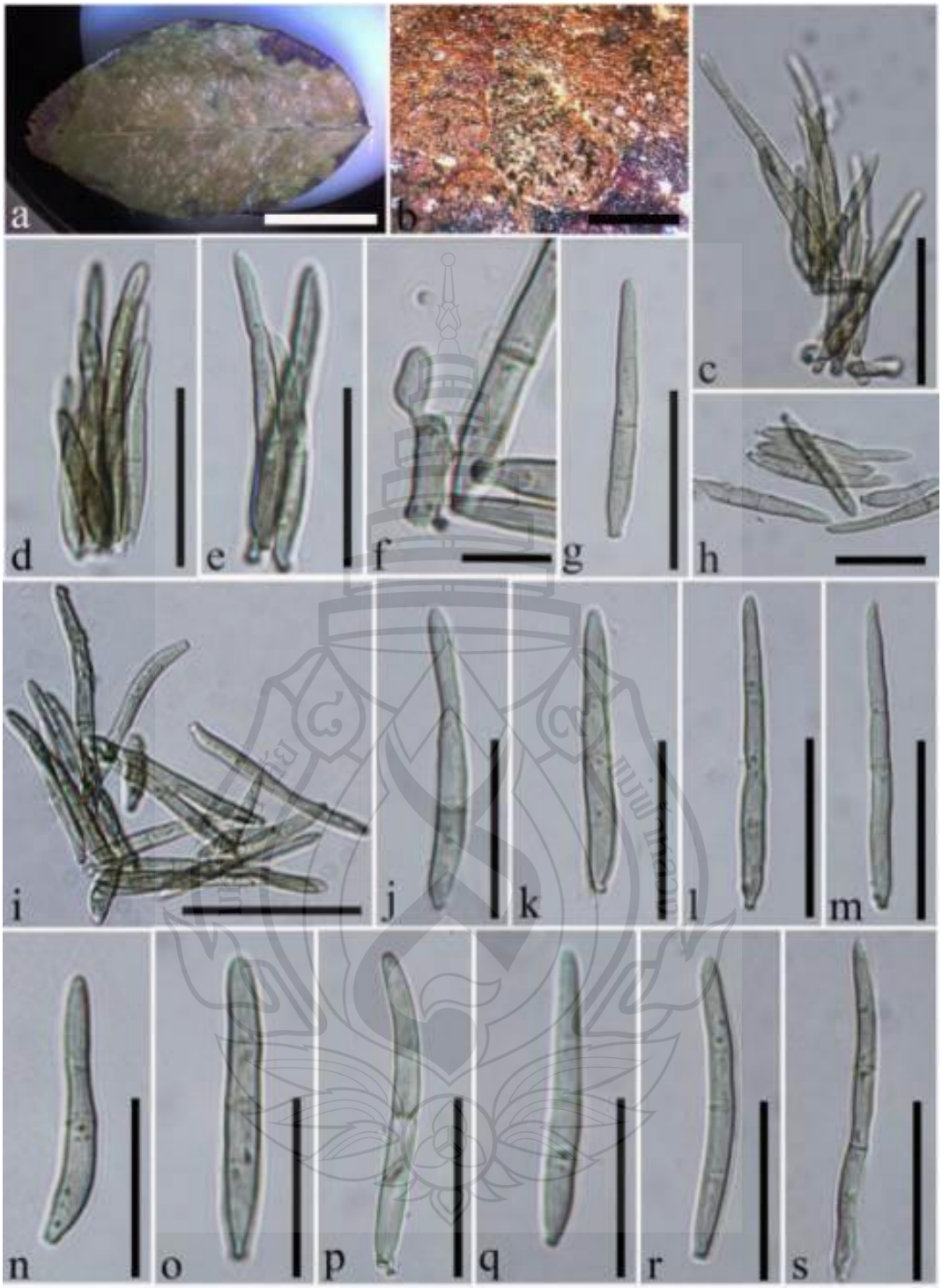
Holotype: HKAS100841.

Pathogenic on leaves of *Rosa canina*. Sexual morph: Unknown. Asexual morph: *Mycelium* superficial on the substrate, composed of septate, branched, smooth, thin-walled, grey to dark grey coloured hyphae. *Stromata* absent. *Conidiophores* (8.8–)9.5–19.4 µm long × 3–4 µm diam ($\bar{x}$ = 14.2 µm × 3.5 µm, n = 10), closely packed on the base. *Conidiogenous cells* (3.2–)3.8–5.5µm long × (2.5–)2.8–3.4 µm diam ($\bar{x}$ = 4.7µm × 3.2 µm, n = 100), monoblastic, integrated, terminal, determinate or percurrent, cylindrical, doliiform, hyaline, smooth, colourless, thin-walled. *Conidia* (75–)84.6–102(–104.5) µm long × (8.5–)8.7–9.6(–10.2) µm diam ($\bar{x}$ =90.2µm× 9.3 µm, n = 100), acrogenous, holoblastic, solitary, hyaline, ellipsoidal, calavate, multiseptate, dry, simple, straight, curved, fusiform, borne in chain, smooth, thin-walled.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from top cells. Colonies growing on PDA, hairy or cottony, grey to dark grey, reaching 5 mm in 20 days at 25ºC, mycelium superficial, effuse, radially striate, with regular edge, grey to dark grey coloured hyphae; Asexual spores and sexual spores were not formed within 60 days.

Material examined: THAILAND, Chiang Mai Province, on leaves of *Rosa canina*, 14th December 2013, Ausana Mapook, H-23 (HKAS100841), living culture = MFLUCC14-0408, KUMCC 17-0264.

Notes: *Pseudocercospora* sp. in recent collection is listed as in Iturriaga & Minter (2006). *Pseudocercospora rosae* described here as a new species within its characters as much shorter conidiophores (up to 20) with non stromata, subhyaline, fusiform, 1–3-septate conidia which differ with other *Pseudocercospora* species in morphology. In phylogenetic analysis, *P. rosae* formed a distinct clade in genus *Pseudocercospora*. This is also the first record to found *Pseudocercospora* sp. on *Rosa canina* in Thailand.



Note a. Dead plant tissue. b. Colonies on dead stem. c–e. Conidiophores. f. Conidiophores with Conidiogenous cells. g–s. Conidia. Scale bars: a = 1cm, b = 500µm, i = 100, c–e = 50µm, j–s = 50µm, f, h = 20µm.

Figure 7.16 Morphology of *Pseudocercospora rosae* (HKAS100841)

7.3.3 Mytilinidiales



Note Phylogram generated from maximum parsimony analysis based on combined LSU, SSU and ITS rDNA, demonstrating the phylogenetic relationships of *Lophium zalerionioides* (MFLUCC 14-0417) is in red bold. Type strains are in black bold.

Figure 7.17 Phylogeny of the genus *Lophium*

Lophium zalerioides J.F. Li, Phookamsak, Campresi & K.D. Hyde sp. nov.

Index Fungorum number: IF553197, Facesoffungi number: FoF03286

Etymology: The specific epithet "zalerioides" refers to its conidia resemble *Lophium arboricola* (*Zalerion arboricola*).

Holotype: MFLU 14-0436

Saprobic on cone of *Cupressus glabra* Sudw. (Cupressaceae). Sexual morph: Undetermined. Asexual morph: *Colonies* effuse, dark brown to black. *Mycelium* partly immersed and partly superficial, smooth, become verrucose when old, thin-walled, sub-hyaline to dark brown hyphae. *Conidiophores* 11.1–14.8 µm long × 2.1–2.4 µm diam ($\bar{x}$ = 12.9 × 2.5 µm, n = 20) macronematous, mononematous, solitary, straight to flexuous, light brown to brown, branched, thick-walled, septate, smooth, solitary. *Conidiogenous cell* 2.1–2.6 µm long × 1.8–2.2 µm diam ($\bar{x}$ = 2.4 × 2.5 µm, n = 10) monoblastic, integrated, terminal, determinate, pale brown. *Conidia* 13.1–41.6 µm long × 13.5–25.9 µm diam ($\bar{x}$ = 27.9 × 19.5 µm, n = 10), irregularly helicoid or coiled in three planes, forming a knot of multi-cells, solitary, verrucose, sometimes branched on base, dark brown, thick-walled.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from conidial cells. Colonies growing on PDA, reaching 5 cm in 10 days at 30ºC, mycelium partly superficial, partly immersed, slightly effuse, radially striate, with irregular edge, dark brown to black; sexual or asexual spores not formed within 60 days.

Material examined: ITALY, Forlì-Cesena Province, Passo la Calla - Santa Sofia, on cone of *Cupressus glabra* (Cupressaceae), 25 Octorber 2013, Erio Campresi, IT-1490 (MFLU 14-0436, holotype), ex-type living culture at MFLUCC 14-0417, KUMCC (isotype in HKAS).

Notes: *Lophium zalerioides* resemble *L. arboricola* in having monoblastic conidiogenous cells, dark hyphae and zalerion-like conidia (Buczacki, 1972). However, conidia in *Lophium zalerioides* is more multicelled and twisted which is different with *L. arboricola.* In phylogenetic analysis, *Lophium zalerioides* form a single clade differ with *L. arboricola* with a high support value 81% (ML).



Note *Lophium zalerioides* (Holotype, MFLU 14-0346). a. Colonies on cone. b–d. Conidiophores bearing conidia. e. Conidiophores. f–g. Conidiogenous cells. e–f. Conidiophores bearing conidia. h–t. Conidia. *Scale bars* a = 200 µm, b–e = 20 µm, d = 50 µm, e–f = 100 µm, h–t = 10 µm.

Figure 7.18 Morphology of *Lophium zalerioides* (MFLU 14-0346)

7.3.4 Tubeufiales

Pseudohelicomyces menglunicus J.F. Li, Phookamsak & K.D. Hyde, sp. nov.

Index Fungorum number: IF555770; Facesoffungi number: FoF05763

Etymolgy: The specific epithet "*menglunicus*" refers to the Menglun Town, Xishuang Banna, Yunnan, China, where the holotype was collected.

Holotype: KUN-HKAS 102239

Saprobic on unidentified rot seed coat. Sexual morph Undetermined. Asexual morph *Mycelium* immersed on the substrate, composed of septate, branched, smooth, thin-walled, subhyaline to dark brown hyphae. *Conidiophores* (106.3–) 129.3–220 µm long × 9.4–13.2 (–13.5) µm wide ($\bar{x}$ = 188.3 × 10.5 µm, n = 20), macronematous or micronematous, paler towards the apex, hyaline to light brown, thin-walled, smooth, septate, branched, straight or flexuous, cylindrical, tapering distally, apical portion. *Conidiogenous cells* (19.8–) 23.5–32.3 (–35.6) µm long × (3.8–) 4.6–5.5 (–6.1) µm diam ($\bar{x}$ = 4.5 × 5.3 µm, n = 10), holoblastic, integrated, intercalary and sometimes terminal, determinate or sympodial, occasionally small and discrete, denticulate, denticles cylindrical, often narrow, hyaline, smooth. *Conidia* (18.8–) 20–32.8 (–34.3) µm long × (21.5–) 25.8–30.4 (–40.4) µm wide ($\bar{x}$ = 22.5 × 27.2µm, n = 20) pleurogenous or acropleurogenous, solitary, simple, Sub-hyaline to paler yellowish brown, septate, slightly constricted at the septa, planate to cochleate, smooth, thin-walled, hygroscopic.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies growing on PDA, hairy, brown to dark brown, reaching 5 mm in 15 days at 23ºC, mycelium partly superficial, partly immersed, slightly effuse, radially striate, with irregular edge, subhyaline todark brown; Asexual spores were sporulated within 15 days on PDA; Sexual spores not formed within 60 days.

