FUNGAL ENDOPHYTES FROM *DENDROBIUM*

XIAOYA MA

DOCTOR OF PHILOSOPHY
IN
BIOSCIENCES

SCHOOL OF SCIENCE
MAE FAH LUANG UNIVERSITY
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ABSTRACT

Dendrobium, as one of the largest genera in Orchidaceae, is spread in Atlantic Islands and Asia. They are well known for commercial, horticultural, medicinal, and ornamental values. For decades, the general compositions, function, and secondary metabolites of culturable fungal endophytes of *Dendrobium* species have been investigated. However, due to the lacking morphological characteristics and ambiguous phylogenetic analysis resulting from the single gene ITS amplification for most research, the species composition of fungal endophytes in *Dendrobium* remains unclear. Furthermore, the species resolution for the taxonomy of fungal endophytes contributes to discovering more cryptic taxa and providing further information for fungal resources. This study aims at identifying fungal endophytes with morph-molecular methods to give a general conclusion for fungal endophytes composition of 65 *Dendrobium* individuals (Including 25 known species and other repeat or not identified species) in southwestern China and northern Thailand. Meanwhile, the cytotoxic activities screening of selected fungal endophytes of *Dendrobium* species were carried out in this study.

To achieve a natural classification, the endophytic species resolution was implemented by sporulation with various media including CMA, MEA, OA, PDA, SDA, SNA, WA (with toothpicks/sample debris/slide culture/pine needle) and multi-gene phylogenetic analysis with partial genes of ACT, ITS, CAL, GAPDH, HIS, LSU, RPB1, RPB2, SSU, TEF -1α, TUB2. A total of 750 culturable fungal endophytic strains were isolated from 65 *Dendrobium* samples and identified to 110 species with 25 novel taxa and 50 new host records were identified. These endophytic species are distributed in 70 genera of 52 families belonging to 24 orders of 8 classes mainly in Ascomycota (94.6%) and less in Basidiomycota (5.4%). In Sordariomycetes, 20 novel species (8 in *Colletotrichum*, 3 in *Xylaria*, 2 in *Diaporthe*, 2 in *Fusarium*, 2 in *Hypoxylon*, 1 in *Annulohypoxylon*, 1 in *Neopestalotiopsis*, 1 in *Nemania*) and 40 new host records were reported. Four and one novel species were discovered from Dothideomycetes (*Aureobasidium*, *Epicoccum*, *Mycosphaerella*, *Phyllosticta*) and Leotiomycetes (*Pezicula*), respectively. The rest isolates were categorized to Agaricomycetes (3.4%), Eurotiomycetes (3.2%), Pezizomycetes (1.4%), Saccharomycetes (5.2%) and Ustilagiomycetes (0.1%). *Colletotrichum*, *Diaporthe* and *Fusarium* were the three frequently isolated genera in Sordariomycetes also in this phylum. *Colletotrichum siamense* is the most frequently isolated species. *Phyllosticta* species are the most isolated group in Dothideomycetes. *Meyerozyma* and *Penicillium* take the majority of Saccharomycetes and Eurotiomycetes, respectively. In Basidiomycota, 13 genera were discovered in Agaricomycetes. *Pseudozyma tsukubaensis* is the solo species identified in Ustilaginomycetes. The mycorrhizal fungi *Tulasnella* sp. represented by several strains were isolated from roots but grow extremely slowly and died very soon.

In this study, there is no universal media or cultivation method for all endophytes sporulation. The whole sporulation rate is 5%. The cultivation with sterilized host debris or pine needles failed to improve the sporulation rates. The media with low concentrations were recommended for enhancing the sporulation.

The highest endophyte isolation rate 31.3% is from *Dendrobium* stems followed by 30.9% of leaves. The separation rates of roots and flowers are 28.4% and 9.2% respectively. Most species identified in this study are phylogenetically nested

within known pathogens that have been reported from other plant hosts including some orchids. The host, geographic and organ specificity between fungal endophytes and *Dendrobium* species were rarely observed. Only the well-known leaf inhabiting endophyte *Phyllosticta capitalensis* was mostly separated from the *Dendrobium* leaves.

In this study, cytotoxic assays were carried out with 153 crude extracts including some further fractions. The results showed that 10 species possess cytotoxic potentials that vary from medium to strong degrees. Two newly introduced species in this study–*Colletotrichum doitungense* and *Neopestalotiopsis dendrobii* performed well in inhibiting K562 and PC3 growth. The crude extracts of *Hypoxylon investiens* (MFLUCC 15-1188), *Neopestalotiopsis dendrobii* (MFLUCC 14-0106) and *Xylaria venosula* (MFLUCC 21-0013) performed the highest inhibitory rates towards A549 (64.8%), K562 (89%) and PC3 (55.8%) cell lines, respectively. Several endophytic xylarialean strains stand out of other species which perhaps indicate that they contain natural compounds for inhibiting tumor cell growth.

This study firstly provides a fungal endophytic species list, which supplies a further knowledge of the diversity of fungal endophytes associated with *Dendrobium* species. Meanwhile, the taxonomy of fungal endophytes showed some cryptic taxa and facilitate phylogenetic stability. Therefore, they should have a place in the fungal taxonomic world. However, to give strong evidence, more type or epitype specimens of fungal endophyte with more protein gene annotation data are expected in future work. With interdisciplinary exploration, we may break through the dilemma caused by method limitations and be free from the laborious and low effective endophyte identification work. The results also indicate these fungal assemblages can be a promising repertoire to produce interesting bioactive compounds.

Keywords: Ascomycotina, Bioactivity, Biodiversity, Checklist, Endophytic, Multi-gene, Orchids, Phylogeny, Taxonomy

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ABBREVIATIONS AND SYMBOLS

diam.	diameter
et al.	and others
hr.	hour
gen.	genus/genera
i.e.	that is
mg	milligram
min	minute
mm	millimetre
nov.	novum (Latin for new thing)
rpm	revolutions per minute
s	second
sp.	species
spp.	Species (plural)
$\bar{x}$	average
°C	degree centigrade
µg/ml	microgram per milliliter
µM	micromolar
µm	micrometer
µl	microliter
%	percentage

CHAPTER 1

INTRODUCTION

1.1 General Introduction of Fungal Endophyte

The term “endophyte” was introduced by De Bary and was used for describing any organisms occurring within the plant tissues (de Bary, 1866). Fungal endophytes are normally defined as fungi that colonize in a plant without causing any visible disease symptoms at any special moment (Schulz & Boyle, 2005). The fossil revealed the coenocytic hyphae and recorded that fungi probably inhabited initially in the vascular plant from the early Devonian (Taylor et al., 1995; Taylor & Krings 2005). Fungal ultrastructure in plants showed that endophytes colonized in the epithelial cells and multivesicular of tall fescue (with no obvious alternation in host cell morphology) (Hinton & Bacon, 1985), in the subterranean gametophytes of pteridophytes of thalloid liverworts and Phycomycetes (Pocock & Duckett, 1984). Almost every living plant is associated with fungal endophytes on earth from the arctic to the tropics (Aly et al., 2011). Fungal endophytes normally transmitted by surrounding fungal conidia/spores in the air (horizontally) or seed infection (vertically) (Hamilton & Bauerle, 2012). The fungal endophytes include obligate and facultative endophytes, dark septate endophytes (DSE) – a group of ascomycetous anamorphic fungi colonizing root tissues intracellularly and intercellularly (Jumpponen, 2001). Rodriguez et al. (2009) classified fungal endophytes into four different groups according to symbiotic criteria as shown in Table 1.1. Over the years, endophytes are extremely well-studied fungi (Hyde & Soytong, 2008).

The majority of fungal endophytes belong to Ascomycota and Basidiomycota, especially Dothideomycetes and Sordariomycetes in Ascomycota (Sieber, 2007; Rodriguez et al., 2009; Hyde et al., 2013; Maharachchikumbura et al., 2016). Fungal endophytes can be parasitic, dormant saprotrophs and latent pathogens (Fisher & Petrini, 1992; Tao et al., 2008; Promputtha et al., 2007). The symbiotic relationship

Table 1.1 Symbiotic criteria used to characterize fungal endophytic classes

Criteria	**Clavicipitaceous**	**Nonclavicipitaceous**		
	Class 1	**Class 2**	**Class 3**	**Class 4**
Host range	Narrow	Broad	Broad	Broad
Tissue(s) colonized	Shoot and rhizome	Shoot and rhizome	Shoot	Root
In planta colonization	Extensive	Extensive	Limited	Extensive
In planta biodiversity	Low	Low	High	Unknown
Transmission	Vertical and horizontal	Vertical and horizontal	Horizontal	Horizontal
Fitness benefits	NHA	NHA and HA	NHA	NHA

Note *Nonhabitat-adapted (NHA) benefits such as drought tolerance and growth enhancement are common among endophytes regardless of the habitat of origin. Habitat-adapted (HA) benefits result from habitat-specific selective pressures such as pH, temperature and salinity.

The source is from: ‘Fungal endophytes: diversity and functional roles’ (Rodriguez et al., 2009) shift from mutualism to parasitism in a seamless manner (Kogel et al., 2006). There is a compromising relation between endophytic infections and host plant fitness after long coevolution (Lehtonen et al., 2005). The mode of transmission, infectious pattern, environmental conditions, plant age and genetic background will affect the plant-endophyte interactions (Saikkonen et al., 1999; Aly et al., 2011; Eaton et al., 2011). It is reported that climate changes convert endophytic fungi into weak pathogens or opportunistic invaders of senescing and indeed healthy trees (Moricca & Ragazzi, 2008).

Fungal endophytes can probably contribute to protecting the plant from pathogen invasion, confer plant stress tolerance via screening certain secondary metabolites or transfer nutrients like mycorrhizal fungi (Jumpponen, 2001; Arnold et al., 2003; Rodriguez et al., 2008). They can also be saprobes or latent pathogens to accelerate the death of host plants (Tao et al., 2008; Promputtha et al., 2007). Fungal endophytes are also regarded as potential resources for discovering alternative drugs fighting against various kinds of diseases (Bills et al., 2012; Wittstein et al. 2020; https://www.scynexis.com/pipeline).

1.1.1 Fungal Endophytes Biodiversity and Specificity

Fungal endophytes have not been largely quantified and investigated the biodiversity (Arnold et al., 2001). However, many studies showed that a lot of plants possess highly diverse fungal endophytes especially in tropical and subtropical areas (Fröhlich & Hyde, 1999; Photita et al., 2001; Promputtha et al., 2005; Hyde et al., 2018). Fungal endophytes turn out to be extremely diverse in a wide array of tropical angiosperms (Arnold et al., 2001). But when there is not strong ecological gradients and host turnover, the beta diversity of endophyte communities could be low in large areas of contiguous forests (Vincent et al., 2016). Class 1 (clavicipitaceous fungi of grasses) and class 2 (non- clavicipitaceous fungi colonized on both shoot and rhizome) fungal endophytes are found to be low biodiversity which is likely to be restricted to the transmission methods (Rodriguez et al., 2009).

There are lacking data to support that most fungal endophytes are host-specific except for several Acremonium species and Neotyphodium uncinatum in Fescue Pasture grass (Leuchtmann, 1993; Ekanayake et al., 2012). However, *Colletotrichum*, *Diaporthe*, *Fusarium*, *Phyllosticta* and xylariaceous fungi are dominant or frequently isolated fungal endophytes in many kinds of plants (Hyde & Soytong 2008; Wikee et al., 2013; Jayawardena et al., 2016).

1.1.2 The Speculative Roles of Fungal Endophytes

The roles of fungal endophytes have been poorly studied (Rodriguez et al., 2009). Fungal endophytes as saprobes can occur in the biological degradation of dead or dying host plants after undergoing a dormant pe;riod like Xylaria in *Magnolia liliifera* (Whalley, 1996; Aly et al., 2011). Fungal saprotrophs may appear when plant substrata decay (Hyde et al., 2007). Taxonomic consistency between endophytes and saprotrophs was observed in tropical palms (Frohlich & Petrini 2000). What’s more, some fungal endophytes were reported to secrete decay lignocellulolytic enzymes related to wood biodegradation (Jordaan et al., 2006; Oses et al., 2006). Strains of common endophytes *Colletotrichum*, *Fusarium*, *Guignardia* and *Phomopsis* isolated from *Magnolia liliifera* turned out to be high sequence similarity and phylogenetically related to their saprotrophic counterparts (Promputtha et al., 2007).

Latent pathogenic fungal endophytes are group pathogens that can make plant disease latterly (Photita et al., 2004). They are limitedly and closely related to pathogens (Carroll, 1988). The disease symptoms may occur when plants are under stress or non-optimal growth conditions (Slippers & Wingfield, 2007).