Material examined: CHINA, Yunnan Provice, Xishuang Bana, on rot coat of unidentified seed, 15 August 2014, Junfu Li, H-26 (KUN-HKAS 85795, holotype), ex-type living culture, MFLUCC14-0689, KUMCC.

GenBank numbers: ITS = MK335914, LSU = MK335915, TEF1-α = MK335916.

Notes: *Pseudohelicomyces menglunicus* resembles species of *Helicosporium* Nees and *Neohelicosporium* Y.Z Lu et al. in morphological characters, but is obviously

unique in conidiophores and conidia. *Pseudohelicomyces menglunicus* differs from *Helicosporium* and *Neohelicosporium* species in having flexuose, branched and hyphae-like conidiophores, and smooth-walled conidia. In the phylogenetic analysis (Figure 7.53), *P. menglunicus* forms a separated lineage, sister to *P. aquaticus* Y.Z. Lu et al. with moderate support (76% ML and 0.97 BYPP). Jayasiri et al. (2019) introduced a new sexual morph species, *P. quercus* Jayasiri et al. on fruit of *Quercus* sp. from Thailand. The species also formed a clade with *P. aquaticus* and *P. menglunicus* (pre-analysis, data not shown). A comparison of ITS and TEF1-α nucleotide bases shows that *P. menglunicus* differs from *P. quercus* in 37/569 bp (6.5%) of ITS and 28/912 bp (3.1%) of TEF1-α. Hence, *P. menglunicus* is introduced as a new asexual morph species.



Note *Helicomyces menglunicus* (KUN-HKAS 85793). a. Colonies on seed. b. Colonies on seed. c. Conidiophores with Conidiogenous cells. d–e. Conidiophores. f. Conidiophoresbearing conidia. g. Conidiogenous cells. h–s. Conidia. t. Germinated spores. Scale bars: a = 0.5 cm, b, d, e = 100 µm, f, t = 50 µm, c, g = 20 µm, h–s = 20 µm.

Figure 7.19 Morphology of *Pseudohelicomyces menglunicus* (KUN-HKAS 85793)

7.3.5 Hypocreales

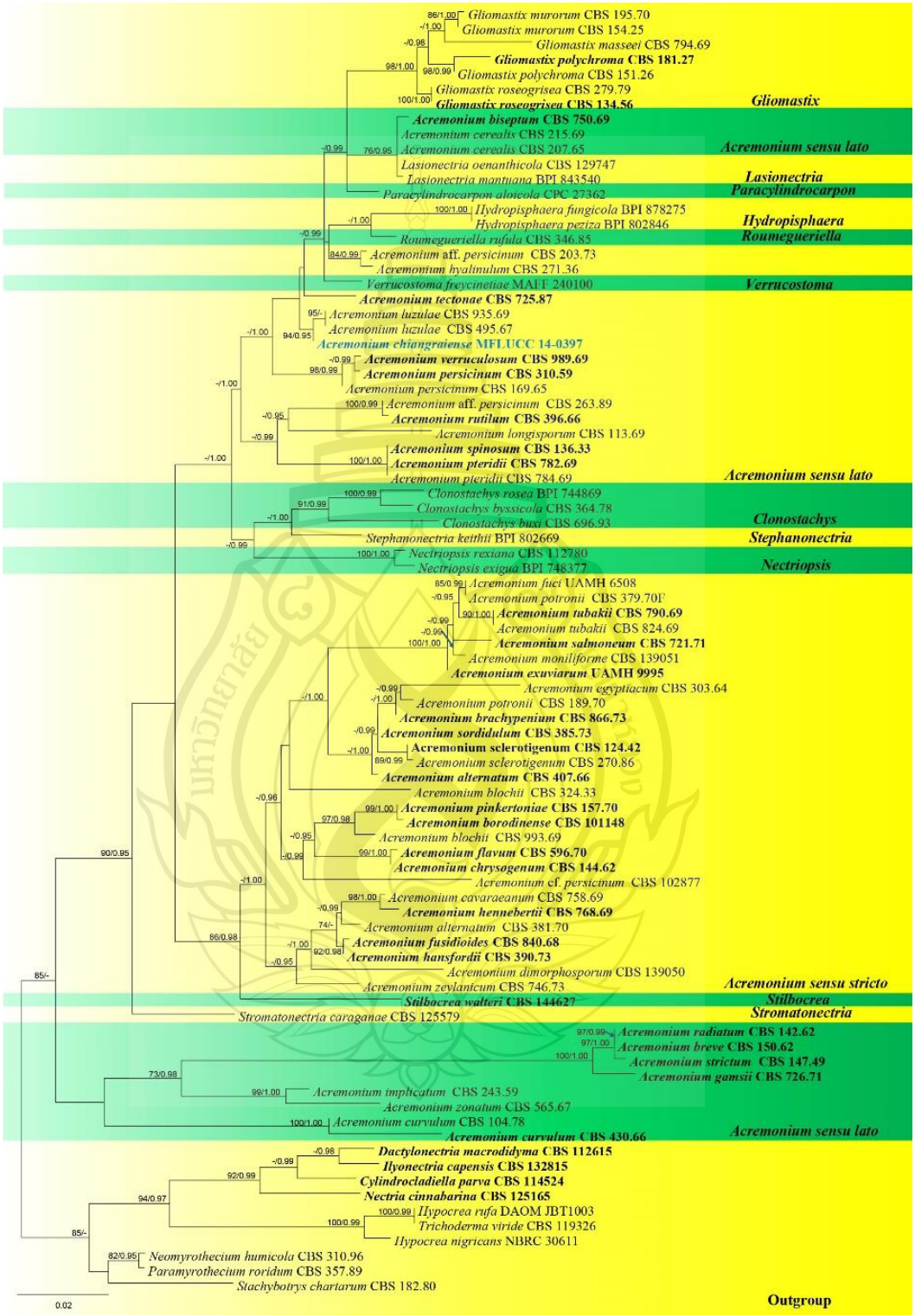


Figure 7.20 Phylogeny of the family Bionectriaceae (Hypocreales)

Figure 7.20 Phylogram generated from the best scoring of the RAxML tree based on LSU sequenced data of taxa in *Acremonium sensu strict* and related genera in Bionectriaceae. Taxa in *Dactylonectria macrodidyma* (CBS 112615), *Ilyonectria capensis* (CBS 132815), *Cylindrocladiella parva* (CBS 114524), *Nectria cinnabarina* (CBS 125165), *Hypocrea rufa* (DAOM JBT1003), *Trichoderma viride* (CBS 119326), *Hypocrea nigricans* (NBRC 30611), *Neomyrothecium humicola* (CBS 310.96), *Paramyrothecium roridum* (CBS 357.89) and *Stachybotrys chartarum* (CBS 182.80) are selected as the outgroup taxa. The best RAxML tree with a final likelihood value of -5389.526823 is presented. RAxML analysis yielded 261 distinct alignment patterns and 5.49% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.243131, C = 0.224240, G = 0.309793, T = 0.222836, with substitution rates AC = 1.062627, AG = 2.954134, AT = 1.259961, CG = 0.466986, CT = 7.967948, GT = 1.000000. The gamma distribution shape parameter alpha = 0.146261. Tree-Length = 1.834559. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with final average standard deviation of split frequencies = 0.008123. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 60% is given above or below the nodes. BYPP (red) equal to or greater than 0.95 are given above or below the nodes. Type sequences are in black bold and newly generated sequences are indicated in blud bold.

7.3.5.1 *Acremonium chiangraiense* J.F. Li & R. Phookamsak, sp. *nov.*

Index Fungorum number: IF556891; Facesoffungi number: FoF 07055.

Etymology: Name reflects the location from which it was collected, Chiang Rai, Thailand.

Holotype: MFLU 14-0202.

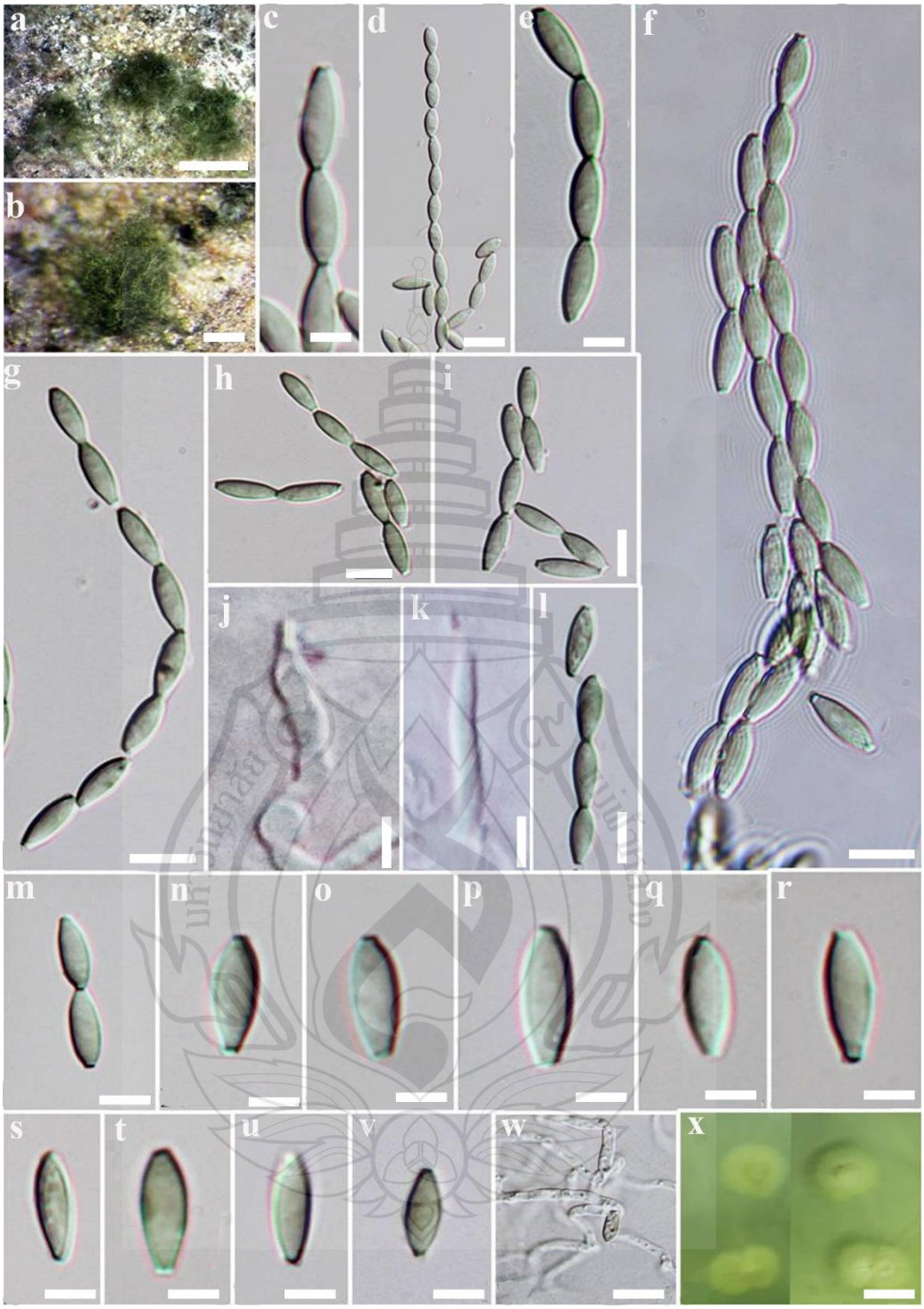
Saprobic on wet leaves of palm. *Colonies* effuse, white to light grey, velvety. *Mycelium* mostly immersed, partly superficial, consisting of septate, branched, thin-walled, white racquet hyphae. *Conidiophores* 71.2–83 × 8.6–11.2 µm ($\bar{x}$ = 75.1 × 10 µm, n = 5) micronematous, ampulliform. subhyaline, smooth, thin-walled. *Conidiogenous cells* 2.5–2.6 × 2.1–2.5 µm ($\bar{x}$ = 2.4 × 2.3 µm, n = 5), monoblastic, holoblastic, globose, subhyaline, smooth. *Conidia* 11.94–15.27 × 2.94–4.52 µm ($\bar{x}$ = 13.97 × 3.93 µm, n = 100), catenate, in branched chains, abundant, fusiform, narrow and truncate at both ends, hyaline to green-yellow, smooth, thick-walled, aseptate.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from all cells. Colonies growing on PDA, hairy, pale white, reaching 5 mm in 15 days at 30ºC, mycelium partly superficial, partly immersed, slightly effuse, radially striate, with irregular edge, pale white; Asexual spores and sexual spores not formed within 60 days.