Plants face environmental stress when they move from aquatic habitats onto lands, which force symbiotic fungi to confer drought tolerance (Pirozynski & Malloch, 1975). All endophytic fungi can help defend drought stress and grow on diverse species (Rodriguez & Redman, 2008). The drought tolerance in plants is achieved by increasing osmotic potential or reducing water consumption with fungal strategies (Redman et al., 2011). What's more, endophytes may secrete certain secondary metabolites when their hosts suffer salinity (Waller et al., 2005). The fungal endophytic cell wall can produce melanin in planta to dissipate heat under thermal conditions (Redman et al., 2002). *Fusarium culmorum* was reported to be a specific colonizer in coastal plants, which helps its host survive exposure to heat (Rodriguez et al., 2008). Diene et al. (2014) confirmed that DSEs inoculating with Chinese cabbage could enhance phytoremediation of Cs in contaminated surroundings and if cultivating them with tomatoes, accumulation of Cs in plants under contaminated circumstances could be decreased.

Fungal endophytes in grasses are normally viewed as mutualists (Saikkonen et al., 2004) and produce physiologically alkaloids in their hosts, which makes plants toxic to domestic mammals and increase resistance to insect herbivory (Clay, 1988). Meanwhile, the infection can also decrease host fitness via reducing competitive properties in short term and be a trade-off benefit in source limited conditions (Ahlholm et al., 2002; Faeth et al., 2004). The response of aphids to plants is subjected to alkaloids including ergovaline, lolitrem B, N-formyl, N-acetyl loline and peramine produced by *Acremonium* spp. and Epichloe typhina (Siegel et al., 1990). *Fusarium oxysporum* and

Cryptosporiopsis sp. were reported to confer disease resistance against virulent pathogens in barley (Hordeum vulgare) and larch (Larix decidua) respectively (Schulz et al., 1999). DSE fungi probably play a role in deterring pathogens through minimizing the carbon available carbon in the host rhizosphere environment and their high melanin levels can protect the host from pathogenic attacking (Mandyam & Jumpponen, 2005). Besides, dual infection with *Cladosporium cladosporioides* and *Trichoderma viride* previously isolated from their host leaves of *Cirsium arvense* markedly reduced beetle feeding (Gange et al., 2012). In addition, endophytes colonization can promote the stability of meadow fescue so that protect hosts from weed invasion (Saikkonen et al., 2013). The endophytes-mediated protection in mature plant leaves perhaps involve less intrinsic defence against fungal pathogens than do young leaves (Arnold et al., 2003).

Diverse endophytic fungi could form novel protoplasts called mycosomes that cultured from plants and phylogenetically diverse endophytic fungi possess common morphological characteristics (Atsatt & Whiteside, 2014). Endophytes symbiosis on various life stages may contribute to niche partitioning between the two congeneric plant species (Kazenel et al., 2015).

1.1.3 Fungal Endophytes Research Dilemma

Although some foliar fungal endophytes may serve as pathogens and saprotrophs, the current speculation of frequency and nature of overlaps among these communities is limited by methodological issues and the ecological variability of many fungi (Arnold, 2007). Fungal endophytes research is a method-dependent process (Guo et al., 2001). There have no standard principles for surface sterilization, the isolation results would be largely subjected to the research method (Frohlich & Petrini, 2000; Hyde & Soytong, 2008). Therefore, to make leaf imprints on the agar surface was proposed and should be highlighted for testing protocols for fungal endophyte isolation in every study (Schulz et al., 1999; Hyde & Soytong, 2008).

In traditional research, fungal endophytes can be only obtained from artificial media(Guo et al., 2001). However, many fungi can not grow or grow very slowly on those media (Tao et al., 2008; Zhu et al., 2008). It is estimated that only 0.1-10% of microorganisms can grow on artificial media (Muyzer et al., 1993; Jeewon et al., 2018).

Therefore, we could observe only fast-growing fungal endophytes and get a biased result about endophytic components and biodiversities (Hyde & Soytong, 2008).

The sporulation rate of fungal endophytes is low (Promputtha et al., 2005; Jeewon et al., 2017). Although the second and third-generation sequencing technologies improve fungal species resolution, potential errors in extracting DNA and lacking morphological characteristics make fungal endophytes identification difficult especially at the species level (Hyde & Soytong, 2008; Jeewon & Hyde, 2016; Jeewon et al., 2017).

Based on the above restriction, it is difficult to evaluate the fungal endophyte biodiversity and study their life cycle, infection mechanisms and roles in host plants.

1.1.4 *Dendrobium* Orchids

Dendrobium Swartz established by the Swedish botanist Olaf Swartz in 1799, alias "Shi hu" in Chinese medical plants, is one of the biggest genera and belongs to the tribe Dendrobieae of the subfamily Epidendroideae in *Orchidaceae* (Chen et al., 2011; Xiang et al., 2013), which comprises over 1550 species including around 50 varieties, 8 subspecies and 14 hybrid species recorded in 'The Plant List 2013' (Cheng et al., 2019). More than 50 species were discovered within 4 years. The highest diversity with over 150 species appears in New Guinea (Lokho, 2013). *Dendrobium* is also the largest orchid genus with more than 150 native species in Thailand (Peyachoknagul et al., 2014). Like other orchids, *Dendrobium* seeds germination rely on mycorrhizal fungi and their growth is associated with fungal endophytes (Rodriguez et al., 2009; Smith & Read, 2010). Most *Dendrobium* orchids are epiphytic (Liu et al., 2014). Some of them grow from rock joints such as *Dendrobium officinale* K. found at an elevation over 1000m. *Dendrobium* species distributed in tropical areas from the south, east, southeast of Asia to Australasia Oceania (Lavarack et al., 2000; Liu et al., 2014). They tend to grow in a half-shade, moist and ventilative environment but low productive (Gogoi 2005). Melaleuca L.and Ficus woodlands are common hosts of *Dendrobium* (Bartareau & Skull, 1994; O'Donnell & Parrella, 2005).

"Shi hu" was first recorded as rare herbs on one of the Chinese traditional medical masterpieces 'Shen Nong Herbal Classic' around 2300 years ago (Journal of Traditional Chinese Medicine, 2010). There have been 41 *Dendrobium* species used in traditional Chinese medicine but many of them confronted with extinction (Cheng et al., 2019). The chemical constituents of *Dendrobium* spp. were firstly reported in the 1930s by Suzuki and Keimatsu (1932) (Suzuki, 1932). *D. thyrsiflrum* was regarded as standard medicine by the Chinese Pharmacopoeia and affirmed its active component is coumarin existing in stem (Zhang et al., 2005). There are about 190 compounds that have been isolated from *D. officinale* in the past decades (Tang et al., 2017). Angiogenesis inhibitory, anticancer, anti-inflammatory and antimutagenic compounds have been discovered from *Dendrobium* extracts including alkaloids, bibenzyls, fluorenones, phenanthrenes, phenylpropanoids, sesquiterpenoids and polysaccharides as listed in table 1.2-1.7 (Song et al., 2012; Gong et al., 2013; Lin et al., 2013; Xu et al., 2013; Chen et al., 2014).

Coumarin takes effect on smoothing muscle, relaxing vascular and anticoagulation. It was also reported as a potent anti-HIV agent (Yu et al., 2003). *D. chrysanthum* Wall., *D. fimbriatum* Hook., *D. loddigesii* Rolfe., and *D. nobile* Lindl. can be used as medicine (Chinese Pharmacopoeia). Their stems are sweet flavor with function of tonifying stomach and increasing fluid, nourishing yin (traditional Chinese medical balance-yin and yang) and clearing heat. Therefore, many researchers explored the active principle in *Dendrobium* (Sritularak et al., 2011; Song et al., 2012; Yang et al., 2015; Wang et al., 2016). Sesquiterpene alkaloid dendrobine has been regarded as the quality standard of D. nobile (Li et al., 2017).

Do et al. (2005) found that antimicrobial and antioxidant activities in phenolic substances from *D. nobile*. Antibacterial effect in dihydrostibene and polysaccharides was also found in *D. amoenum*, *D. hookerianum* and *D.officinale* (Venkateswarlu et al., 2003; Li et al., 2011). The ethanolic extract of *D. formosum* and water-soluble polysaccharides extracted from *D. denneanum* possess antitumor activity (Luo & Fan, 2011; Prasad & Koch, 2014). Some interesting compounds such as Liriodendrin - a kind of glucoside grease, Dendronone – a new phenanthrenequinoe, Dendrowardols A, B, C – 3 new sesquiterpenoids were found in *Dendrobium* (Chen et al., 2008, 2014; Fan et al., 2013a, 2013b). Although *Dendrobium* orchids are famous medicinal herbs, few products of them have been subject to proper clinical study because the weakness of our conclusions is that Chinese herbal medicines do not have the rigorous characterization and standardization necessary for clinical study (Bulpitt et al., 2007).

Table 1.2 Alkaloids isolated from *Dendrobium* species

Alkaloids			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Dendroparine	*D. anosmum; D. parishii*	-	Leander and Luning (1968)
cis- and trans-dendrochrysine	*D. chrysanthum*	-	Ekevag et al. (1973)
cis- and trans-dendrochrysanines	*D. chrysanthum*	-	Yang et al. (2005)
Hygrine	*D. chrysanthum; D. primulimun*	-	Ye et al. (2003)
Crepidine	*D. crepidatum*	-	Elander et al. 1973; Xu et al. (2019)
Crepidamine	*D. crepidatum*	-	Elander et al. 1973; Xu et al. (2019)
Isocrepidamine	*D. crepidatum*	Hypoglycemic effect	Elander et al. 1973; Xu et al. (2019)
Crepidatumines (C-D)	*D. crepidatum*	-	Xu et al. (2019)
1-((5S 6R 7S 8aR)-6-hydroxy-7-methyl-6-phenyl-octahydroindolizin-5-yl) propan-2-one	*D. crepidatum*	-	Zhao et al. (2011)
Dendrocrepine	*D. crepidatum*	-	Elander et al. (1973)
Isodendrocrepine	*D. crepidatum*	-	Elander et al. (1973)
Dendrobine	*D. findlayanum; D. nobile*	Effects on liver glucose /lipid metabolism and antioxidant gene expressions; Improve learning and memory impairment; Alter hepatic lipid homeostasis	Granelli et al. (1970); Okamoto et al. 1972; Li et al. (2011); Nie et al. (2016); Wang et al. (2016); Xu et al. (2017); Huang et al. (2019)
Dendrobine-N-oxide	*D. nobile*	Effects on liver glucose /lipid metabolism and antioxidant gene expressions; Improve learning and memory impairment	Nie et al. (2016); Li et al. (2019); Xu et al. (2017)
6-Hydroxy-dendrobine	*D. nobile; D. friedricksianum; Dendrobium hildebrandii*	-	Hedman et al. (1971); Okamoto et al. (1972); Wang et al. (2016)
10-hydroxydendrobine	*D. findlayanum*	-	Qin et al. (2011)
Nobiline	*D. findlayanum; D. nobile*	-	Qin et al. (2011); Hayakawa et al. (1970)
Nobilonine	*D. nobile*	Effects on liver glucose /lipid metabolism and antioxidant gene expressions; Improve learning and memory impairment	Okamoto et al. (1972); Li et al. (2011) (2019); Nie et al. (2016); Wang et al. (2016); Xu et al. (2017)

Table 1.2 (continued)

Alkaloids			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Dendramine	*D. friedricksianum; D. hilderbrandii; D. nobile*	Improve learning and memory impairment	Hedman et al. (1971); Elander and Leander (1971); Okamoto et al. (1966); Li et al. (2011, 2019); Wang et al. (2016)
N-isopentenyldendroxinium	*D. friedricksianum; D. hilderbrandii; D. nobile*	-	Hedman et al. (1971); Wang et al. (2016)
N-isopentenyldendrobinium	*D. nobile*	-	Wang et al. (2016)
N-isopentenyl-6-hydroxydendroxinium	*D. friedricksianum; D. hilderbrandii; D. nobile*	-	Hedman et al. (1971);
6-hydroxy-nobiline	*D. friedricksianum; D. hilderbrandii*	-	Hedman et al. (1971)
Shihunidine	*D. loddigessi*	Inhibitors of Na+ K+-ATPase of the rat kidney	Li et al. (1991)
Shihunine	*D. loddigessi; D. lohohense; D. pierardii*	Inhibitors of Na+ K+-ATPase of the rat kidney	Li et al. (1991); Inubushi et al. (1964); Leete and Bodem (1976)
Monoline	*D. moniliforme*	-	Liu et al. (2007)
Dendrine	*D. nobile*	-	Granelli et al. (1970)
Dendronobiline A	*D. nobile*	-	Inubushi et al. (1965) Suzuki and Keimatsu (1932)
Dendroxine	*D. nobile*	Effects on liver glucose /lipid metabolism and antioxidant gene expressions; Improve learning and memory impairment	Okamoto et al. (1972); Nie et al. (2016); Xu et al. (2017); Li et al. (2019)
3-hydroxy-2-oxodendrobine	*D. nobile*	-	Wang et al. (1985)
13-Hydroxy-14-oxodendrobine	*D. nobile*	Effects on liver glucose /lipid metabolism and antioxidant gene expressions; Improve learning and memory impairment	Nie et al. (2016); Xu et al. (2017); Li et al. (2019)
6-hydroxy-dendroxine	*D. nobile*	-	Okamoto et al. (1966)
6-Hydroxy-nobilonine	*D. nobile*	Effects on liver glucose /lipid metabolism and antioxidant gene expressions; Improve learning and memory impairment	Nie et al. (2016); Xu et al. (2017); Li et al. (2019)