GenBank numbers: ITS: MN648324, LSU: MN648329.

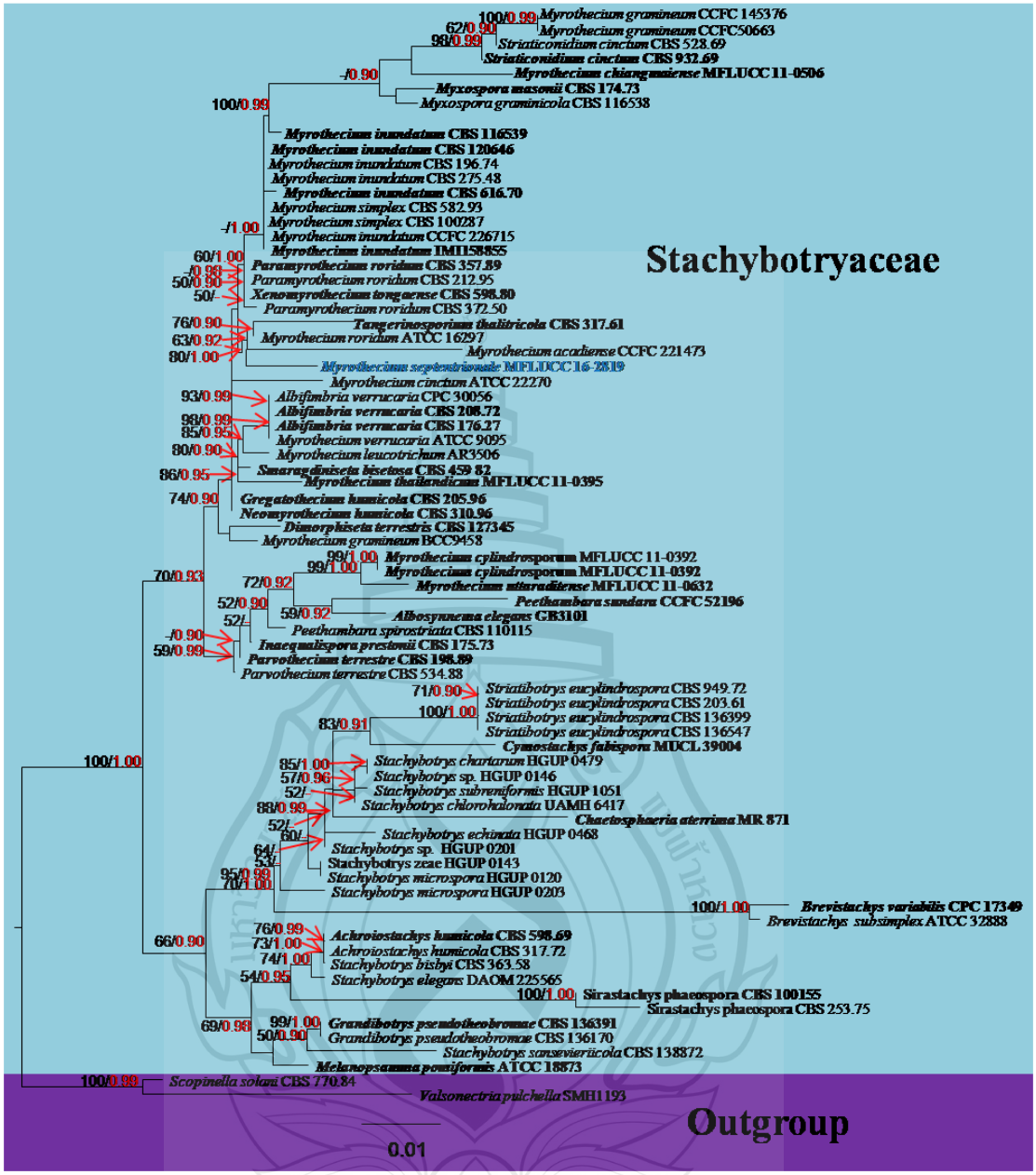
Material examined: THAILAND, Chiang Rai Provice, Khun Kron water fall, on dead wet leave of palm, 9 January 2014, Junfu Li, H-10b (MFLU MFLU 14-0202, holotype; *ibid*. HKAS, isotype). ex-type, living culture at MFLUCC 14-0397, KUMCC.

Notes: *Acremonium chiangraiense* phylogenetically close to *A. luzulae*. However, *A. chiangraiense* has 2.8% difference in ITS gene with *A. luzulae* (16 difference in 568bp nuclearbases). On the other hand, *A. chiangraiense* is unique in having ampulliform, smooth conidiophores with monoblastic conidiogenous cells, conidia unseptated and bearing in chains, while *A. luzulae* with verrucosa conidiophores and septated conidia (Matsushima, 1975). In phylogenetic based on LSU gene and ITS gene, *Acremonium chiangraiense* form a separated clade differ with *A. luzulae* in *Acremonium sensu lato* (Bionectriaceae).



Note a–b. Colonies on palm leaves. c–i, l–m. Conidia in chains. j–k. Conidiophores with conidiogenous cells. n–v. Conidia. w. Germinated conidia. x. Culture. Scale bars: a = 500 µm, b = 100 µm, d, f, w = 20 µm, e–l = 10µm, c, m, n–v = 5 µm, x= 0.5 cm.

Figure 7.21 Morphology of *Acremonium chiangraiense* (MFLU 14-0202)



Note Phylogenetic construction using RAxML-based analysis of a combined LSU and SSU, dataset. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 50% and Bayesian posterior probabilities (PP, red) equal to or greater than 0.95 are shown above the nodes. The tree is rooted to *Valsonectria pulchella* and *Scopinella solani***.** The type strains are in black bold and the newly generated sequences are indicated in red bold.

Figure 7.22 Phylogeny of the family Stachybotryaceae

7.3.5.2 *Myrothecium septentrionale* J.F. Li, Phookamsak & K.D. Hyde sp. nov.

Indexfungorum number: IF552737, Facesoffungi number: FoF02482.

Etymology: Named after the location where the species collected, Chiang Mai, northern Thailand.

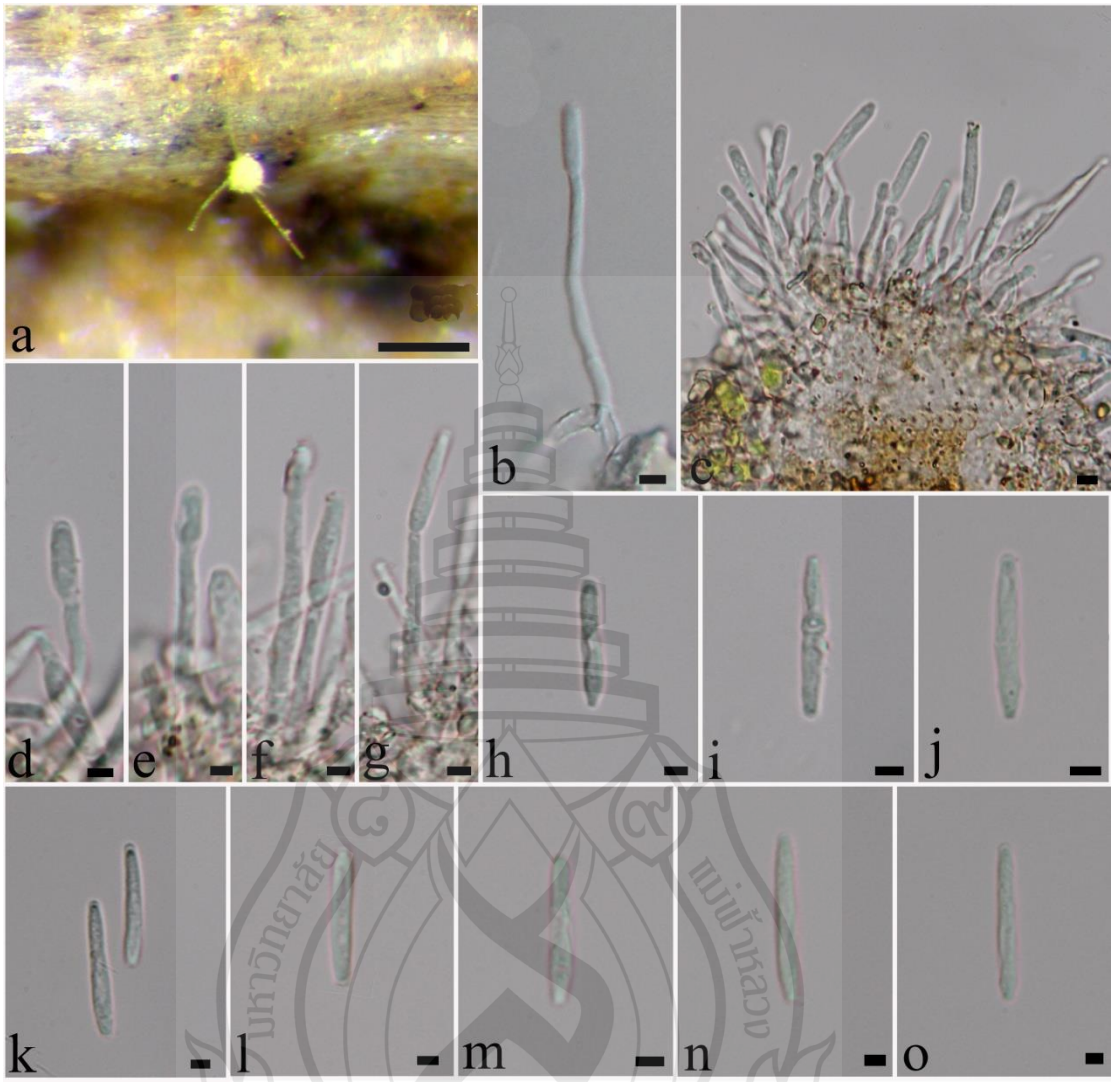
Holotype: MFLU 16-2889

Saprobic on dead (not land) leaves of Pandanus. Sexual morph: Undetermined. Asexual morph: *Colonies* effuse on host, grey. *Mycelium* superficial on the substrate, composed of unseptate, unbranched, smooth, sub-hyaline hyphae. *Conidiophores* 8–11 µm long × 1–1.6 µm diam ($\bar{x}$ = 9.8 × 1.2 µm, n = 20), maronematous, mononematous, solitary, erect, scattered, straight to curved, sub-hyaline to hyaline, smooth, thin-walled, unseptate, unbranched, arising from a stromatic base. *Conidiogenous cells* 1–1.5 µm long × 1–1.6 µm diam ($\bar{x}$ = 1.3 × 1.3 µm, n = 20), phialidic, terminal, glodose to ellipsoid, hyaline, smooth, thin-walled. *Conidia* 8.6–12 µm long × 1.2–2.1 µm diam ($\bar{x}$ = 10.5 × 1.6 µm, n = 20) solitary, acrogenous, hyaline, smooth, 0–1-septate, ampulliform to doliiform, chiefly subcylindrical. *Conidial secession* rhexolytic.