Table 1.2 (continued)

Alkaloids			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
3-hydroxy-2-oxoden-drobine	*D. nobile*	Improve learning and memory impairment	Li et al. (2011)
8-hydroxy-dendroxine	*D. nobile*	-	Inubushi et al. (1965) Suzuki and Keimatsu (1932) Inubushi and Nakano (1965)
N-methyldendrobinium	*D. nobile*	-	Wang et al. 2016
Nisopentenyldendrobinium	*D. nobile*	-	Inubushi et al. (1965) Suzuki and Keimatsu (1932) Inubushi and Nakano (1965)
Dendrobine N-oxide	*D. nobile*	-	Inubushi et al. (1965) Suzuki and Keimatsu (1932) Inubushi and Nakano (1965); Xu et al. (2017)
Nisopentenyldendroxinium	*D. nobile*	-	Inubushi et al. (1965) Suzuki and Keimatsu (1932) Inubushi and Nakano (1965)
N-isopentenyl-6-hydroxydendroxinium	*D. nobile; D. friedricksianum; D. hilderbrandii*	-	Wang et al. (2016); Hedman et al. (1971)
Pierardine	*D. pierardii*	-	Elander et al. (1971)
Dendroprimine	*D. primulimun*	-	Blomqvist et al. (1972)
Mubironine (A-B)	*D. snowflake*	-	Morita et al. (2000) Wang et al. (2016)
Dendrowardine	*D. wardianum*	-	Glomqvist et al. (1973)
4´-Hydroxy-transcinnamates	*D. clava var.aurantlacum*	-	Chang et al. (2001)
Campesterol	*D. clava var.aurantlacum*	-	Chang et al. (2001)
Stigmasterol	*D. clava var.aurantlacum; D. brymerianum*	-	Chang et al. (2001); Chen et al. (2014)
β-sitosterol	*D. clava var.aurantlacum; D. polyanthum; D. crepidatum; D. moniliforme; D. brymerianum; D. aduncum; D. trigonopus; D. chrysotoxum*	-	Chang et al. (2001); Hu et al. (2009); Li et al. (2013); Xin et al. (2011); Zhao et al. (2015); Chen et al. (2014); Bi et al. (2006; Zhang et al. (2005); Ma et al. (1994)
Alkyltrans-ferulates	*D. clava var.aurantlacum*	-	Chang et al. (2001)

Table 1.2 (continued)

Alkaloids			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Stigmast-4-en-3-one	*D. clava var.aurantlacum*	-	Chang et al. (2001)
Aliphatic alcohols	*D. clava var.aurantlacum*	-	Chang et al. (2001)
369-Trihydroxy-34-dihydroanthracen-1(2H)-one	*D. polyanthum*	Cytotoxic toxic: against human tumor cell lines (A549 and HL-60)	Hu et al. (2008)
Homocrepidine A	*D. crepidadum*	Anti-inflammatory	Hu et al. (2015)
2-Hydroxydendrobine	*D. findlayanum*	-	Granelli et al. (1970)

Table 1.3 Bibenzyls isolated from *Dendrobium* species

Bibenzyls			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Moscatilin (44'-dihydroxy-33'5-trimethoxybibenzyl)	*D. amoenum; D. aphyllum; D. aurantiacum var. denneanum; D. chrysanthum; D. crepidatum; D. densiflorum; D. fimbriatum; D. gratiosissimum; D. infundibulum; D. loddigessi; D. longicornu; D. moniliforme; D. moscatum; D. nobile; D. polyanthum; D. secundum; D. thyrsiflorum; D. trigonopus; D. moniliforme*	Antioxidant. Cytotoxic: against human tumor cell lines (A549 and HL-60); Anti-platelet aggregation; Antioxidant; Inhibited nitric oxide production in macrophage RAW 264.7 cells	Majumder et al. (1999); Miyazawa et al. (1999); Fan et al. (2001); Zhang et al. (2004); Zhang et al. (2005); Zhang et al. (2008); Hu et al. (2009); Hwang et al. (2010); Xin et al. (2010); Zhao et al. (2011); Sritularak et al. (2011b); Chanvorachote et al. (2013); Li et al. (2013); Zhao et al. (2015); Ranong et al. (2018);
910-Dihydromoscatin	*D. polyanthum*	-	Hu et al. (2009)
Chrysotobibenzyl	*D. amoenum; D. aurantiacum var. denneanum; D. chrysanthum; D. chrysotoxum; D. fimbriatum; D.nobile; D. pulchellum*	Facilitate anoikis and inhibit the growth of lung cancer cells	Li et al. (2011); Chanvorachote et al. (2013)
Batatasin III (53'-dihydroxy-3-methoxybibenzyl)	*D. amoenum; D. amplum; D. aphyllum; D. cariniferum; D. chrysotoxum; D. crystallinum; D. draconis; D. gratiosissimum;D. infundibulum; D. loddigessi; D. longicornu; D. nobile; D. plicatile; D. polyanthum; D. rotundatum*	Antioxidant and antifungal activity; Inhibit α-glucosidase	Sritularak et al. (2011); Majumder et al. (2008); Chen et al. (2008a); Zhang et al. (2008); Zhou et al. (2016); Ranong et al. (2018)

Table 1.3 (continued)

Bibenzyls			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
34'-dihydroxy-5-methoxybibenzyl	*D. amoenum; D. crystallinum; D. gratiosissimum; D. moniliforme; D. officinale;*	-	Majumder et al. (1999); Zhang et al. (2008)
Amoenylin (4-hydroxy-34'5-trimethoxybibenzy)	*D. amoenum*	-	Majumder et al. (1999)
Isoamoenylin	*D. amoenum*	-	Majumder et al. (1999)
Gastrochilinin	*D. amoenum*	-	Majumder and Bandyopadhyay. (2010)
Gastrochilin	*D. amoenum*	-	Majumder and Bandyopadhyay. (2010)
Gigantol (54'-dihydroxy-33'-dimethoxybibenzyl)	*D. amplum; D. aphyllum; D. aurantiacum var. denneanum; D. cariniferum; D. chrysanthum; D. chrysotoxum; D. crystallinum; D. densiflorum; D. draconis; D. gratiosissimum; D. loddigessi; D. lohohense; D. nobile; D. officinale; D. polyanthum; D. scabrilingue; D. sonia; D. thyrsiflorum; D. trigonopus; D. moniliforme; D. brymerianum*	Antioxidant; Inhibit the proliferation of HSC-T6 cells	Huang et al. (2000); Fan et al. (2001); Zhang et al. (2004); Zhang et al. (2005); Chen et al. (2008); Hu et al. (2009); Chen et al. (2014); Zhao et al. 2015); Majumder et al. (2008); Yang et al. (2008); Zhang et al. (2008); Sritularak et al. 2011; Sarakulwattana et al. (2018)
Tristin (53'4'-trihydroxy-3-methoxybibenzyl)	*D. aphyllum; D. cumulatum; D. densiflorum; D. gratiosissimum; D. longicornu; D. nobile; D. thyrsiflorum; D. trigonopus*	Antifungal activity	Fan et al. 2001); Zhang et al. (2008); Zhang et al. (2004 2005); Zhou et al. (2016)
34'5-Trihydroxybibenzyl	*D. aphyllum; D. gratiosissimum; D. officinale*	-	Wang et al. (2007); Zhang et al. (2008); Chen et al. (2012)
44'-Dihydroxy-35-dimethoxybibenzy	*D. aphyllum; D. crystallinum*	-	Chen et al. (2012)
Chrysotoxine	*D. aurantiacum var. denneanum; D. chrysanthum; D. chrysotoxum; D. fimbriatum; D. nobile; D.pulchellum*	Facilitate anoikis and inhibit the growth of lung cancer cells/cancer stem cell	Ma et al. (1994, 1996); Zhang et al. (2009); Chanvorachote et al. (2013); Bhummaphan et al. (2018)
Crepidatin	*D. aurantiacum var. denneanum; D. chrysanthum; D. crepidatum; D. fimbriatum; D. nobile;*	Facilitate anoikis and inhibit the growth of lung cancer cells	Li et al. (2013); Xin et al. (2010); Zhao et al. (2011); Chanvorachote et al. (2013)
33'5-Trihydroxybibenzyl	*D. cariniferum; D. nobile*	Antifungal acticity	Liu et al. (2009); Zhou et al. (2016)
Erianin	*D. chrysotoxum; D. gratiosissimum; D. crepidatum*	-	Li et al. (2011, 2013); Ma et al. (1994)
Trigonopol B	*D. chrysotoxum; D. trigonopus*		Hu et al. (2008); Hu et al. (2012)

Table 1.3 (continued)

Bibenzyls

Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
3-O-methylgigantol	*D. crystallinum; D. nobile; D. officinale; D. plicatile; D. sonia;*	-	Hwang et al. (2010); Huang et al. (2000)
33'-Dihydroxy-5-methoxybibenzyl	*D. crystallinum*	-	Miyazawa et al. (1997 1999); Li et al. (2010); Ye and Zhao (2002); Zhang et al. (2006 2007 2008); Yang et al. (2007)
3-hydroxy-5-methoxybibenzyl	*D. nobile*	Antifungal activity	Zhou et al. (2016)
35'-Dihydroxy-3'4-dimethoxybibenzy	*D. crystallinum*	-	Wang et al. (2008 2009; Hu (2008)
4'-Hydroxy-33'5-trimethoxybibenzyl	*D. crystallinum*	-	Wang et al. (2008 2009; Hu (2008)
Dencryol (A-B)	*D. crystallinum*	-	Wang et al. (2009)
Cumulatin	*D. cumulatum*	-	Majumder and Pal (1993)
Densiflorol A	*D. densiflorum*	-	Fan et al. (2001)
Dendrofalconerol B	*D. falconeri*	-	Sritularak and Likhitwitayawuid (2009)
34-Dihydroxy-4'5-dimethoxy bibenzyl	*D. gratiosissimum*	-	Wang et al. (2007)
Isomoniliformine A	*D. gratiosissimum*	-	Wang et al. (2007)
Dengraols (A-B)	*D. gratiosissimum*	Cytotoxic to HL60 cell	Zhang et al. (2008)
Loddigesiinol (A-D)	*D. loddigessi*	AB: Inhibited NO production	Ito et al. (2010)
Aloifol I	*D. infundibulum; D. longicornu*	-	Hu (2008); Li et al. (2009); Ranong et al. (2018)
33'4-Trihydroxybibenzyl	*D. longicornu*	-	Hu (2008); Li et al. (2009)
Longicornuol B	*D. longicornu*	-	Hu et al. (2010)
34'-Dihydroxy-3'45-trimethoxybibenzyl	*D. longicornu*	-	Hu (2008); Li et al. (2009)
33'-Dihydroxy-45-dimethoxybibenzyl	*D. longicornu*	-	Hu (2008); Li et al. (2009)
33'-Dihydroxy-45-dimethoxybibenzyl	*D. nobile*	Antifungal activity	Zhou et al. (2016)
Cannabistilbene II	*D. longicornu*	-	Xu et al. (2013)

Table 1.3 (continued)

Bibenzyls			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Longicornuol A	*D. longicornu; D. moniliforme*	-	Zhao et al. (2015)
Trigonopol A	*D. longicornu; D. trigonopus*	-	Hu et al. (2010)
34'-Dihydroxy-3'5-dimethoxybibenzyl	*D. moniliforme; D. nobile*	-	Zhao et al. (2003); Zhou et al. (2016)
Dendromoniliside E	*D. moniliforme; D. officinale*	-	Zhao et al. (2003)
Dendrobin A	*D. nobile*	-	Shu et al. (2009)
4-Hydroxy-33'5-trimethoxybibenzyl	*D. nobile*	-	Gao et al. (2015)
Nobilin (A-E)	*D. nobile*	A-B: Antioxidant.C: weak Antioxidant	Zhang et al. (2008)
Dendronophenol (A-B)	*D. nobile*	-	Yang et al. (2007)
Dendrophenol	*D. officinale*	-	Lin et al. (2010)
Dendrocandin (A-G)	*D. officinale*	-	Yang and Xin (2006); Ye et al. (2002); Zhao et al. (2001)
Dendrocandin (I-K)	*D. officinale*	-	Yang and Xin (2006); Ye et al. (2002); Zhao et al. (2001)
Dendrocandin (M-R)	*D. officinale*	-	Yang and Xin 2006); Ye et al. (2002); Zhao et al. (2001)
Dendrocandin (T and U)	*D. officinale*	Neurite outgrowth-promoting activity	Yang et al. (2014)
Dendroscabrol (A-B)	*D. scabrilingue*	B: Inhibit a-glucosidase	Sarakulwattana et al. (2018)
454‘-Trihydroxy-33’-dimethoxybibenzyl	*D. secundum; D. ellipsophyllum*	Antioxidant; Inhibition of lung cancer cell migration	Sritularak et al. (2011); Chen et al. (2015); Chaotham and Chanvorachote (2015)
Crepidatuols A	*D. crepidatum*	Enhancing nerve growth factor in PC12	Li et al. (2013)
Crepidatuols B	*D. crepidatum*	-	
Aphyllone (B-D)	*D. aphyllum*	B: Antioxidant	Yang et al. (2015)
354'-Trihydroxybibenzyl	*D. gratiosissimum*	-	Zhang et al. (2008); Wang et al. (2007)
3453'4'-Pentamethoxybibenzyl	*D. Chrysotoxum*	-	Ma et al. (1994)
3'4-Dihydroxy-55'dimethoxybibenzyl	*D. sonia*	-	Huang et al. (2000)