Cultural characteristics: Conidia germinating on PDA within 14 h and germ tubes produced from the apex. Colonies growing on PDA, reaching 5 cm in 14 days at 30 ºC, mycelium partly superficial, partly immersed, slightly effuse, powdery, vertical, with regular edge, maroon to yellowish-red; sexual or asexual spores not formed within 60 days.

Material examined: THAILAND, Chiang Mai, Mae Taeng (M.R.C.), dead (not land) leaves of Pandanus, 25 March 2016, Junfu Li, H-MRC41B (MFLU 16-2889, holotype), ex-type living culture MFLUCC 16-2819, KUMCC; (isotype in HKAS).

Notes: *Myrothecium septentrionale* is close to *M. chiangmaiense* D.Q.Dai & K. D. Hyde in morphology. However, *M. septentrionale* and *M. chiangmaiense* are distinct in the phylogenic analysis. *Myrothecium septentrionale* is close relationship with *M. roridum*, but *M. septentrionale* differ with *M. roridum* in having bigger (8.6–12 × 1.2–2.1 µm vs. 5–6 × 1–1.2 µm), ampulliform conidia while is cylindrical in *M. roridum*.



Note a Colonies on dead leave of Pandanus. b–g Conidiophores with conidiogenous cells. h–o Conidia. Scale bars a. 100 µm, b–o. 1 µm

Figure 7.23 *Myrothecium septentrionale* (MFLU 16-2889)

7.3.6 Xylariales

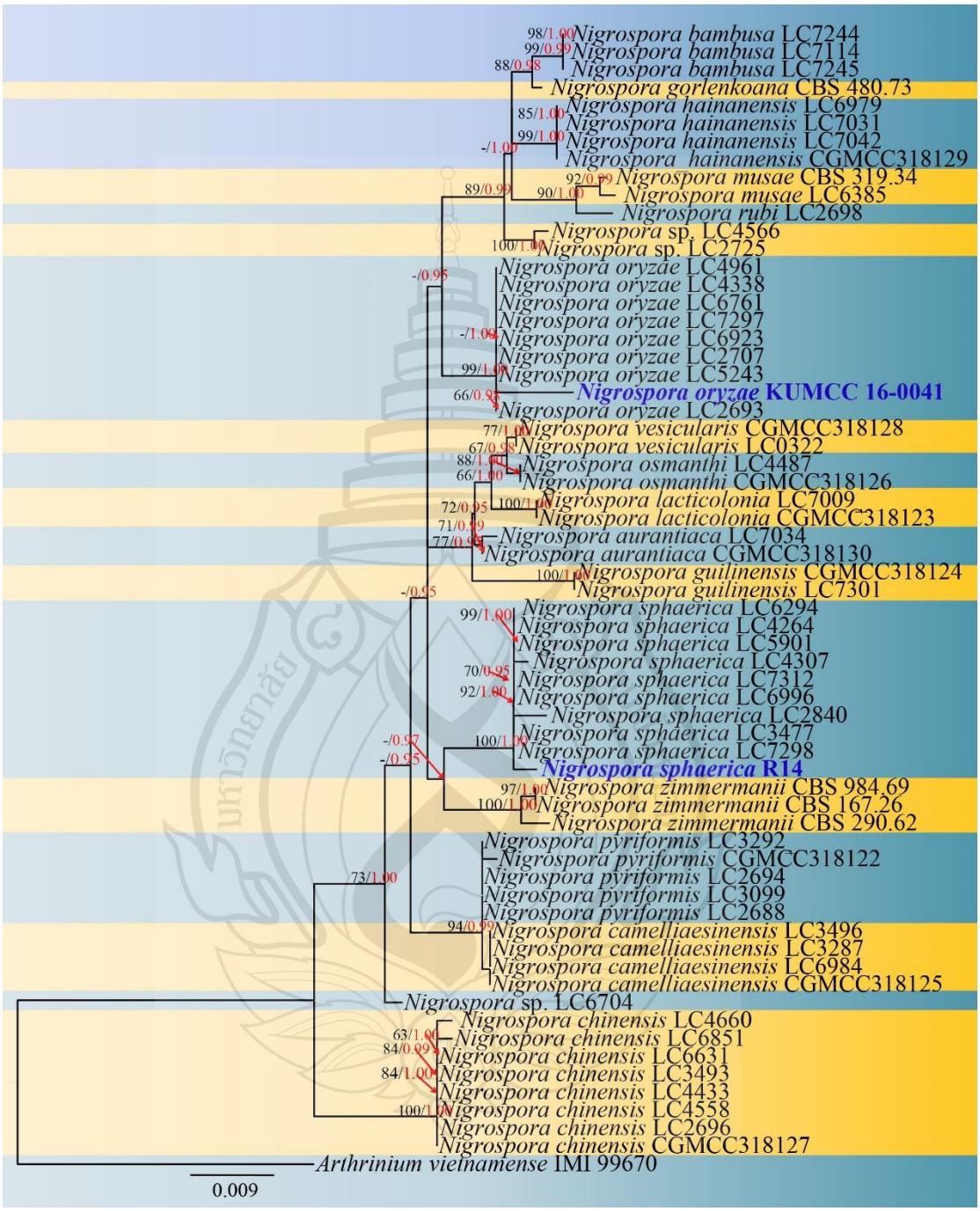


Figure 7.24 Phylogeny of the genus *Nigrospora* (Apiosporaceae, Hypocreales)

Figure 7.24 Phylogram generated from the best scoring of the RAxML tree based on combined ITS, TEF1-α and TUB-2 sequenced data of taxa in *Nigrospora*. Taxa in *Arthrinium vietnamense* (IMI 99670) were selected as the outgroup taxa. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 60%. BYPP (red) equal to or greater than 0.95 are given above or below the nodes. Newly generated sequences are indicated in blue

7.3.6.1 *Nigrospora oryzae* (Berk. & Broome) Petch, J. Indian bot. Soc. 4: 24 (1924)

Basionym: *Monotospora oryzae* Berk. & Broome, J. Linn. Soc., Bot. 14: 99. 1873.

Synonym: *Khuskia oryzae* H.J. Huds., Trans. Brit. Mycol. Soc. 46: 358. 1963.

Facesoffungi number: FoF06596

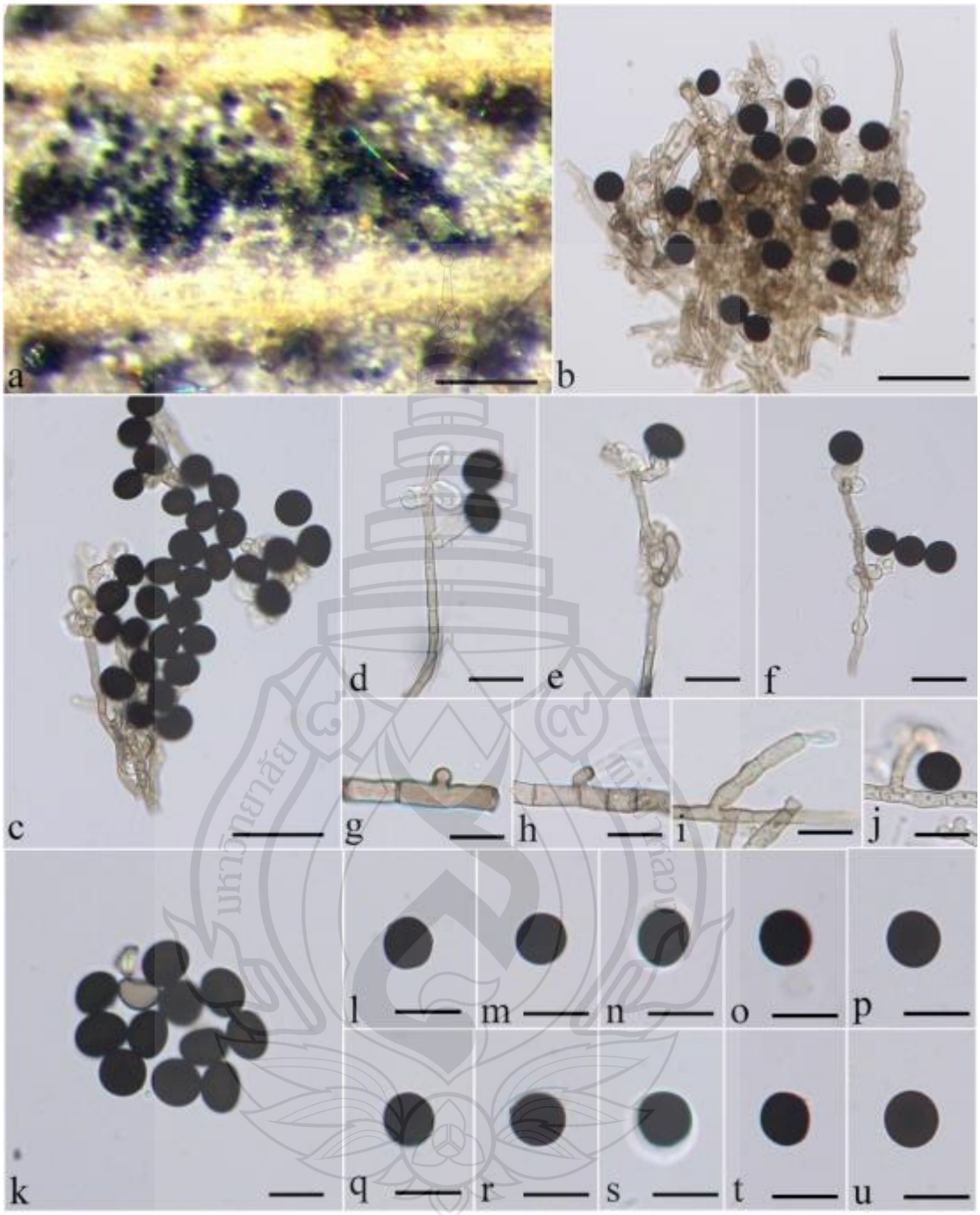
Type: Sri Lanka, Jaffra, H.S.O. Russell, Esq. Government Agent of the Northern Provinces, from rice leaves, 1873, Berk. & Broome (IMI 99832, slide of holotype).

Saprobic on fallen dead leaves of bamboo. Sexual state: Undetermined. Asexual state: hyphomycetes. *Conidiophores* 2–3 × 24–32 µm ($\overline{x}$ = 2.5 × 28 µm, n = 10) mononematous, flexuous, smooth, hyaline to pale brown, branched. *Conidiogenous cells* 2–3 × 3.2–5.6 µm ($\overline{x}$ = 2.6 × 4.9 µm, n = 20) monoblastic, solitary, determined, hyaline to subhyaline, ampliform to subspherical, discret, smooth. *Conidia* 4–5.5 × 4.2–5µm ($\overline{x}$ = 5.2 × 4.8 µm, n = 100) dry, solitary, acrogenous, simple, spherical to broadly ellipsoidal, aseptate, black, smooth, velvety.

Cultural characteristics: Conidia germinating on PDA within 12 hours and germ tubes produced from all cells. Colonies growing on PDA, hairy, black, reaching 5 mm in 7 days at 30ºC, mycelium partly superficial, partly immersed, slightly effuse, radially striate, with irregular edge, black; Asexual spores were formed after 30 days and sexual spores not formed within 60 days.

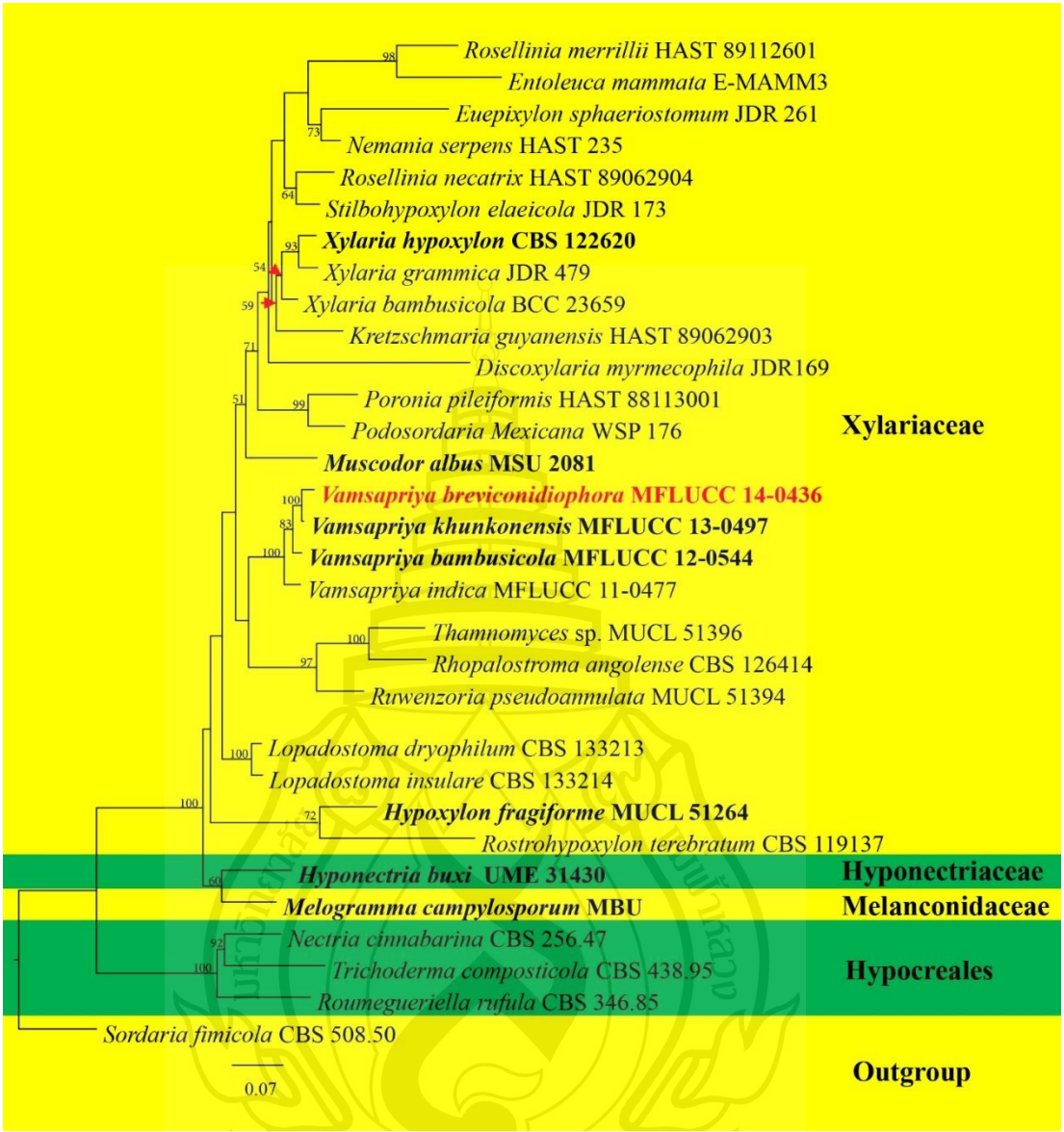
Material examined: THAILAND, Chiang Rai Province, Doi Mae Salong, on fallen dead leaves of bamboo, 18 May 2016, Junfu Li, DMS12-2 (MFLU 17–0199**)**. Living culture at KUMCC 16-0041; MFLUCC.

Notes: *Nigropora oryzae* have been reported as with a cosmopolitan distribution and wide host range (Wang et al., 2017). In this study, our isolation (KUMCC 16-0041) collected from Doi Mae Sai Long, Thailand, clustered in one single clade with *N. oryzae* and their morphological characteristics were in good accordance with *N. oryzae*. Therefore, we regard this isolation (KUMCC 16-0041) as *N. oryzae* which collected from Doi Mae Sai Long District, Thailand for the first time.



Note a. Colonies on bamboo leaves. b–c. Conidia with conidiophores. d–j. Conidiophores with conidiogenous cells. k–u. Conidia. Scale bars: a=200 µm, b=50 µm, c=20µm , d–f= 10µm, g–u=5µm.

Figure 7.25 Morphology of *Nigrospora oryzae* (MFLU 17–0199)



Note Phylogenetic tree of Xylariales order level generated from maximum-parsimony (MP) analysis based on combined data set of ITS and LSU sequence data set. Bootstrap support (BS) values above 50% are shown at the nodes. The original isolate numbers or GenBank codes are noted after the species names. Ex-type and the type species are indicated in bold. The tree is rooted with *Sordaria fimicola* (CBS 508.96).

Figure 7.26 Phylogeny of the family Xylariaceae

7.3.6.2 *Vamsapriya breviconidiophora* J.F. Li, Phookamsak & K.D. Hyde sp. nov

Index Fungorum number: IF553196, Facesoffungi number: FoF03287.

Etymology: Name after its short conidiophres.

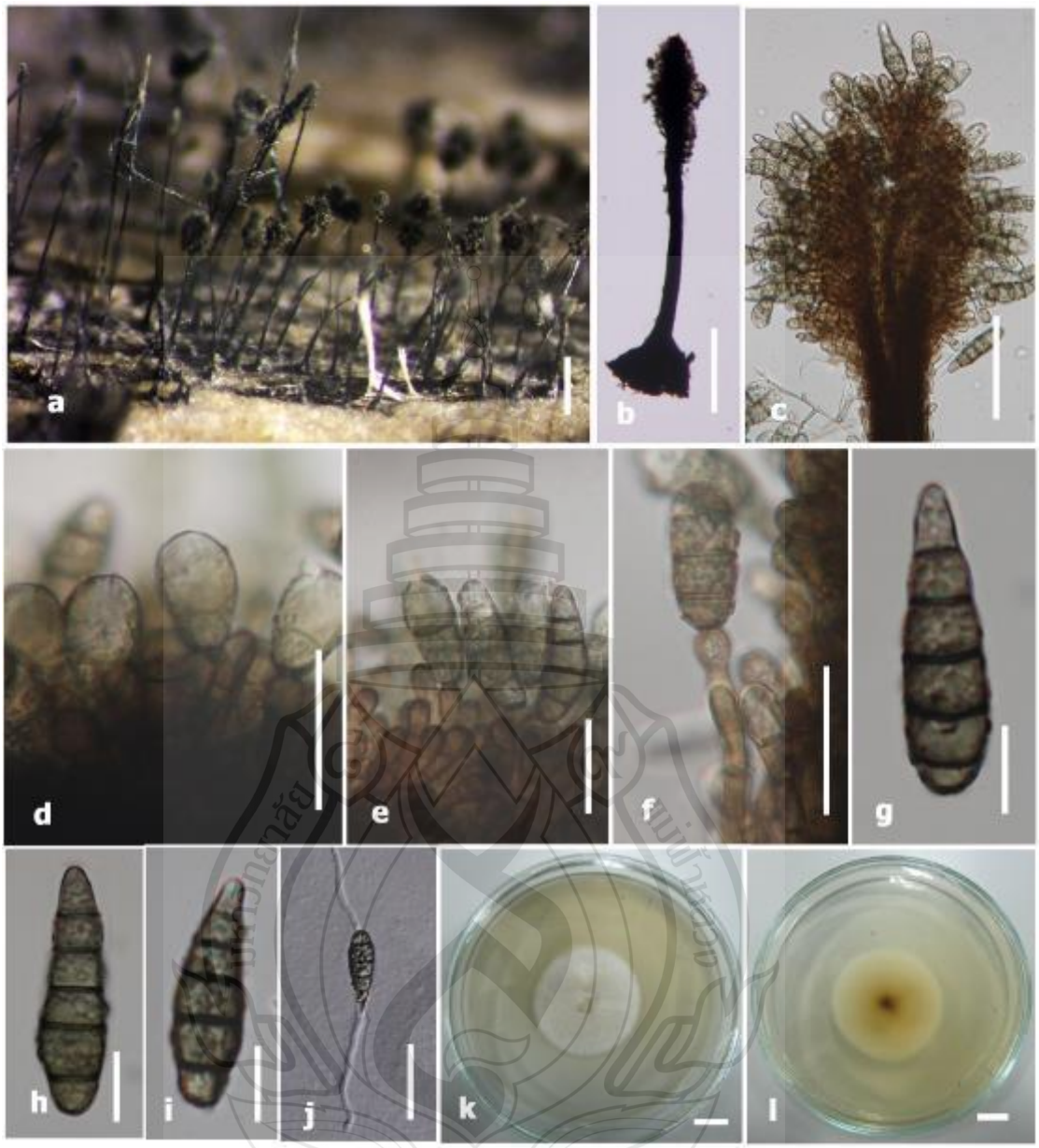
Holotype: MFLU 16-0271

Saprobic on dead stem of bamboo. Sexual morph: Undetermined. Asexual morph: *Colonies* superficial on the substrate, composed of septate, branched, black-colored hyphae. *Conidiophores* 340–450 µm long × 13–21 µm diam ($\bar{x}$ = 381.2× 16.5 µm, n = 10), mononematous, scatted, dark brown to black, pale at the apex, smooth, thick-walled, septate, branched. *Conidiogenous cells* 4–5.5 µm long × 5.2–6.8 µm diam ($\bar{x}$ = 4.7 × 5.8 µm, n = 40), polyblastic, holoblastic, integrated, terminal, cylindrical to clavate, wider at the apex, pale brown to brown, smooth. *Conidia* 25.2–32.4 µm long × 7.9–10.2 µm diam ($\bar{x}$ =27.8 × 8.7, n = 40) solitary, dry, simple, grey to dark brown, broadly fusiform, oval at immature with ovoid to inequilateral, expend at the base, narrow at the upper part, verrucose.