Table 1.3 (continued)

Bibenzyls			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
33’-dihydroxy-45-dimethoxybibenzyl	*D. infundibulum*	-	Ranong et al. (2018)
54’-dihydroxy-343’-trimethoxybibenzyl	*D. infundibulum*	-	Ranong et al. (2018)

Table 1.4 Fluorenones isolated from *Dendrobium* species

Fluorenones			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Chrysotoxone (2 5 8-Trihydroxy-1 4-dimethoxy-9-fluorenone)	*D. aduncum; D. chrysotoxum*	-	Bi et al. (2006); Ma et al. (1998)
Dengibsin	*D. aduncum; D. aurantiacum var. denneanum; D. chrysanthum; D. chrysotoxum; D. densiflorum; D. farmerii; D. gibsonii; D. nobile; D. thyrsiflorum*	-	Bi et al. (2006); Fan et al. (2001); Majumder and Chakraborti (1989); Zhang et al. (2000)
Dengibsinin	*D. aduncum; D. gibsonii;*	-	Bi et al. (2006)
Dendroflorin	*D. aurantiacum var. denneanum; D. chrysanthum; D. chrysotoxum; D. densiflorum; D. nobile; D. thyrsiflorum; D. trigonopus; D. brymerianum*	Cytotoxic against human leukaemia cell lines K562 and HL-60 human lung adenocarcinoma A549 human hepatoma BEL-7402 and human stomach cancer SGC-7901	Chen et al. (2014); Fan et al. (2001); Zhang et al. (2004); Chen et al. (2008)
Denchrysan A	*D. chrysanthum; D. chrysotoxum; D. nobile; D. thyrsiflorum*	Cytotoxic against human leukaemia cell lines K562 and HL-60 A549 BEL-7402 and SGC-7901	Zhang et al. (2004); Chen et al. (2008)
35-Dihydroxy-24-dimethoxy-9H-fluoren-9-one	*D. chrysanthum*	-	Wang et al. (2009)
35-Dihydroxy-4-methoxy-9H-fluoren-9-one	*D. chrysanthum*	-	Wang et al. (2009)
Denchrysan B	*D. chrysanthum; D. chrysotoxum;D. thyrsiflorum*	-	Zhang et al. (2004)

Table 1.4 (continued)

Fluorenones			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
145-Trihydroxy-7-methoxy-9H-fluoren-9-one	*D. chrysotoxum*	Cytotoxic against human leukaemia cell lines K562 and HL-60 human lung adenocarcinoma A549 human hepatoma BEL-7402 and human stomach cancer SGC-7901	Chen et al. (2008)
247-Trihydroxy-15-dimethoxy-9H-fluoren-9-one	*D. chrysotoxum*	–	Gong et al. (2006)

Table 1.5 Phenanthrenes isolated from *Dendrobium* species

Phenanthrenes			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Confusarin (27-dihydroxy-348-trimethoxyphenanthrene)	*D. amoenum; D. aurantiacum var. denneanum; D. chrysotoxum; D. fimbriatum; D. nobile; D. officinale; D. sonia; D. crepidatum*	Enhancing nerve growth factor in PC12	Li et al. (2013); Li et al. (2011); Majumder and Bandyopadhyay (2010); Huang et al, (2000; Zhou et al. (2016)
27-Dihydroxy-346-trimethoxyphenanthrene	*D. amoenum; D. amplum; D. chrysotoxum; D. rotundatum;*	-	Majumder et al. (2008); Majumder and Pal. (1992)
Imbricatin	*D. amoenum*	-	Majumder et al. (1999)
Amoenumin	*D. amoenum*	-	Majumder et al. (1999)
237-Trihydroxy-46-dimethoxyphenanthrene	*D. amplum*	-	Majumder et al. (2008)
237-Trihydroxy-46-dimethoxy-910-dihydrophenanthrene	*D. amplum*	-	Zhang et al. (2008)
27-Dihydroxy-346-trimethoxy-910-dihydrophenanthrene	*D. amplum*	-	Zhang et al. (2008)
Coelonin	*D. amplum; D. aphyllum; D. longicornu; D. nobile;*	Inhibited nitric oxide production in macrophage RAW 264.7 cells	Chen et al. (2008a; Hwang et al. (2010)
Amplumthrin (22'77'-tetrahydroxy-33'44'66'hexamethoxy-99'1010'-tetrahydro-11'-biphenanthryl)	*D. amplum*	-	Majumder et al. (2008)
44’77’-tetrahydroxy-22’-dimethoxy-99’1010’-tetrahydro-11’-phenanthrene	*D. nobile*	-	Zhou et al. (2016)

Table 1.5 (continued)

Phenanthrenes			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Flavanthrin	*D. amplum; D. aphyllum; D. nobile;*	-	Chen et al. (2008a)
Moscatin	*D. aphyllum; D. aurantiacum var. denneanum; D. chrysanthum; D. chrysotoxum; D. densiflorum; D. loddigessi; D. moscatum; D. nobile; D. plicatile; D. trigonopus; D. thyrsiflorum*	-	Fan et al. (2001); Chen et al. (2008a; Zhang et al. (2004, 2005); Zhou et al. (2016)
247-Trihydroxy-910-dihydrophenanthrene	*D. aphyllum; D. chrysotoxum; D. moniliforme; D. officinale; D. polyanthum;*	-	Li et al. (2011)
lhridinusiant	*D. aphyllum; D. densiflorum; D. loddigessi; D. nobile; D. plicatile; D. sonia;*	-	Zhao et al. (2001 Shu et al. (2004 Luo et al. (2006)
Dendronone (5-hydroxy-7-methoxy-910-dihydro-14-phenanthrenequinone)	*D. cariniferum; D. longicornu;*	-	Chen et al. (2008)
25-Dihydroxy-49-dimethoxyphenanthrene	*D. chrysanthum; D. chrysotoxum; D. nobile*	-	Zhang et al. (2008
Denthyrsinin (3-(5'6'-dimethoxybenzofuran-2'-yl)-67-dimethoxy-2H-chromen-2-one)	*D. chrysanthum; D. densiflorum; D. thyrsiflorum*	Cytotoxic to HL60 K562 and MCF-7 cell lines	Zhang et al. (2005)
Denchryside A	*D. chrysanthum*		Ye et al. (2003)
Dendrochrysanene	*D. chrysanthum*	Anti-inflammatory	Yang et al. (2006a)
Cypripedin	*D. chrysanthum; D. densiflorum;*	-	Fan et al. (2001)
Chrysotoxene	*D. chrysotoxum*	-	Ma et al. (1994)
26-Dihydroxy-57-dimethoxyphenanthrene	*D. chrysotoxum; D. nobile; D. plicatile*	-	Zhang (2005);
Nudol (27-dihydroxy-34-dimethoxyphenanthrene)	*D. chrysotoxum; D. nobile; D. officinale; D. rotundatum; D. sonia*	-	Li et al. (2011); Huang et al. (2000; Zhou et al. (2016)
Fimbriatone	*D. chrysotoxum; D. fimbriatum; D. nobile*	-	Miyazawa et al. (1997, 1999) Li et al. (2010)
Chrysotoxol (A B)	*D. chrysotoxum*	-	Ma et al. (1994)
Densiflorol B	*D. chrysotoxum; D. densiflorum; D. thyrsiflorum; D. brymerianum*	-	Chen et al. (2014)
Dendroscabrols (A-B)	*D. scabrilingue*	-	Sarakulwattana et al. (2018)
Cypripedin	*D. densiflorum; D. chrysanthum;*	-	Fan et al. (2001);
257-Trihydroxy-4-methoxy-910-dihydrophenanthrene	*D. draconis; D. longicornu*	-	Li et al. (2009, 2013);
Hircinol	*D. draconis; D. loddigessi; D. longicornu; D. nobile; D. plicatile; D. polyanthum; D. thyrsiflorum; D. trigonopus; D. crepidatum; D. moniliforme; D. brymerianum*	Antioxidant; Inhibited nitric oxide production in macrophage RAW 264.7 cells	Sritularak et al. (2011); Li et al. (2013); Zhao et al. (2015); Chen et al. (2014); Zhang et al. (2004); Zhou et al. (2016)

Table 1.5 (continued)

Phenanthrenes			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
5-Methoxy-7-hydroxy-910-dihydro-14-phenanthrenequinone	*D. draconis*	Antioxidant	Sritularak et al. (2011)
5-Hydroxy-24-dimethoxyphenanthrene	*D. loddigessi*	-	Sritularak et al. (2011)
Plicatol C	*D. loddigessi; D. plicatile; D. rotundatum;*	-	Yamaki and Honda (1996) Honda and Yamaki (2000 2001)
Ephemeranthoquinone	*D. longicornu*	-	Yamaki and Honda (1996) Honda and Yamaki (2000 2001)
7-Hydroxy-56-dimethoxy-14-phenanthrenequinone	*D. moniliforme*	-	Sritularak et al. (2011)
Denbinobin (5-hydroxy-37diniethoxy-14-phenanthraquinone)	*D. moniliforme; D. nobile; D. officinale; D.sonia*	Antiinflammatory; Cytotoxic against A549 (human lung carcinoma) SK-OV-3 (human ovary adenocarcinoma) and HL-60 (human promyelocytic leukemia) cell lines; Inhibit the proliferation of HSC-T6 cells	Lin et al. (2001); Lee et al. (1995); Huang et al. (2000); Yang et al. (2008)
Moniliquinone	*D. moniliforme;*	-	Zhao et al. (2003)
25-Dihydroxy-348-trimethoxyphenanthrene	*D. nobile*	-	Honda and Yamaki (2000, 2001)
28-Dihydroxy-347-trimethoxyphenanthrene	*D. nobile*	-	Yang et al. (2008)
235-Trihydroxy-49-dimethoxyphenanthrene	*D. nobile*	Antifibrotic	Yang et al. (2011)
3-Hydroxy-247-trimethoxyphenanthrene	*D. nobile; D. plicatile*	-	Yamaki and Honda (1996)
25-Dihydroxy-34-dimethoxyphenanthrene	*D. nobile; D. officinale;*	-	Zhang (2010)
Flavanthrinin	*D. nobile*	Antioxidant	Majumder et al. (1990; Zhang et al. (2008)
Bulbophyllanthrin	*D. nobile; D. officinale*	-	Li et al. (2008, 2009, 2010); Li (2009); Sritularak and Likhitwitayawuid (2009)
Fimbriol B	*D. nobile*	Inhibited nitric oxide production in macrophage RAW 264.7 cells	Hwang et al. (2010)
Plicatol A	*D. nobile; D. plicatile*	-	Yamaki and Honda (1996) Honda and Yamaki (2001)
45-Dihydroxy-37-dimethoxy-910-dihydrophenanthrene	*D. nobile*	-	Ye et al. 2002 (Zhao) et al. 2001
28-Dihydroxy-347-trimethoxy-910-dihydrophenanthrene	*D. nobile*	-	Yang et al. (2008)
2-Hydroxy-47-dimethoxy-910-dihydrophenanthrene	*D. nobile*	-	Yang et al. (2008)
2-Hydroxy-347-trimethoxy-910-dihydrophenanthrene	*D. nobile*	-	Luo et al. (2006); Xu et al. (2008)
45-Dihydroxy-2-methoxy-910-dihydrophenanthrene	*D. nobile*	-	Wang et al. (2007); Zhou et al. (2016)

Table 1.5 (continued)

Phenanthrenes			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Ephemeranthol A	*D. infundibulum; D. nobile*	Inhibited nitric oxide production in macrophage RAW 264.7 cells	Hwang et al. (2010); Zhou et al). (2016); Ranong et al. (2018)
Epheneranthol C	*D. nobile*	Inhibited nitric oxide production in macrophage RAW 264.7 cells	Hwang et al. (2010)
Flavanthridin	*D. nobile*	Inhibited nitric oxide production in macrophage RAW 264.7 cells	Hwang et al. 2010); Zhou et al. (2016)
22'-Dihydroxy-33'44'77'-hexamethoxy-99'1010'-tetrahydro-11'-biphenanthrene	*D. nobile*	-	Yang and Xin 2006); Ye et al. (2002); Zhao et al. (2001)
2347-Tetramethoxyphenanthrene	*D. officinale*	-	Li et al. (2008 2009 2010)
Dendrocandin H	*D. officinale*	-	Sritularak and Likhitwitayawuid (2009); Wang et al. (2010)
Dendrocandin L	*D. officinale*	-	Sritularak and Likhitwitayawuid (2009); Wang et al. (2010)
2-hydroxy-567-trimethoxy-910-dihydrophenanthrene	*D. plicatile*	-	Yamaki and Honda (1996) Honda and Yamaki (2000, 2001)
44'77'-Tetrahydroxy-22'-dimethoxy-99'1'10'-tetrahydro-110-biphenanthrene	*D. plicatile*	-	Yamaki and Honda (1996) Honda and Yamaki (2000, 2001)
Ephemeranthoquinone	*D. plicatile; D. longicornu*	-	Yamaki and Honda (1996) Honda and Yamaki (2000, 2001)
27-Dihydroxy-346-trimethoxy-910-dihydrophenanthrene	*D. amplum; D. rotundatum; D. hongdie*	-	Yamaki and Honda (1996) Honda and Yamaki (2000, 2001); Chen et al. (2015
Denthyrsinol and Denthyrsinol (A-C)(45'-dimethoxy-[11']biphenanthrenyl-254'7'-tetraol)	*D. nobile; thyrsiflorum*	Cytotoxic to HL60 K562 and MCF-7 cell lines	Zhang et al. (2005); Zhou et al. (2016)
Denthyrsinone (74'7'-trihydroxy-22'8'-trimethoxy-[51']biphenanthrenyl-14-dione)	*D. thyrsiflorum*	Cytotoxic to HL60 K562 and MCF-8 cell lines	Zhang et al. (2005)
7-Methoxy-910-dihydrophenanthrene-245-triol	*D. draconis*	Antioxidant	Sritularak et al. (2011)
15-Dihydroxy-347-trimethoxy-910-dihydrophenanthrene	*D. moniliforme*	-	Zhao et al. (2015)
34'-Dihydroxy-3'45-trimethoxybibenzyl	*D. moniliforme*	-	Zhao et al. (2015)
Alloaromadendrane	*D. moniliforme*	-	Zhao et al. (2015)
Protocatechuic acid	*D. moniliforme*	-	Zhao et al. (2015)
Picrotoxane	*D. moniliforme*	-	Zhao et al. (2015)
Aphyllone	*D. aphyllum*	-	Yang et al. (2015)

Table 1.5 (continued)

Phenanthrenes			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Lusianthridin	*D. aphyllum; D. nobile; D. sonia*	Inhibited nitric oxide production in macrophage RAW 264.7 cells	Chen et al. (2008a); Hwang et al. (2010); Huang et al. (2000); Zhou et al. (2016)
34'-Dihydroxy-54'-dimethoxy bibenzyl	*D. gratiosissimum*	-	Zhang et al. (2008)
2-Hydroxy-1567-tetramethoxyphenanthrene	*D. chrysotoxum*	-	Ma et al. (1994)
26-dihydroxy-157-trimethoxyphenanthrene	*D. densiflorum*	Anti-platelet aggregation	Fan et al. (2001)
47-Dihydroxy-2-methoxy-910-dihydrophenanthrene	*D. nobile*	Cytotoxic against A549 (human lung carcinoma) SK-OV-3 (human ovary adenocarcinoma) and HL-60 (human promyelocytic leukemia) cell lines; Antitumor	Lee et al. (1995)
25-Dihydroxy-4910-trimethoxyphenanthrene	*D. Plicatile*	-	Yamaki and Honda (2000)
25-Dihydroxy-4-methoxyphenanthrene	*D. Plicatile*	-	Yamaki and Honda (2000)
259-Trihydroxy-4-methoxy-910-dihydrophenanthrene	*D. Plicatile*	-	Yamaki and Honda (2000)
Dendroinfundin (A-B)	*D. infundibulum*	-	Ranong et al. (2018)

Table 1.6 Sesquiterpenoids isolated from *Dendrobium* species

Sesquiterpenoids			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Aduncin	*D. aduncum; D. officinale*	—	Gawell and Leander (1976); Wang et al. (2010); Bi et al. (2006)
Amotin	*D. amoenum*	—	Dahme´n and Leander (1978)
Amoenin	*D. amoenum*	—	Dahme´n and Leander (1978)
Dendronobilin (A-N)	*D. crystallinum; D.noble*	—	Wang et al. (2009); Zhang (2005); Zhang et al. (2007); Gao et al. (2007); Liu et al. (2007)
Dendronobilosides	*D.noble*		Zhao et al. (2001)
Crystallinin	*D. crystallinum; D. findlayanum*	—	Wang et al. (2009); Qin et al. (2011)
Dendrodensiflorol	*D. densiflorum; D. nobile*	—	Tang et al. (2004); Zhang et al. (2007)

Table 1.6 (continued)

Sesquiterpenoids			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Denhydroshizukanolide	*D. fimbriatum*	—	Qing et al. (2009)
Findlayanin	*D. findlayanum*	—	Qing et al. (2011)
Dendromoniliside (A-D)	*D. moniliforme*	AC: stimulate the proliferation of B cells and inhibit the proliferation of T cells in vitro	Zhao et al. (2003)
Dendroside F	*D. moniliforme; D. nobile*	-	Zhao et al. (2003); Okamoto et al. (1966); Ye et al. (2002)
α-dihydropicrotoxinin	*D. moniliforme*	-	Zhao et al. (2004)
Picrotin	*D. moniliforme*	-	Bi et al. (2004)
Dendrobiumane E	*D. moniliforme*	-	Bi et al. (2004)
Dendrobiumane C	*D. moniliforme*	-	Bi et al. (2004)
Dendrobiumane A	*D. moniliforme; D. nobile*	-	Lin et al. (2007); Zhang et al. (2007)
trihydroxyalloaromadendrane	*D. moniliforme; D. nobile*	-	Zhao et al. (2001); Zhang et al. (2007)
Dendroside	*D. moniliforme; D. nobile*	Inhibit the growth of HeLa MCF-7 and A549 cells	Ye et al. (2002); Zhao et al. (2001); Zhou et al. (2016); Zhou et al. (2017)
Dendrowardol C	*D. wardianum*	-	Fan et al. (2013)
Dendroside G	*D. nobile*	-	Ye et al. (2002)
Nobilomethylene	*D. nobile*	-	Okamoto et al. (1972)
712-Dihydroxy-5-hydroxymethyl-11-isopropyl-6-methyl-9-oxatricyclo[6.2.1.026]undecan-10-one-15-O-b-D-glucopyranoside	*D. nobile*	-	Shu et al. (2004)
(7S8R) dehydrodiconiferyl alcohol 9’-β-glucopyranoside	*D. nobile*	Inhibit the growth of HeLa MCF-7 and A549	Zhou et al. (2017)
Dendronobiloside (A-E)	*D. nobile*	-	Ye et al. (2002); Zhao et al. (2001); Shu et al. (2004); Luo et al. (2006) Xu et al. (2008)
6a1012-Trihydroxypicrotoxane	*D. nobile*	Inhibit the proliferation of mouse T and/or B lymphocytes in vitro	Zhao et al. (2001); Zhang (2005)
1012-Dihydroxypicrotoxane	*D. nobile*	Stimulated significantly the proliferation of mouse T and/or B lymphocytes in vitro	Zhao et al. (2001); Zhang et al. (2007)
Bullatantriol	*D. nobile*	-	Zhang et al. (2007)
Dendrosides (B-E)	*D. nobile; D.moniliforme*	D–G: stimulated significantly the proliferation of mouse T and/or B lymphocytes in vitro	Zhang et al. (2007); Zhao et al. (2003); Ye et al. (2002)
Dendrobane A	*D. nobile*	-	Zhang et al. (2007)
Corchoionoside C	*D. polyanthum; D. primulimun*	-	Hu et al. (2009)
Flakinin (A-B)	*D. snowflake*	-	Morita et al. (2000)
Dendrowardols (A-B)	*D. wardianum*	B: weak promoting effect on the proliferation of D-galactose-induced HLECs	Fan et al. (2013)

Table 1.6 (continued)

Sesquiterpenoids			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
10ß1214-Trihydroxy alloaromadendrane	*D. wardianum*	Stimulate the proliferation of murine T and B lymphocytes	Zhao et al. (2001); Fan et al. (2013)
Moniliformin (26-Dimethoxy-1458-phenanthraquinone)	*D. moniliforme*	-	Lin et al. (2001)
ß-Patchoulene	*D. parishii*	-	Julsrigival et al. (2013)
α-Guaiene	*D. parishii*	-	Julsrigival et al. (2013)
Dihydropseudoionone (Monoterpene)	*D. parishii*	-	Julsrigival et al. (2013)
α-Humulene	*D. parishii*	-	Julsrigival et al. (2013)
γ-Gurjunene	*D. parishii*	-	Julsrigival et al. (2013)
γ-Selinene	*D. parishii*	-	Julsrigival et al. (2013)
ß-Selinene	*D. parishii*	-	Julsrigival et al. (2013)
α-Panasinsen	*D. parishii*	-	Julsrigival et al. (2013)
Hexahydrofarnesyl acetone	*D. parishii*	-	Julsrigival et al. (2013)
Sandaracopimaradiene (Diterpene)	*D. parishii*	-	Julsrigival et al. (2013)
Isodensifloside	*D. thyrsiflorum*	-	Zhang et al. (2005)
cis-Melilotoside	*D. aurantiacum var. denneanum*	Antioxidant	Yang et al. (2007)
Dihydromelilotoside	*D. aurantiacum var. denneanum*	Antioxidant	Yang et al. (2007)
trans-Melilotoside	*D. aurantiacum var. denneanum*	Antioxidant	Yang et al. (2007)
(−)-(1R2S3R4S5R6S9S11R)-11-carboxymethyldendrobine	*D. nobile*	-	Meng et al. (2017)
(+)-(1R2S3S4R5R6S9R)-2411-Trihydroxypicrotoxane-3(15)-lactone	*D. nobile*	Angiogenesis	Meng et al. (2017)
(+)-(1R2S3R4S5R6S9R)-21112-Trihydroxypicrotoxane-3(15)-lactone	*D. nobile*	-	Meng et al. (2017)

Table 1.7 Other compounds isolated from *Dendrobium* species

Other compounds			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Monoaromatics	*D. aduncum; D. aphyllum; D. aurantiacum var. denneanum; D. chrysanthum; D. chrysotoxum; D. crepidatum; D. crystallinum; D. densiflorum; D. falconeri; D. fimbriatum; D. fuscescens; D. gratiosissimum; D. huoshanense; D. longicornu; D. moniliforme; D. nobile; D. officinale; D. thyrsiflorum*	-	Sritularak and Likhitwitayawuid (2009); Chen et al. (2010); Hu (2007); Bae et al. 2004); Lin et al. (2000, 2001); Li et al. (2008, 2009, 2010); Zhang et al. (2004, 2005); Liu et al. (2011); Wrigley (1960)
Steroids	*D. aduncum; D. aurantiacum var. denneanum; D. cariniferum; D. chrysanthum; D. chrysotoxum; D. crepidatum; D. crystallinum; D. fimbriatum; D. gratiosissimum; D. loddigessi; longicornu; D. moniliforme; D. ochreatum; D. officinale; D. polyanthum; D. primulimun; D. thyrsiflorum; D. trigonopus*	-	Zhang et al. (2008); Li et al. (2009, 2011); Yang et al. (2001, 2002, 2004); Zhao et al. (2011); Majumder and Chatterjee (1989); Li et al. (2007); Wang et al. (2008, 2009, 2011); Bi et al. (2001, 2003); Zhang et al. (2007, 2008); Wang et al. (2007)
Flavonoids	*D. aphyllum; D. aurantiacum var. denneanum; D. chrysotoxum; D. crepidatum; D. crystallinum; D. densiflorum; D. gratiosissimum; D. huoshanense; D. longicornu; D. nobile; D. officinale; D. thyrsiflorum; D. trigonopus*	Antioxidant activity (Stem and leaf)	Sritularak et al. (2011); Zhang et al. (2007, 2008); Wang et al. (2007); Chang et al. (2010); Hu et al. 2008, (2010); Chen et al. (2010); Hu (2007); Sritularak and Likhitwitayawuid (2009) Wang et al. (2010); Zhang et al. (2017)
Lignans	*D. aphyllum; D. chrysanthum; D. chrysotoxum; D. crystallinum; D. loddigessi; D. moniliforme; D. nobile; D. officinale; D. trigonopus*	-	Sritularak and Likhitwitayawuid (2009) Wang et al. (2010); Glomqvist et al. (1973); Li et al. (2010c); Ye and Zhao (2002); Zhang et al. (2006, 2007, 2008)
Coumarin	*D. aurantiacum var. denneanum; D. densiflorum; D.thyrsiflorum*	-	Zhang et al. (2005)
Terpenes	*D. aurantiacum var. denneanum; D. crepidatum; D. crystallinum; D. nobile; D. officinale; D. thyrsiflorum*	Anti-inflammatory	Barlocco (2006); Majumder et al. (2010); Zhang et al. (2008, 2013)
Anthraquinones	*D. chrysanthum; D. fimbriatum; D. nobile; D. thyrsiflorum*	-	Xu et al. (2008); Zhang et al. (2004, 2005); Qing et al. (2009); Talapatra et al. (1992)
Nucleosides	*D. crystallinum*	-	Wang et al. (2009)
Scoparone	*D. densiflorum; D. farmerii; D. fimbriatum; D. thyrsiflorum*	Anti-platelet aggregation	Fan et al. (2001); Majumder and Chakraborti (1989)
Scopoletin	*D. densiflorum; D. thyrsiflorum*	-	Zhang et al. (2005); Fan et al. (2001)
Scopolin	*D. densiflorum; D. thyrsiflorum*	-	Zhang et al. (2005)
Ayapin	*D. densiflorum; D. fimbriatum; D. thyrsiflorum*	-	Zhang et al. (2005)
Dihydroayapin (67-methylenedioxy-34-dihydrobenzopyran-2-one)	*D. densiflorum*	-	Zheng et al. (2000a)
Xeroboside	*D. thyrsiflorum*	-	Zhang et al. (2005)
Denthyrsin	*D. thyrsiflorum*	-	Zhang et al. (2005)