Cultural characteristics: Conidia germinating on PDA within 14 hours and germ tubes produced from conidial cells. Colonies growing on PDA, reaching 5 cm in 10 days at 30ºC, cottony, mycelium partly superficial, partly immersed, slightly effuse, radially striate, with irregular edge, white to brownish-red; sexual or asexual spores not formed within 60 days.

Material examined: THAILAND, Chiang Rai provice, M.F.L.U. (Mae Fah Luang University), on dead stem of bamboo, 31 October 2013, Junfu Li, H-6 (MFLU 16-0271, holotype), living culture at MFLUCC 14-0436, KUMCC (isotype in HKAS).

Notes: *Vamsapriya breviconidiophora* decribed here as a new species. Morphologically, *Vamsapriya breviconidiophora* resemble *V. khunkonensis* in having fusiform 5-septated conidia and wide apex conidiophores (Dai et al., 2014). However, *Vamsapriya breviconidiophora* is characterized by its prominent short conidiophores (conidiophores is above 600 µm in other *Vamsapriya* species vels 340–450 µm in *V. breviconidiophora*). In phylogenetic analysis, *Vamsapriya breviconidiophora* form a clade differ with *V. khunkonensis*.



Note a. Superficial hyphomycetes on dead bamboo tissues. b. Conidiophores. c–f. Conidiogenous cells. g–i. Conidia. j. Germinated conidia. k–l. Culture. *Scale bars* a = 500 µm, h = 100µm, b, g, m = 100µm, c–g = 20 µm, i–l = 10 µm, k–l = 1 cm.

Figure 7.27 Morphology of *Vamsapriya breviconidiophora* (MFLU 16-0271)

CHAPTER 8

CONCLUSIONS

8.1 Overall Conclusions

This study mainly dealt with the taxonomy of hyphomycetous fungi on plant issue. In addition, 50 new taxa, with two new genera and one new family in Dothideomycetes, Sordariomycetes and Eurotiomycetes were identified based on morphological characteristics and phylogenetic analyses from different gene regions (ITS, LSU, SSU, TEF1, RPB2, GAPDH and Alt-a1).

8.1.1 Taxonomic Studies of Hyphomyctous Fungi on Plant Litters

The number of species hyphomyctous fungi on plant litters has increased their diversity in the past years. However, the taxonomy and classification of hyphomyctous fungi was confused and overlooked in early stage, and thus need to be emended and re-generalized hyphomyctous species in modern method, which analyzed based on morphological and molecular evidence. This study provides descriptions and illustrations on new taxa and new records on plant litters from China, Italy, and Thailand, belonging to Dothideomycetes, Eurotiomycetes and Sordariomycetes based on analyzed by the charactors of morphology and phylogeny. The results show: one new families (Zygosporiaceae); three new genera (*Fusiconidium*, *Marinophialophora*, *Sporidesmioides*); 24 new taxa (*Acremonium chiangraiensis*, *Curvularia palmicola*, *Corynespora doipuiensis*, *Dendryphion hydei*, *Fusiconidium mackenziei*, *F. aquaticum*, *Helicomyces menglunicus*, *Lophium zalerioides*, *Marinophialophora garethjonesii*, *Myrothecium septentrionale*, *Periconia palmicola*, *Pseudocercospora maetaengensis*, *Pse. rosacearum*, *Pseudopithomyces palmicola*, *Pse. chartarum*, *Pse. maydicus*, *Torula chiangmaiensis*, *T. chiangmaiensis*, *T. hydei*, *T. mackenziei*, *T. pluriseptata*, *Seifertia shangrilaensis*, *Sporidesmioides thailandica*, *Vamsapriya breviconidiophora*); The family Zygosporiaceae

placed in Xylariales; the genera *Seifertia* and *Fusiconidium* placed in Melanommataceae; *Sporidesmioides* replaced in Torulaceae; *Marinophialophora* placed in Herpotrichiellaceae.

This study provides full description and photo plates of morphology, type specimens and molecular sequence data of the novel taxa. It is hoped the data of these interesting species which provided in this study will be avaluable and helpful in future mycological studies.

8.1.2 Taxonomic and Evolutionary Explaintation of *Alternaria*

As a key pathogenic hyphomyctous genus, *Alternaria* contains more than 300 species were found on animal and plant hosts, which could be a good model to analyze the taxonomy, phylogeny and evolution of hyphomycetes, especially pathogenic hyphomycetes fungi. The multi-gene phylogeny and morphology of the new collection of *Alternaria* on diverse definite plant hosts, from China, Italy and Russia, yielded 26 new taxa (*Alternaria arctoseptata, A. arundinis, A. baoshanensis, A. breviconidiophora, A. brevirostra, A. doliconidium, A. ellipsoconidia, A. eupatoriicola, A. falcata, A. italica, A. lathyri, A. macilenta, A. macroconidia, A. minimispora, A. muriformispora, A. nodulariconidiophora, A. oblongoellipsoidea, A. obpyriconidia, A. orobanches, A. ovoidea, A. phragmiticola, A. phytolaccae, A. pseudoinfectoriae, A. rostroconidia, A. salicicola* and *A torilis*) and over 40 plant host records within *Alternaria*. This study contributes the updated taxonomy and phylogeny of *Alternaria* species complex, preliminary estimated evolutionary divergence and sundry plant hosts, more taxa in *Alternaria* based on morphology, phylogenetic data and available literature.

8.1.3 Diversity of Plant Hosts Species

The collections in this study were collected indiscriminately from various plant host species, which widely placed in monocotyledon and dicotyledonous, from China, Italy, Russia and Thailand. Over 30 host species belongs in more ten botanic families were definitely recorded, which a clear hosts information of plant-host hyphomycetes is provided.

8.2 Research Advantages

The hyphomyctous fungi is diversity, most recent known hyphomycetes are the asexual morph of species in Ascomycota and new taxa were added in the study. However, there are still various taxa expect to be discovered from the natural environment, especially in the Topical and Subtropical regions, whereas Hyde et al. (2018) indicated that up to 96% of fungi may be new taxa. In addition, the modern phylogenetic methods intend to link hyphomyctous species with their expected sexual morphs, and classify the taxa *incertea sedis* in their correct classifications. Adapter from this, the relevance between fungi and plant host species, could be provided as basic knowledge on the Botany, Agronomy, and Conservation biology. This study on morphology and taxonomy of hyphomycetes on plant hosts in Dothideomycetes, Sordariomycetes and Eurotiomycetes to provide their descriptions and illustrations, and coupled with available molecular data to determine their classification, and their hosts species records. Therefore, this study completes the basic information of hyphomyctous fungi from various plant hosts, which will hope to be the value literatures on mycological research. However, the discovery of potential novel taxa and new host records of hyphomycetes, fungal evolutionary exploration, and chemical secondary metabolism research are still strongly expected in future studies.

8.3 Limitations of this Study

This study is expected to explore hyphomyctous fungal species in the natural environments, and then fresh samples were indiscriminately collected from China, Italy, Russia, and Thailand.

8.3.1 Hyphomyctous species are diversity, which could be find on biomass, as well on abiotic material, such as soil, waste, water and air etc., in terrestrial and aquatic habitats. In this study, it is unable to investigate all the natural habitats, the fungal collections are almost focus on terrestrial plant issue and litters, for the hyphomyctous fungi on abiotic material, animal hosts and in aquatic habitats are less provided in this study.

8.3.2 In this study, some hosts species of our collections unable identified. This is because the decaying woods and fallen plant litters were lost their specific characters, and some host plants are hardly to identify, such as, withered plant without the leaves or other organs. Thus, some specimens are marked as unidentified hosts or host species identified as their genus/family rank.

8.3.3 The evolutionary estimated divergence analysis is only tentatively provided for *Alternaria* complex. Due to lacking of fossil information, it is unable to convince analysis of evolutionary estimated divergence in suitable rank for the all collections in this study. However, the evolutionary estimated divergence analysis in a high rank are expected for some minor hyphomyctous family, such as Torulaceae.

8.4 Future Studies on Hyphomycetes

In the research of Mycology, the discovery of new taxa is not the only score on Mycology, to record the statues of fungal species, such as their express descriptions and correct phylogenetic classification, the host species, the location, species abundance in microhabitat and potential application as well important. However, the precursory investigation for fungal species from natural environments is necessary as the basic knowledge for above-mentioned aspects. Therefore, this study surveyed the hyphomycetous fungi on various plant hosts from Asia and Europe. This study found that the results of molecular and morphological classification of some taxa are indicated as unknown species or classification *incertea sedis*, and morphological characters of some taxa is overlooked. Therefore, investigation of novel species and new records, completion of accurate species information should be provided to better understanding of fungal species on earth. Furthermore, fresh collections are expected to identify the species, unique characters and be deposited to used in other studies. Many hyphomycetes is reported as potential biological-control which produce important secondary metabolism. It is meaningful to the future research.

8.5 Publications

8.5.1 First Author Publications

Li, J., Phookamsak, R., Mapook, A., Boonmee, S., . . . Lumyong, S. (2016) *Seifertia shangrilaensis* sp. nov. (Melanommataceae), a new species from Southwest China. *Phytotaxa*, *273*(1), 34-42.

Li, J. F., Bhat, D. J., Phookamsak, R., Mapook, A., . . . Hyde, K. D. (2016) *Sporidesmioides thailandica* gen. et sp. nov. (Dothideomycetes) from northern Thailand. *Mycological Progress*, *15*(10), 1169-1178.

Li, J., Jeewon, R., Luo, Z., Phookamsak, R., . . . Hyde, K. D. (2017) Morphological characterization and DNA based taxonomy of *Fusiconidium* gen. nov. with two novel taxa within Melanommataceae (Pleosporales). *Phytotaxa*, *308*(2), 206-218.

Li, J. F., Phookamsak, R., Jeewon, R., Bhat, D. J., . . . Hyde, K. D. (2017) Molecular taxonomy and morphological characterization reveal new species and new host records of *Torula* species (Torulaceae, Pleosporales). *Mycological progress*, *16*(4), 447-461.

Li, J. F., Phookamsak, R., Jeewon, R., Tibpromma, S., . . . McKenzie, E. H. C. (2017) Establishment of Zygosporiaceae fam. nov. (Xylariales, Sordariomycetes) based on rDNA sequence data to accommodate *Zygosporium*. *Mycosphere*, *8*(10), 1855-1868.

Li, J., Jeewon, R., Phookamsak, R., Bhat, D. J., . . . McKenzie, E. H. C. (2018) *Marinophialophora garethjonesii* gen. et sp. nov.: a new hyphomycete associated with *Halocyphina* from marine habitats in Thailand. *Phytotaxa*, *345*(1), 1-12.

Li, J., Jeewon, R., Mortimer, P. E., Doilom, M., . . . Promputtha, I. (2020) Multigene phylogeny and taxonomy of *Dendryphion hydei* and *Torula hydei* spp. nov. from herbaceous litter in northern Thailand. *PloS one*, *15*(2), e0228067.