Table 1.7 (continued)

Other compounds			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Monosaccharide (arabinose xylose mannose glucose galactose)	*D. denneanum; D. huoshanense; D. officinale*	Antioxidant; Improve the intestinal physical barrier function and regulate the microbiota; Enhance macrophage-mediated immune responses	Fan et al. (2009); Xie et al. (2019)
Brittonin A	*D. chrysotoxum; D. moniliforme; D. secundum; D. trigonopus*	Antioxidant	Zhang et al. (2005); Gong et al. (2006); Sritularak et al. (2011); Zhao et al. (2015)
Ferulic acid	*D. secundum*	Antioxidant	Sritularak et al. (2011)
Syringic acid	*D. nobile; D. aphyllum; D. crystallinum*	Antioxidant	Zhang et al. (2008); Chen et al. (2008); Wang et al. (2009)
2-Hydroxyphenylpropanol	*D. nobile*	Antioxidant	Zhang et al. (2008)
Vanilli	*D. nobile*	Antioxidant	Zhang et al. (2008)
Apocyni	*D. nobile*	Antioxidant	Zhang et al. (2008)
Conifery laldehyde	*D. nobile*	Weak antioxidant	Zhang et al. (2008)
Syringaldehyde	*D. nobile*	Antioxidant	Zhang et al. (2008)
Syringylethanone	*D. nobile*	Antioxidant	Zhang et al. (2008)
P-hydroxybenzaldehyde	*D. nobile; D. crepidatum; D. moniliforme*	Antioxidant	Zhang et al. (2008); Zhao et al. (2015); Zhao et al. (2005)
3-Hydroxy-4-methoxyphenylethanol	*D. nobile*	Antioxidant	Zhang et al. (2008)
α-Hydroxysyringylethanone	*D. nobile*	Weak antioxidant	Zhang et al. (2008)
Dihydroxyconiferylalcohol	*D. nobile*	Weak antioxidant	Zhang et al. (2008)
P-hydroxybenzoic acid	*D. nobile*	Antioxidant	Zhang et al. (2008)
P-hydroxyphenylpropionic acid	*D. nobile; D. moniliforme*	Weak antioxidant	Zhang et al. (2008); Zhao et al. (2015)
10-Dihydrophenanthrene-247-triol	*D. polyanthum*	-	Hu et al. (2009)
Daucosterol	*D. polyanthum; D. crepidatum; D. moniliforme; D. aduncum; D. trigonopus; D. longicornu*	-	Hu et al. (2009); Li et al. (2013); Xin et al. (2011); Zhao et al. (2015); Bi et al. (2006); Zhang et al. (2005); Hu et al. (2010)
3-(2-Acetoxy-5-methoxy)-phenylpropanol	*D. crepidatum*	Enhancing nerve growth factor in PC12	Li et al. (2013)
46-dihydroxyisobenzofuran-1(3H)-one	*D. crepidatum*	-	Li et al. (2013)
Ergostan-7922-trien-35-diol	*D. crepidatum*	-	Li et al. (2013)
Ergosterol	*D. crepidatum*	-	Li et al. (2013)
Syringaldazine	*D. crepidatum*	-	Li et al. (2013)
Kaempferol	*D. crepidatum*	-	Li et al. (2013)
Vanillin	*D. crepidatum; D. moniliforme*	-	Xin et al. (2011); Zhao et al. (2015); Zhao et al. (2011)
N-tetracosyl ferulate	*D. crepidatum*	-	Xin et al. 2011; Zhao et al. (2011)

Table 1.7 (continued)

Other compounds			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
4'57-Trihydroxy-flavanone	*D. crepidatum*	-	Xin et al. (2011); Zhao et al. (2011)
Friedelin	*D. crepidatum; D. thyrsiflorum*	-	Xin et al. (2011); Zhang et al. (2005)
58-Epidioxy-24 (R)-methylcholesta-622-dien-3-ol	*D. crepidatum*	-	Xin et al. (2010)
(2R*3S*)-3-hydroxymethyl-9-methoxy-2-(4'-hydroxy-3'5'-dimethoxyphenyl)-2367-tetrahydrophenanthro [43-b] furan-511-diol	*D. moniliforme*	-	Zhao et al. (2015)
Diospyrosin	*D. moniliforme*	-	Zhao et al. (2015)
N-trans-cinnamoyltyramine	*D. moniliforme*	-	Zhao et al. (2015)
Paprazine	*D. moniliforme*	-	Zhao et al. (2015)
N-trans-feruloyl 3'-O-methyldopamine	*D. moniliforme*	-	Zhao et al. (2015)
Moupinamide	*D. moniliforme*	-	Zhao et al. (2015)
Dihydroconiferyl dihydro-p-coumarate	*D. moniliforme*	-	Zhao et al. (2015)
3-Isopropyl-5-acetoxycyclohexene-2-one-1	*D. moniliforme*	-	Zhao et al. (2015)
Vanillic acid	*D. moniliforme; D. chrysotoxum*	-	Zhao et al. (2015); Gong et al. (2006)
Protocatechuic acid	*D. moniliforme*	-	Zhao et al. (2015)
Alloaromadendrane	*D. moniliforme*	-	Zhao et al. (2015)
Protocatechuic acid	*D. moniliforme*	-	Zhao et al. (2015)
Picrotoxane	*D. moniliforme*	-	Zhao et al. (2015)
Copacamphane	*D. moniliforme*	-	Zhao et al. (2003)
Hexanal	*D. parishii*	-	Julsrigival et al. (2013)
2-trans-Hexenal	*D. parishii*	-	Julsrigival et al. (2013)
Hexanol	*D. parishii*	-	Julsrigival et al. (2013)
1-Octen-3-ol	*D. parishii*	-	Julsrigival et al. (2013)
2-Pentylfuran	*D. parishii*	-	Julsrigival et al. (2013)
3-Ethyl-2-methyl-13-hexadiene	*D. parishii*	-	Julsrigival et al. (2013)
2-trans-Octenal	*D. parishii*	-	Julsrigival et al. (2013)
Linalool	*D. parishii*	-	Julsrigival et al. (2013)
Camphor	*D. parishii*	-	Julsrigival et al. (2013)
(2-trans6-cis)-Nonadienal	*D. parishii*	-	Julsrigival et al. (2013)
2-trans-Nonenal	*D. parishii*	-	Julsrigival et al. (2013)
Benzenepropanoic acid ethyl ester	*D. parishii*	-	Julsrigival et al. (2013)
Dodecanoic acid ethyl ester	*D. parishii*	-	Julsrigival et al. (2013)
Tetradecanal	*D. parishii*	-	Julsrigival et al. (2013)
Pentadecanal	*D. parishii*	-	Julsrigival et al. (2013)

Table 1.7 (continued)

Other compounds			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Tetradecanoic acid ethyl ester	*D. parishii*	-	Julsrigival et al. (2013)
Pentadecanoic acid ethyl ester	*D. parishii*	-	Julsrigival et al. (2013)
Hexadecanoic acid ethyl ester	*D. parishii; D. thyrsiflorum*	-	Julsrigival et al. (2013); Zhang et al. (2005)
Liriodendrin	*D. brymerianum*	-	Chen et al. (2014)
n-Docosyl trans-ferulate	*D. brymerianum*	-	Chen et al. (2014)
β-(1-4)-Man linkage	*D. officinale*	Antioxidant	Huang et al. (2015)
O-acetyl	*D. officinale*	Antioxidant	Huang et al. (2015)
(-)-(8R70E)-4-hydroxy-330550-tetramethoxy-840-oxyneolign-70-ene-990-diol 49-bis-O-b-D-glucopyranoside	*D. aurantiacum var. denneanum*	-	Li et al. (2014)
(-)-(8S70E)-4-hydroxy-330550-tetramethoxy-840-oxyneolign-70-ene-990-diol 49-bis-O-b-D-gluco-pyranoside	*D. aurantiacum var. denneanum*	-	Li et al. 2014)
(-)-(8R70E)-4-hydroxy-33055090-pentamethoxy-840-oxyneolign-70-ene-9-ol 49-bis-O-b-D-glucopyranoside	*D. aurantiacum var. denneanum*	-	Li et al. (2014)
n-Triacontyl p-hydroxy-cis-cinnamate	*D. aduncum*	-	Bi et al. (2006)
Defuscin	*D. aduncum; D. thyrsiflorum*	-	Bi et al. (2006); Zhang et al. (2005)
4-Hydroxy-3-methoxy benzoic acid	*D. aduncum*	-	Bi et al. (2006)
Dibutyl phthalate	*D. aphyllum*	-	Chen et al. (2008a)
Diisobutyl phthalate	*D. aphyllum*	-	Chen et al. (2008a)
p-Hydroxyphenylpropionic methyl ester	*D. aphyllum*	-	Chen et al. (2008a)
4'-Methoxyl-tricin	*D. aphyllum*	-	Shao et al. (2008)
Tricin	*D. aphyllum; D. thyrsiflorum*	-	Shao et al. (2008); Zhang et al. (2004)
73'5'-Tri-O-methyl-tricetin	*D. aphyllum*	-	Shao et al. (2008)
(+)-Syring-aresinol	*D. aphyllum*	-	Shao et al. (2008)
D-allitol	*D. aphyllum*	-	Shao et al. (2008)
Sucrose	*D. aphyllum*	-	Shao et al. (2008)
Icariside D2	*D. aphyllum*	-	Shao et al. (2008)
Naringenin (4'57-trihydroxyflavanone)	*D. trigonopus; D. densiflorum; D. thyrsiflorum*	-	Zhang et al. (2005); Fan et al. (2001); Zhang et al. (2004)
Chrysophanein	*D. thyrsiflorum*	-	Zhang et al. (2005)

Table 1.7 (continued)

Other compounds			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
24(R)-6β-hydroxy-24-ethlcholest-4-en-3-one	*D. thyrsiflorum*	-	Zhang et al. (2005)
Hexadecanoic acid 2 3-dihydroxypropyl ester	*D. thyrsiflorum*	-	Zhang et al. (2005)
157-Trimethoxyphenanthrene-26-diol	*D. thyrsiflorum*	-	Zhang et al. (2005)
5α 8α-epidioxy-24(R)-methycholesta-6 22-dien-3beta-ol	*D. chrysotoxum*	-	Gong et al. (2006)
trans-3-(4-hydroxy-3-methoxyphenyl)-acrylic acid octacosyl ester	*D. chrysotoxum*	-	Gong et al. (2006)
Defusin	*D. chrysotoxum.*	-	Gong et al. (2006)
3 4-Dihydroxy benzoic acid	*D. chrysotoxum.*	-	Gong et al. (2006)
3 4-Dimethoxy-benzoic acid	*D. chrysotoxum.*	-	Gong et al. (2006)
3 4-Dimethoxy-benzoic acid methyl ester	*D. chrysotoxum.*	-	Gong et al. (2006)
3 5-Dibromo-2-aminobenzaldehyde	*D. chrysotoxum.*	-	Gong et al. (2006)
Heptadecanoic acid 2 3-dihydroxy-propyl ester	*D. chrysotoxum.*	-	Gong et al. (2006)
Crystalltone	*D. crystallinum*	-	Wang et al. (2009)
3-Hydroxy-2-methoxy-56-dimethylbenzoic acid	*D. crystallinum*	-	Wang et al. (2009)
Apigenin	*D. crystallinum*	-	Wang et al. (2009)
Isoviolanthin	*D. crystallinum*	-	Wang et al. (2009)
6'''-Glucosyl-vitexin	*D. crystallinum*	-	Wang et al. (2009)
Palmarumycin JC2	*D. crystallinum*	-	Wang et al. (2009)
5-[2'-(3''-Hydroxy-5''-methoxyphenyl)-ethyl]-13-benzodioxol	*D. densifiorum*	-	Fan et al. (2009)
Homoeriodictyol	*D. densifiorum; D. thyrsiflorum*	Anti-platelet aggregation	Fan et al. (2009); Zhang et al. (2004)
Densifloroside	*D. thyrsiflorum; D. densiflorum*	-	Zhang et al. (2005)); Dahmen et al. (1975)
2-Hydroxyethyl caffeate	*D. fimbriatum*	-	Qing et al. (2009)
(25R)-22α-O-spirost-5-ene-3β16α17α-triol	*D. fimbriatum*	-	Talapatra et al. (1992)
34'-Dihydroxy-55'-dimethoxydihydrostilbene	*D. nobile*	-	Hwang et al. (2010)
57-Dimethoxyphenanthrene-26-diol	*D. nobile*	Antioxidant; Inhibited nitric oxide production in macrophage RAW 264.7 cells	Hwang et al. (2010)
Chrysoeriol	*D. thyrsiflorum*	-	Zhang et al. (2004)
Spirolactone	*D. chrysanthum*	Anti-inflammatory	Barlocco (2006)
DNPE6(4)	*D. nobile*	Defense against TMV	Li et al. (2019)
DHPD1[(1 →(3)(4)6)-linked-Glcp]	*D. huoshanense*	Prevent H_2O_2 induced apoptosis of HLE cells	Zha et al. (2017)

Table 1.7 (continued)

Other compounds			
Compounds	***Dendrobium* spp.**	**Bioactivity**	**Ref.**
Spirodiketones (as (+)- and (−)- denobilone A)	*D. nobile*	Inhibit the growth of HeLa MCF-7 and A549 cells	Zhou et al. (2016)
Denobilones B and C	*D. nobile*	-	Zhou et al. (2016)
7-hydroxy-910-dihydro-14-phenanthrenedione	*D. nobile*	-	Zhou et al. (2016)
67-Dihydroxy-2-methoxy-14-phenanthrenedione	*D. nobile*	-	Zhou et al. (2016)
Lusianthrin	*D. nobile*	-	Zhou et al. (2016)
Phochinenin D & G	*D. nobile*	-	Zhou et al. (2016)
Isorhamentin-3-O-β-d-rutinoside	*D. nobile*	Inhibit the growth of HeLa MCF-7 and A549	Zhou et al. (2017)
Adenosine	*D. nobilea*	-	Zhou et al. (2017)
4-methoxy-259R-trihydroxy-910-dihydrophenanthrene-2-O-β-d-glucopyranoside	*D. nobile*	-	Zhou et al. (2017)
Koaburaside	*D. nobile*	-	Zhou et al. (2017)
Juniperoside	*D. nobile*	-	Zhou et al. (2017)
Dehydrodiconiferylalcohol-4-β-d-glucoside	*D. nobile*	Inhibit the growth of HeLa MCF-7 and A549	Zhou et al. (2017)
Mannose and glucose (with linkages of 14-Manp 14-Glcp 146-Manp and 1-Glcp)	*D. huoshanense; D. officinale*	Inhibit CS-induced enhancement in TNF-α and IL-1βsecretion;Immunomodulatory effects on RAW 264.7; Ameliorative effect on constipation	Ge et al. (2017); Liu et al. (2018); Luo et al. (2017)
2-O-acetylglucomannan	*D. officinale*	Treat experimental acute ulcerative colitis	Hua et al. (2004); Liang et al. (2018)
Dinaphthalenone RF-3192C	*D. scabrilingue*	Inhibit a-glucosidase	Sarakulwattana et al. (2018)
Coelonin	*D. scabrilingue*	Inhibit a-glucosidase	Sarakulwattana et al. (2018)
Lusianthridin	*D. scabrilingue*	Inhibit a-glucosidase	Sarakulwattana et al. (2018)
Dendrosinen B	*D. infundibulum*	Inhibit a-glucosidase and pancreatic lipase	Ranong et al. (2018)

1.1.5 Fungal Endophytes Associated with *Dendrobium* Species

Fungal endophytes in *Dendrobium* distributed over 50 genera and 23 orders mainly categorized into Sordariomycetes and Dothideomycetes of Ascomycota (Table 1.1.8 Fungal endophytes isolated from *Dendrobium* species). There are a few fungal endophytes isolated from *Dendrobium* spreading in Agaricomycetes Saccharomycetes and Tremellomycetes (Chen et al., 2011). *Colletotrichum*, *Fusarium*, *Pestalotiopsis* and *Xylaria* are common culturable fungal endophytes in other plants and are also the most frequent fungal endophytes of *Dendrobium* (Yuan et al., 2009; Yang et al., 2011; Chen et al., 2013; Manamgoda et al., 2013; Maharachchikumbura et al., 2014). The components of fungal endophytes in *Dendrobium* were found to be subjected to host trees and habitats (Wang et al., 2017; Wang et al., 2018). Molecular phylogenetic analysis is currently the main method for fungal identification (Jeewon et al., 2017). Multi-gene sequencing is normally used to solve fungal taxonomic problems (Hyde et al., 2014). However some fungal species isolated from *Dendrobium* spp. such as *Scolecobasidium* sp. *Evlachovaea* sp. and *Xylohypha* sp. remain unresolved in species level because of the lack of both morphological and molecular data (Mangunwardoyo, 2012; Boddington & Dearnaley, 2008). Furthermore a fungal genus reported from *Dendrobium Westerdikella* is missing in the database of Index Fungorum (http://www.indexfungorum.org/names/Names.asp). It is also important to note that compared to their diversity specificity between species/tissues/locality of *Dendrobium* and fungal endophytes have even been less well studied (Ma et al., 2015).

Even though approximately 150 fungal endophytes (mainly distributed in Sordariomycetes and Dothideomycetes of Ascomycota including *Xylaria*, *Colletotrichum* and *Fusarium* species in *Xylariaceae Glomerellaceae* and *Nectriaceae* respectively) from *Dendrobium* orchids have been reported and identified (Yuan et al., 2009; Chen et al., 2012, 2013). However these studies account for only 60 *Dendrobium* species or 5% of the total number of species of *Dendrobium* orchids (Ma et al., 2015). Therefore further investigation of fungal endophytes from this orchid genus should result in more number and highly diverse fungal endophytes (Ma et al., 2018). The findings should be beneficial to not only the understanding of orchid physiology but also fungal taxonomy phylogeny and diversity. Furthermore the isolated fungal endophytes from *Dendrobium* orchids will be valuable materials for bioactive compound exploration (Cui et al., 2012).

Some fungal endophytes isolated from *Dendrobium* orchids can promote seed germination or development in hosts as mycorrhizal fungi (Nontachaiyapoom et al., 2010; Khamchatra et al., 2016). The elicitors produced by fungal endophytes of *D. officinale* could stimulate biomass and active ingredients accumulation in the host plants (Zhu et al., 2018). Antimicrobial antioxidant and antitumor bioactivities have been found in some *Dendrobium* fungal endophytes (Jin et al., 2017; Shi et al., 2018; Bungtongdee et al., 2019). However the function of most fungal endophytes in *Dendrobium* orchids remain unknown (Ma et al., 2015).

1.1.6 Bioactive Compounds from Fungal Endophytes of *Dendrobium* spp.

Orchid fungal endophytes are potential sources of bioactive natural compounds (Bungtongdee et al., 2019). Zhang et al. (2008) separated and purified polysaccharides and water-soluble proteins from two *Xylaria* strains isolated from the stem of *D. fimbiratum* and leaf of *D. brymerianum* and showed that they had bacteriostatic activity. Xing et al. (2011) revealed that components in the extracts of *Phoma* sp. *Fusarium* sp. and *Epicoccum nigrum* isolated from *D. thyrsiflorum* and *D. devonianum* and had antimicrobial activity. Cytotoxic metabolites - alternariol alternariol 5-O-methyl ether and altenusin isolated from one fungal endophyte *Aternaria* sp. have been identified the same as the constituents in leaves of its host plant *Polygonum senegalense* (Aly et al., 2008). Recently it has been found that a new polysaccharide isolated from one fungal endophyte *Fusarium solani* possessed immune enhancement activity.

The genus *Pestalotiopsis* has been reported to be the source of 130 different compounds including alkaloids terpenoids isocoumarin derivatives coumarins chromones quinones semiquinones peptides xanthones xanthone derivatives phenols phenolic acids and lactones isolated from various species in (Xu et al., 2010). Although there is no report that fungal endophytes from *Dendrobium* possess the same substances as their plant hosts. Therefore it is interesting to investigate the bioactive compounds in the extracts of *Dendrobium* fungal endophytes and those reported from this orchid genus. However there is still little report about fungal endophytes metabolites in *Dendrobium* spp. (Yuan et al., 2011; Ma et al., 2015).

1.2 Research Objectives

1.2.1 To study the taxonomy and phylogeny of fungal endophytes isolated from *Dendrobium* orchids sampled from southwestern China and northern Thailand.

1.2.2 To identify and describe the fungal endophytes in *Dendrobium* orchids and document the new taxa.

1.2.3 To investigate the host/tissue/locality-specificity of the isolated fungal endophytes.

1.2.4 To screen extracts of some fungal endophytes for their anticancer activity.

1.3 Research Contents

This thesis is divided into eight chapters.

Chapter 1 is the general introduction which provides a background of the fungal endophytes *Dendrobium* orchids fungal endophytes of *Dendrobium* orchids and the research objectives and the outline of the thesis.

Chapter 2 Non-mycorrhizal endophytic fungi from Orchidaceae

Chapter 3 Endophytic *Colletotrichum* species from *Dendrobium* spp. in China and Northern Thailand

Chapter 4 Endophytic pestalotiod fungi in *Dendrobium* orchids

Chapter 5 Endophytic Botryosphaeriales species in *Dendrobium* orchids (*Neofusicoccum Phyllosticta*)

Chapter 6 Endophytic *Pezicula* (Discomycota) fungi in *Dendrobium* orchids.

Chapter 7 Endophytic Xylariales fungi and their bioactivities in *Dendrobium* orchids from southwestern China and northern Thailand

Chapter 8 Overall conclusions. This part presents a general conclusion for this study and put forward future perspectives.

Table 1.8 Fungal endophytes isolated from *Dendrobium* species

Fungal endophytes name	Family	Order	Class	Phylum	Host	Location	Ref.
Acremonium nepalense	-	Hypocreales	Sordariomycetes	Ascomycota	*D. loddigesii*	Xingyi Guizhou China	Chen et al. (2010)
Acremonium spp.	-	Hypocreales	Sordariomycetes	Ascomycota	*D. loddigesii. D. candidum; D. nobile; D. falconeri; D. primulinum; D. christyanum; D. hancockii; D. moniliforme; D. officinale*	Xingyi Guizhou; Puwen and Xishuangbanna Yunnan China	Chen et al. (2010); Chen et al. (2011); Liu et al. (2017)
Acremonium stromaticum	-	Hypocreales	Sordariomycetes	Ascomycota	*D. loddigesii*	Xingyi Guizhou China	Chen et al. (2010)
Alternaria spp.	Pleosporaceae	Pleosporales	Dothideomycetes	Ascomycota	*D.nobile; D. chrysanthum. D. loddigesii. D. candidum; D. falconeri; D. hancockii; D. huoshanense; D. officinale*	Chishui and Xingyi Guizhou; Xishuangbanna Yunnan; An Hui China	Chen et al. (2010); Chen et al. (2012); Liu et al. (2017); Chen et al. (2019)
Ampelomyces sp.	Phaeosphaeriaceae	Pleosporales	Dothideomycetes	Ascomycota	*D. loddigesii; D. candidum; D. nobile; D. falconeri; D. christyanum;*	Xingyi Guizhou China	Chen et al. (2010)

Table 1.8 (continued)