Li, J., Jiang, H.B., Jeewon, R., Hongsanan, S., . . . Phookamsak, R. (2021) *Alternaria*: update on species limits, evolution, multi-locus phylogeny, and classification. *Mycosphere* (In prep).

8.5.2 Co-author Publications

Singtripop, C., Hongsanan, S., Li, J., de Silva, N. I., . . . Hyde, K. D. (2016) *Chaetothyrina mangiferae* sp. nov., a new species of Chaetothyrina. *Phytotaxa*, *255*(1), 21-33.

Mapook, A., Hyde, K. D., Dai, D. Q., Li, J., . . . Boonmee, S. (2016) Muyocopronales, ord. nov., (Dothideomycetes, Ascomycota) and a reappraisal of Muyocopron species from northern Thailand. *Phytotaxa*, *265*(3), 225-237.

Mapook, A., Hyde, K. D., Hongsanan, S., Phukhamsakda, C., . . . Boonmee, S. (2016) Palawaniaceae fam. nov., a new family Dothideomycetes, Ascomycota to accommodate *Palawania* species and their evolutionary time estimates. *Mycosphere*, *7*(11), 1732-1745.

Tibpromma, S., Hyde, K. D., Jeewon, R., Maharachchikumbura, S. S., . . . Karunarathna S. C. (2017). Fungal diversity notes 491–602: taxonomic and phylogenetic contributions to fungal taxa. *Fungal diversity*, *83*(1), 1-261.

Hyde, K. D., Norphanphoun, C., Abreu, V. P., Bazzicalupo, A., . . . Mortimer, P. E. (2017) Fungal diversity notes 603–708: taxonomic and phylogenetic notes on genera and species. *Fungal Diversity*, *87*(1), 1-235.

Phookamsak, R., Lu, Y. Z., Hyde, K. D., Jeewon, R., . . . Promputtha, I. (2018). Phylogenetic characterization of two novel *Kamalomyces* species in Tubeufiaceae (Tubeufiales). *Mycological Progress*, *17*(5), 647-660.

Wanasinghe, D. N., Phukhamsakda, C., Hyde, K. D., Jeewon, R., . . . Karunarathna, S. C. (2018) Fungal diversity notes 709–839: taxonomic and phylogenetic contributions to fungal taxa with an emphasis on fungi on Rosaceae. *Fungal diversity*, *89*(1), 1-236.

Phookamsak, R., Hyde, K. D., Jeewon, R., Bhat, D. J., . . . Xu, J. C. (2019). Fungal diversity notes 929–1035: taxonomic and phylogenetic contributions on genera and species of fungi. *Fungal diversity*, *95*(1), 1-273.

Jayawardena, R. S., Hyde, K. D., Chethana, K. W. T., Daranagama, D. A., . . . Yan, J. Y. (2018). Mycosphere notes 102-168: Saprotrophic fungi on *Vitis* in China, Italy, Russia and Thailand. *Mycosphere*, *9*(1), 1-114.

Luo, Z. L., Hyde, K. D., Liu, J. K. J., Maharachchikumbura, S. S., . . . Su, H. Y. (2019) Freshwater sordariomycetes. *Fungal diversity*, *99*(1), 451-660.

Hyde, K. D., Dong, Y., Phookamsak, R., Jeewon, R., . . . Sheng, J. (2020). Fungal diversity notes 1151-1276: taxonomic and phylogenetic contributions on genera and species of fungal taxa. *Fungal Diversity*, *100*(1), 5-277.

Tang, X., Goonasekara, I. D., Jayawardena, R. S., Jiang, H. B., . . . Kang, J. C. (2020). *Arthrinium bambusicola* (Fungi, Sordariomycetes), a new species from *Schizostachyum brachycladum* in northern Thailand. *Biodiversity Data Journal*, *8*, e58755.

Hyde, K. D., de Silva, N. I., Jeewon, R., Bhat, D. J., . . . Bulgakov, T. S. (2020). AJOM new records and collections of fungi: 1–100. *Asian Journal of Mycology 3*(1), 22–294.

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Ahmadpour, A. (2019). *Alternaria caricicola*, a new species of *Alternaria* in the section *Nimbya* from Iran. *Phytotaxa*, *405*, 65–73.

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APPENDICES

APPENDIX A

CHEMICAL REAGENTS AND MEDIA

1. India ink used for observing mucilaginous sheath or appendage of conidia in some species

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

3. Lactoglycerol used for maintaining semi-permanent slides

Lactic acid 10 ml

Glycerol 10 ml

Distilled water 10 ml

Mix 10 ml lactic acid, 10 ml glycerol, and add 10 ml distilled water

4. 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

5. Lactophenol-Cotton Blue used to highlight fungal structures for viewing with the compound light microscope. Cotton blue is the most popular stain for observe septate. 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.

6. 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

7. Water Agar (WA) used for single spore isolation of fungi

Agar 15 g

Dissolve 15 g agar in distilled water then mixed and add volume to 1000 ml of water. Heath until dissolved and autoclave at 121ºC for 15 minutes

8. Malt Extract Agar (MEA) used for fungal cultivation

Agar 15 g

Peptone 0.78 g

Glycerol 2.35 g

Dextrin 2.75 g

Maltose, Technical 12.75 g

Suspend 33.6 g of malt extract agar in distilled water and mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the power and bring volume to 1000 ml. Autoclave at 121ºC for 15 minutes

9. 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 power and bring volume to 1000 ml. Autoclave at 121ºC for 15 minutes.

APPENDIX B

PUBLICATIONS

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Seifertia shangrilaensis sp. *nov.* (Melanommataceae), a new species from Southwest China

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Abstract

A new *Seifertia* species was isolated from hanging rachides of *Rhododendron decorum* in Yunnan Province, Southwest China. The new taxon was compared with the type species, *S. azaleae* and differs in having wider conidiophores, with hyaline to subhyaline and smaller conidia, while *S. azaleae* has olive-brown to brown, rarely branched conidiophores, and pale brown or olive-brown, very rarely septate conidia. Phylogenetic analyses of combined LSU, SSU and TEF1-α sequence data show that *S. shangrilaensis* forms a robust clade with *S. azaleae* nested among the species of Melanommataceae in the order Pleosporales. A new species, *S. shangrilaensis* is introduced in this study, and *Seifertia* should be placed in Melanommataceae (Pleosporales, Dothideomycetes) based on phylogenetic analysis. Description and illustration of *Seifertia shangrilaensis* are provided with notes and its introduction is supported by molecular data.

Keywords: Dothideomycetes, hyphomycetous fungi, Melanommataceae, phylogeny, taxonomy

Introduction

Seifertia was introduced as a monotypic genus by Partridge and Morgan-Jones (2002) and is typified by *S. azaleae* (Peck) Partridge & Morgan-Jones which was originally described as *Periconia azaleae* Peck. with the names *Briosia azaleae* (Peck) Deam, *Cephalotrichum azaleae* (Peck) Kuntze, *Pycnonysanus azaleae* (Peck) E.W. and *Sporocybe azaleae* (Peck) Sacc. treated as synonyms (Partridge & Morgan-Jones 2002, Glawe & Hummel 2006, Index Fungorum 2016).

Seifertia azaleae has been reported as a cosmopolitan taxon causing bud blast and twig blight of azaleas and rhododendrons in Japan, Europe and North America (Mason 1941, Ellis 1976, Farr *et al.* 1996, Partridge & Morgan-Jones 2002, Glawe & Hummel 2006). However, *Seifertia* species are relatively poorly studied worldwide, and have not been studied in the southwest of China. Based on phylogenetic analysis of LSU sequence data, Seifert *et al.* (2007) treated *Seifertia azaleae* in Dothideomycetes, as closely related to *Mycosphaerella mycopappi* A. Funk & Dorworth, but unrelated to *Mycosphaerella sensu stricto*. Crous *et al.* (2009, 2013) placed *Seifertia* in Pleosporales which was shown to be allied to *Xenostigmina*, a synanamorph of *Mycopappus*, based on combined ITS and LSU phylogenetic analyses. There is little molecular data to resolve the natural placement of *Seifertia* in Dothideomycetes.

The aim of this study is to introduce a new species, *Seifertia shangrilaensis*, collected from Yunnan Province, Southwest China. The species is described and illustrated with phylogenetic support. *Seifertia shangrilaensis* is the first record of *Seifertia* for China.

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ORIGINAL ARTICLE

Sporidesmioides thailandica gen. et sp. nov. (Dothideomycetes) from northern Thailand

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Abstract *Sporidesmioides thailandica* gen. et sp. nov., isolated from herbaceous litter in northern Thailand, is described and illustrated. The new genus is characterised by sporidesmium-like, septate, solitary, acrogenous, pale brown to brown, obclavate, conidia, which taper towards the apex and are truncate at the base and septate conidiophores similar to those of *Sporidesmium* species in Sporidesmiaceae (Sordariomycetes). It differs from similar genera by the presence of a stromatic base, polyblastic conidiogenous cells and a distinct, colourless sheath at the conidial apex. Phylogenetic analyses of combined LSU, SSU, TEF1-α and RPB2 sequence data show that the new taxon, *Sporidesmioides thailandica*, forms a separate, single clade with Torulaceae in Pleosporales, Dothideomycetes, and is distinct from Sporidesmiaceae (Sordariomycetes).

Keywords Asexual fungi · Hyphomycetes · Pleosporales · Torulaceae

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Introduction

Sporidesmium-like taxa are a fungal group that include several dematiaceous hyphomycetous genera, taxonomically segregated under *Sporidesmium sensu lato* (Shenoy et al. 2006), which produce holoblastic phragmoconidia on proliferating or non-proliferating conidiophores, with schizolytic conidial secession (Ellis 1958, 1971, 1976; Kirk 1982; Subramanian 1992; Hernández-Gutiérrez and Sutton 1997; Shoemaker and Hambleton 2001; Wu and Zhuang 2005). In their asexual phase, they occur predominantly as saprobes on rotten wood, dead branches and decaying leaves of various plant species. Some species are also plant pathogens (Ellis 1971, 1976; Guo 1989; Wu and Zhuang 2005). Su et al. (2016) introduced three new species in Sporidesmiaceae and a novel genus *Pseudosporidesmium*, typified by *Pseudosporidesmium horridum* (Crous) K.D. Hyde & McKenzie, in Sordariomycetes. Morphologically, members of Sporidesmiaceae and *Pseudosporidesmium horridum* holoblastic conidial development. *Acrocondylella arecae* (Berk. & Broome) M.B. Ellis (Ellis 1971) also produces sporidesmium-like conidiophores and conidia, but conidial development of this taxon is polytretic (Bhat 2010).

Réblová and Winka (2001) provided a phylogenetic analysis based on LSU nu-rDNA sequence data to show that *Sporidesmium folliculatum* (Cordo) E.W. Mason & S. Hughes and *S. hormiscioides* Corda are linked to Chaetosphaeriaceae, and the study revealed that sporidesmium-like genera may have

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Morphological characterization and DNA based taxonomy of *Fusiconidium* gen. nov. with two novel taxa within Melanommataceae (Pleosporales)

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Abstract

Fusiconidium machaerinae sp. nov. and *Fusiconidium aquaticum* sp. nov., isolated from a dead branch of *Clematis vitalba* in Italy and a submerged decaying wood in China respectively, are described and illustrated. The new taxa are characterized by having rhexolytic conidial secession, percurrently proliferating conidiophores, with enteroblastic conidiogenous cells and acrogenous, pale brown to dark brown, verruculose, fusiform, cruciate conidia. *Fusiconidium* is introduced as a novel genus to accommodate these novel species base on morphologically distinct from related asexual genera within the Dothideomycetes and multigene phylogenetic analyses. Based on multigene phylogenetic analyses, *Fusiconidium* is referred to the family Melanommataceae (Pleosporales).

Keywords: Asexual fungi, Dothideomycetes, hyphomycetous, multigene phylogeny

Introduction

Many asexual genera were isolated from dead plant materials and have been referred to the order Pleosporales, Dothideomycetes (Wang *et al.* 2007, Zhang *et al.* 2009). They are associated with diverse habitats and associated with various hosts worldwide (Hyde *et al.* 2013, Wijayawardene *et al.* 2014). Pleosporales is the largest order in the class Dothideomycetes (Kirk *et al.* 2008, Zhang *et al.* 2012, Hyde *et al.* 2013, Wijayawardene *et al.* 2014, Phookamsak *et al.* 2015, Tian *et al.* 2015). Over 40 families, including Melanommataceae, were included in the order Pleosporales based on morphological and DNA based phylogenetic studies (Liew *et al.* 2000, Lumbsch & Lindemuth 2001, Zhang *et al.* 2012, Hyde *et al.* 2013, Wijayawardene *et al.* 2014, Ariyawansa *et al.* 2015, Phookamsak *et al.* 2015, Tian *et al.* 2015, Li *et al.* 2016).

The family Melanommataceae was established by Winter (1885) for sexually reproducing ascomycetes, characterized by globose or sunken perithecial ascomata, bitunicate and fissitunicate asci, pigmented and phragmosporous ascospores (Sivanesan 1984, Barr 1990, Zhang *et al.* 2012, Hyde *et al.* 2013, Tian *et al.* 2015). Barr (1983) introduced Melanommatales to accommodate all taxa with trabeculate pseudoparaphyses which are morphologically distinct from cellular pseudoparaphyses in the Pleosporales. However, recent phylogenetic analyses have shown that Melanommatales should be assigned to Pleosporales (Berbee 1996, Liew *et al.* 2000, Lumbsch & Lindemuth 2001, Kruys *et al.* 2006, Schoch *et al.* 2006, Wang *et al.* 2007, Tian *et al.* 2015) and thus Eriksson (2006) transferred Melanommatales to Pleosporales. Currently Melanommataceae stands out as a distinct monophyletic family within the Pleosporales (Tian *et al.* 2015, Li *et al.* 2016).

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ORIGINAL ARTICLE

Molecular taxonomy and morphological characterization reveal new species and new host records of *Torula* species (Torulaceae, Pleosporales)

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Abstract Four new species and two new host records of *Torula* (Torulaceae, Pleosporales) are described and illustrated from herbaceous litter collected in Italy and Thailand. The new species possess colony, conidiophore and conidial characteristics that fit within the generic concept of *Torula*. Detailed morphological observations clearly demarcate four of these from extant species and are hence described as new (*Torula chiangmaiensis* sp. nov., *Torula pluriseptata* sp. nov., *Torula chromolaenae* sp. nov., *Torula wuchengziti* sp. nov.). Details of asexual morphs are described, and justifications for establishing these new species are provided. The nuclear rDNA sequenced ribosomal RNA genes as well as protein coding genes to infer phylogenetic relationships and discuss phylogenetic affinities with morphologically similar species. Our morphological distinction is further supported by phylogenetic discrimination. In particular, phylogenies depict a close relationship of *Torula chiangmaiensis* and *T. pluriseptata* to *T. hollandica*, while *T. chromolaenae* and *T. wuchengziti* constitute an independent phylogenetic lineage basal to *T. herbarum* and *T. ficus*. *Torula ficus* and *T. waltonii* are also described and their phylogeny investigated as new host records from *Rubus pileus* and *Iris germanica*, respectively.

Keywords Asexual fungi · Dothideomycetes · Hyphomycetous · Multigene phylogeny

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Introduction

The family Torulaceae, first introduced by Corda (Sturm 1829), is known only by asexual characters of its erect micro- or macronematous conidiophores, with or without apical branches, and doliiform to ellipsoid or clavate, brown, smooth to verruculose, mono- to polyblastic, often cupulate conidiogenous cells and subcylindrical, phragmosporous, acrogenous, brown, dry, smooth to verruculose conidia, characteristically produced in branched chains (Crous et al. 2015; Su et al. 2016; Hyde et al. 2016). Catenate conidia or branched conidial chains are formed mostly by the terminal cell and sometimes even other cells of the conidia becoming fertile and producing newer conidia in chains (Ellis 1971; Crous et al. 2015). Crous et al. (2015) investigated phylogenetic relationships of the Torulaceae and provided DNA sequence data for four *Torula* species, and accepted

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Marinophialophora garethjonesii gen. et sp. nov.: a new hyphomycete associated with *Halocyphina* from marine habitats in Thailand

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Abstract

Marinophialophora garethjonesii sp. nov., representing a novel genus *Marinophialophora* gen. nov., associated with the basidiomycete fungus, *Halocyphina* on mangrove wood from Phetchaburi, Thailand, is described and illustrated. *Marinophialophora* morphologically resembles *Phialophora* and *Austrosporo* (Herpotrichiellaceae, Chaetothyriales) in having macronematous, unbranched or branched conidiophores, globose to subglobose, solitary, acrogenous, pale to subhyaline, aseptate, conidia in chains and phialidic conidiogenous cells. *Marinophialophora* mainly differs from other related genera due to its conidia borne in basipetally developing branched chains and septate conidiophores. Phylogenetic analyses of a combined ITS, LSU and SSU sequence data show that *Marinophialophora garethjonesii* constitutes an independent lineage with high statistical support basal to the genus *Exophiala* within the family Herpotrichiellaceae (Chaetothyriales). The new marine taxon is described herein with illustrations and relationships inferred from DNA sequence data.

Keywords: Asexual fungi, Chaetothyriales, Herpotrichiellaceae, new genus, phylogenetics

Introduction

Asexual morph of ascomycetes within the order Chaetothyriales comprise morphologically diverse hyphomycetes (Shenoy *et al.* 2006, Liu *et al.* 2015), which are ubiquitous with different ecological lifestyles such as epilithic on rocks, lichenized and lignicolous fungi, saprobic on dead materials and pathogenic on plants, humans and animals (Gueidan *et al.* 2008, Chomnunti *et al.* 2012a, b, Réblová *et al.* 2013, Stillmann *et al.* 2013, Hubka *et al.* 2014, Liu *et al.* 2015). Herpotrichiellaceae is the best known family in the order Chaetothyriales (Nascimento *et al.* 2016). About one-third of the species of this family were described from pathogenic strains isolated from human diseases, and occasionally infections can be fatal (Li *et al.* 2011). Nevertheless, there are poorly known marine fungi within this family. Marine habitats, especially mangroves are well-known to harbour many novel species of fungi (Jones *et al.* 2009, 2015). Species in genus *Halocyphina* are well-known to occur on core mangrove species (Pang & Jheng 2012). Recent collections of *Halocyphina* species on decayed mangrove wood from the Southern parts of Thailand recovered an unusual species. The present study investigates the morphological characterisation and phylogenetic position of this new taxon, *Marinophialophora garethjonesii*, collected from a tropical marine habitat.

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Establishment of Zygosporiaceae *fam. nov.* (Xylariales, Sordariomycetes) based on rDNA sequence data to accommodate *Zygosporium*

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Abstract

Zygosporium species are widespread on hosts and usually associated with monocotyledonous plants including Arecaceae (palms) and Pandanaceae. *Zygosporium* forms colonies as darkened spots on the host surface. This study recovers two *Zygosporium* species from China and Thailand and makes an attempt to clarify the systematic placement of *Zygosporium* based on morphological characteristics and phylogenetic analyses based on rDNA sequence data. Phylogenetic analyses of a combined LSU and ITS sequence data generated under Maximum Likelihood and Bayesian criteria indicate that *Zygosporium* species form a strongly supported monophyletic lineage basal to the Coniocessiaceae and not to the Microdochiaceae as previously assumed. Given the distinct morphological features of *Zygosporium* and its strongly supported monophyletic nature, a new family, Zygosporiaceae is introduced within the Xylariales.

Key words – Asexual fungi – hyphomycetes – multigene phylogeny – new family

Introduction

Zygosporium (Xylariales, Sordariomycetes) species are usually found as saprobes occurring on various angiosperms and some gymnosperms with a cosmopolitan distribution from temperate to tropical regions (Photita et al. 2001, Whitton et al. 2002, Manoharachary et al. 2006, McKenzie et al. 2007, Abbas et al. 2011, Pratibha et al. 2012, Taheriyan et al. 2014, Farr & Rossman 2017). Currently there are 16 described species (Wijayawardene et al. 2017). Sequence data of

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RESEARCH ARTICLE

Multigene phylogeny and taxonomy of *Dendryphion hydei* and *Torula hydei* spp. nov. from herbaceous litter in northern Thailand

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Abstract

During our studies on asexual fungi colonizing herbaceous litter in northern Thailand, we discovered two new fungal species, viz. *Dendryphion hydei* and *Torula hydei* spp. nov. The latter are examined, and their morphological characters are described as well as their DNA sequences from ribosomal and protein coding genes are analysed to infer their phylogenetic relationships with extant fungi. *Torula hydei* is different from other similar *Torula* species in having tiny and catenate conidia. *Dendryphion hydei* can be distinguished from other similar *Dendryphion* species in having large conidiophores and subhyaline to pale olivaceous brown, 2–4(–5)-septate conidia. Multigene phylogenetic analyses of a combined LSU, SSU, TEF1-α, RPB2 and ITS DNA sequence dataset generated from maximum likelihood and Bayesian inference analyses indicate that *T. hydei* forms a distinct lineage and basal to *T. fici*. *Dendryphion hydei* forms a distinct lineage and basal to *D. europaeum*, *D. comosum*, *D. aquaticum* and *D. fuminicola* within Torulaceae (Pleosporales, Dothideomycetes).

Introduction

The family Torulaceae Corda was introduced by Sturm [1] and is typified by *Torula* Pers. Species in Torulaceae are known only by their asexual morphs which are characterized as followed: superficial, effuse, greyish brown to black, powdery colonies; micro- or macronematous conidiophores, with or without apical branches; doliform to ellipsoid or clavate, brown, smooth to verruculose, mono- to polyblastic conidiogenous cells which often remaining cupulate; subcylindrical, phragmosporous, acrogenous, brown, dry, smooth to verrucose conidia characteristically produced in branched chains [2,3,4,5,6,7]. Crous et al. [8] investigated phylogenetic relationships of this family with the inclusion of *Torula* species and accepted *Dendryphion* Wallr., besides *Torula* within Torulaceae in Pleosporales. Su et al. [6] introduced *Neotorula* Arzuaga, Z.L. Luo & K.D. Hyde and two new *Dendryphion* species in Torulaceae

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Alternaria: update on species limits, evolution, multi-locus phylogeny, and classification

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Abstract

Alternaria a genus of ascomycetes comprise major plant pathogens, saprobes and common allergens in humans. There are more than 360 species in the genus, which are currently divided into 28 sections. This paper aims to elaborate the taxonomy of *Alternaria* with multi-locus

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