Fungal endophytes name	**Family**	**Order**	**Class**	**Phylum**	**Host**	**Location**	**Ref.**
Annulohypoxylon sp.	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. chrysotoxum; D. falconeri; D.crystallinum*	Xishuangbanna Yunnan China	Chen et al. (2013)
Apiosporaceae sp.	Apiosporaceae	Xylariales	Sordariomycetes	Ascomycota	*D. nobile; D. chrysanthum*	Chishui Guizhou Xishuangbanna Yunnan China	Chen et al. (2012)
Arthrinium euphorbiae	Apiosporaceae	Xylariales	Sordariomycetes	Ascomycota	*D. nobile; D. chrysanthum*	Chishui Guizhou Xishuangbanna Yunnan China	Chen et al. (2012)
Arthrinium sp.	Apiosporaceae	Xylariales	Sordariomycetes	Ascomycota	*D. candidum*	Xishuangbanna Yunnan China	Chen et al. (2011)
Aspergillus brevipes	Aspergillaceae	Eurotiales	Eurotiomycetes	Ascomycota	*D. moniliforme*	Forest of Suryabinayak Nepal	Shrestha et al. (2018)
A. clavatum	Aspergillaceae	Eurotiales	Eurotiomycetes	Ascomycota	*D. moniliforme*	Forest of Suryabinayak Nepal	Shrestha et al. (2018)
A. flavus	Aspergillaceae	Eurotiales	Eurotiomycetes	Ascomycota	*D. officinale; D. moniliforme*	Planting base Yunnan China; Forest of Suryabinayak Nepal	Jin et al. (2017); Shrestha et al. (2018)
A. fumigatus	Aspergillaceae	Eurotiales	Eurotiomycetes	Ascomycota	*D. moniliforme*	Forest of Suryabinayak Nepal	Shrestha et al. (2018)
A. niger	Aspergillaceae	Eurotiales	Eurotiomycetes	Ascomycota	*D. officinale*	Planting base Yunnan China; Forest of Suryabinayak Nepal	Shrestha et al. (2018)
Aureobasidium sp.	Saccotheciaceae	Dothideales	Dothideomycetes	Ascomycota	*D. candidum*	Xishuangbanna of Yunnan China	Chen et al. (2011)
Beauveria sp.	Cordycipitaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. officinale*	Chanthaburi Thailand	Khamchatra et al. (2016)
Bionectria spp.	Bionectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. loddigesii. D. candidum; D. nobile; D. moniliforme*	Xingyi Guizhou China	Chen et al. (2010)
Biscogniauxia dendrobii	Graphostromataceae	Xylariales	Sordariomycetes	Ascomycota	*Dendrobium aphyllum*	China	Ma et al. (2020)
Biscogniauxia petrinenses	Graphostromataceae	Xylariales	Sordariomycetes	Ascomycota	*Dendrobium harveyanum*	Thailand	Ma et al. (2020)
Botryosphaeria mamane	Botryosphaeriaceae	Botryosphaeriales	Dothideomycetes	Ascomycota	*D. compactum*	Yunnan Chongqing China	Cui et al. (2012)
Botryosphaeria sp.	Botryosphaeriaceae	Botryosphaeriales	Dothideomycetes	Ascomycota	*D. nobile*	Xishuangbanna Yunan province	Yuan et al. (2009)
Candida sp.	-	Saccharomycetales	Saccharomycetes	Ascomycota	*D. candidum*	Xishuangbanna Yunan China	Chen et al. (2011)
Cercophora sp.	Lasiosphaeriaceae	Sordariales	Sordariomycetes	Ascomycota	*D. loddigesii. D. candidum*	Xingyi Guizhou in China	Chen et al. (2010)
Chaetomium globosum	Chaetomiaceae	Sordariales	Sordariomycetes	Ascomycota	*D. longicornu. D. candidum*	Yunnan Chongqing Xishuangbanna Yunan China	Cui et al. (2012); Chen et al. (2011)
Chaetomella sp.	Chaetomellaceae	Helotiales	Leotiomycetes	Ascomycota	*D. loddigesii. D. primulinum; D. moniliforme*	Xingyi and Guizhou China. Xishuangbanna Yuanna China	Chen et al. (2010); Chen et al. (2011)
Chaetophoma sp.	-	-	-	Ascomycota	*D. candidum*	Xishuangbanna Yunnan China	Chen et al. (2011)
Chloridium virescens var. *chlamydosporum*	Chaetosphaeriaceae	Chaetosphaeriales	Sordariomycetes	Ascomycota	*D. nobile; D. chrysanthum*	Chishui Guizhou; Xishuangbanna Yunnan; China	Chen et al. (2012)
Cladosporium cladosporioides	Cladosporiaceae	Capnodiales	Dothideomycetes	Ascomycota	*D. crumenatum; D. longicornu*	Herbarium Bogoriense and Bogor Botanical Garden Indonesia; Yunnan Chongqing China	Mangunwardoyo et al. (2012); Cui et al. (2012)
C. sphaerospermum	Cladosporiaceae	Capnodiales	Dothideomycetes	Ascomycota	*D. crumenatum*	Herbarium Bogoriense and Bogor Botanical Garden Indonesia	Mangunwardoyo et al. (2012)

Table 1.8 (continued)

Fungal endophytes name	Family	Order	Class	Phylum	Host	Location	Ref.
Cladosporium spp.	Cladosporiaceae	Capnodiales	Dothideomycetes	Ascomycota	*D. huoshanense; D. loddigesii. D. nobile; D. gratiosissimum; D. officinale; D. pendulum D. moniliforme*	Xingyi Guizhou; Xishuangbanna Yunnan; An Hui China; Forest of Suryabinayak Center hill Nepal	Chen et al. (2010); Chen et al. (2011); Liu et al. (2017); Shrestha et al. (2018); Chen et al. (2019); Shah et al. (2019)
Clonostachys rosea	Bionectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. nobile*	Xishuangbanna Yunan China	Yuan et al. (2009)
Colletotrichum boninense	Glomerellaceae	Glomerellales	Sordariomycetes	Ascomycota	*D. aqueum*, D. catenatum	Tamil Nadu India	Parthibhan et al. (2017); Ma et al. (2018)
C. chiangraiense	Glomerellaceae	Glomerellales	Sordariomycetes	Ascomycota	*Dendrobium* sp.	Chiang Rai, Thailand	Ma et al. (2018)
C. citricola	Glomerellaceae	Glomerellales	Sordariomycetes	Ascomycota	*Dendrobium* sp.	Chiang Rai, Thailand	Ma et al. (2018)
C. doitungense	Glomerellaceae	Glomerellales	Sordariomycetes	Ascomycota	*Dendrobium* sp.	Chiang Rai, Thailand	Ma et al. (2018)
C. fructicola	Glomerellaceae	Glomerellales	Sordariomycetes	Ascomycota	*Dendrobium* sp.	Chiang Rai, Thailand	Ma et al. (2018)
C. gloeosporioides	Glomerellaceae	Glomerellales	Sordariomycetes	Ascomycota	*D. aqueum; D.nobile; D. chrysanthum*	Chishui Guizhou; Xishuangbanna Yunnan; China. Herbarium Bogoriense and Bogor Botanical Garden Indonesia; Tamil Nadu India	Chen et al. (2012); Mangunwardoyo et al. (2012); Parthibhan et al. (2017)
C.kahawae	Glomerellaceae	Glomerellales	Sordariomycetes	Ascomycota	*D. aqueum*	Tamil Nadu India	Parthibhan et al. (2017)
C. jiangxiense	Glomerellaceae	Glomerellales	Sordariomycetes	Ascomycota	*Dendrobium* sp.	Chiang Rai, Thailand	Ma et al. (2018)
C.orchidophilum	Glomerellaceae	Glomerellales	Sordariomycetes	Ascomycota	*D. harveyanum*	Chiang Rai, Thailand	Ma et al. (2018)
C. watphraense,	Glomerellaceae	Glomerellales	Sordariomycetes	Ascomycota	*Dendrobium* sp.	Chiang Rai, Thailand	Ma et al. (2018)
C.parallelophorum	Glomerellaceae	Glomerellales	Sordariomycetes	Ascomycota	*Dendrobium* sp.	Chiang Rai, Thailand	Ma et al. (2018)
Colletotrichum spp.	Glomerellaceae	-	Sordariomycetes	Ascomycota	*D.nobile. D. loddigesii; D. exile; D. falconeri; D. primulinum; D. officinale; D. moniliforme*	Xishuangbanna Yunan Xingyi Guizhou China; Herbarium Bogoriense and Bogor Botanical Garden Indonesia; Forest of Suryabinayak Center hill Nepal	Yuan et al. 2009); Chen et al. (2010); Mangunwardoyo et al. (2012); Jin et al. (2017); Liu et al. (2017); Parthibhan et al. (2017); Meng et al. (2019); Shah et al. (2019)
C. truncata	Glomerellaceae	-	Sordariomycetes	Ascomycota	*D. nobile; D. chrysanthum*	Chishui Guizhou Xishuangbanna Yunnan China	Chen et al. (2012)
Curvularia brachyspora	Pleosporaceae	Pleosporales	Dothideomycetes	Ascomycota	*D. crumenatum*	Herbarium Bogoriense and Bogor Botanical Garden Indonesia	Mangunwardoyo et al. (2012)
Cylindrocarpon destructans	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. chrysanthum*	Chishui Guizhou Xishuangbanna Yunnan China	Chen et al. (2012)
Cylindrocarpon sp.	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. moniliforme*	Center hill Nepal	Shah et al. (2019)
Daldinia eschscholzii	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. chrysotoxum*	Xishuangbanna Yunan China	Chen et al. (2013)

Table 1.8 (continued)

Fungal endophytes name	Family	Order	Class	Phylum	Host	Location	Ref.
Davidiella sp.	Davidiellaceae	Davidiellaceae	Dothideomycetes	Ascomycota	*D. loddigesii*	Xingyi Guizhou China	Chen et al. (2010)
Epicoccum nigrum	Didymellaceae	Pleosporales	Dothideomycetes	Ascomycota	*D. speciosum (Smith); D. nobile; D. chrysanthum. D. Speciosum*	Southern Queensland rainforests; Redwood Park near Toowoomba Australia; Chishui Guizhou Xishuangbanna Yunnan China	Boddington et al. (2006); Boddington and Dearnaley (2008); Chen et al. (2012)
Evlachovaea sp.	-	-	-	Ascomycota	*D. speciosum (Smith); D. nobile; D. chrysanthum*	Chishui Guizhou Xishuangbanna Yunnan China	Boddington et al. (2006); Chen et al. (2013)
Fusarium chlamydosporum	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. crumenatum*	Faculty Science Universiti Putra Malaysia	Siddiquee et al. (2010)
F. dimerum	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. loddigesii*	Xingyi Guizhou China	Chen et al. (2010)
F. equiseti	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. moniliforme*	Forest of Suryabinayak Nepal	Shrestha et al. (2018)
F. moniliformis	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. gratiosissimum*	Yunnan Chongqing China	Cui et al. (2012)
F. nivale	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. crumenatum*	Herbarium Bogoriense and Bogor Botanical Garden Indonesia	Mangunwardoyo et al. (2012)
F. oxysporum	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. chrysanthum; D.lindleyi; D. officinale*	Chishui Guizhou XishuangbannaYunnan China; Chiang Mai Thailand	Chen et al. (2012); Jin et al. (2017); Bungtongdee et al. (2018)
F. proliferatum	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. minutiflorum; D. gratiosissimum; D. compactum; D. aphyllum. D. chrysanthum*	Yunnan Chongqing Chi shui Guizhou Xishuangbanna Yunnan China	Cui et al. (2012); Chen et al. (2012); Yuan et al. (2009)
F. solani	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. loddigesii; D. chrysanthum. D. nobile*	Xingyi Guizhou China; Herbarium Bogoriense and Bogor Botanical Garden Indonesia	Yuan et al. (2009; Chen et al. (2010); Mangunwardoyo et al. (2012)
Fusarium spp.	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. speciosum (Smith); D. nobile; D. chrysanthum. D. loddigesii. D. speciosum. D. candidum; D. falconeri; D. primulinum; D. gratiosissimum; D. hancockii; D. huoshanense; D. pendulum; D. moniliforme; D. officinale*	Southern Queensland rainforests; Redwood Park near Toowoomba Australia; An Hui Chong Qing Xishuangbannn XingyiChina; Forest of Suryabinayak center hill Nepal	Boddington et al. (2006); Boddington and Dearnaley (2008); Chen et al. (2012); Yuan et al. (2009); Chen et al. (2010); Chen et al. (2011); Liu et al. (2017); Shi et al. (2018); Shrestha et al. (2018); Chen et al. (2019); Shah et al. (2019)
F. stoveri	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. loddigesii*	Xingyi Guizhou China	Chen et al. (2010)
F. tricinctum	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. minutiflorum;D. huoshanense*	Yunnan Chongqing China	Cui et al. (2012)
F. udum	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. loddigesii*	Xingyi Guizhou China	Chen et al. (2010)
F. venfricosum	Nectriaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. loddigesii*	Xingyi Guizhou China	Chen et al. (2010)
Fusicoccum arbuti	Botryosphaeriaceae	Botryosphaeriales	Dothideomycetes	Ascomycota	*D. nobile*	Yunnan Chongqing China	Cui et al. (2012)

Table 1.8 (continued)

Fungal endophytes name	Family	Order	Class	Phylum	Host	Location	Ref.
Glomerularia sp.	Ceratostomataceae	Melanosporales	Sordariomycetes	Ascomycota	*D. candidum; D. moniliforme*	Xishuangbanna Yunnan China	Chen et al. (2011)
Guignardia endophyllicola	Phyllostictaceae	-	Dothideomycetes	Ascomycota	*D. crumenatum*	Herbarium Bogoriense and Bogor Botanical Garden Indonesia	Mangunwardoyo et al. (2012)
Guignardia mangiferae	Phyllostictaceae	-	Dothideomycetes	Ascomycota	*D. nobile*	Xishuangbanna Yunan China	Yuan et al. (2009)
Helminthosporium sp.	Massarinaceae	Pleosporales	Dothideomycetes	Ascomycota	*D. transparens*	Forest of Suryabinayak Nepal	Shrestha et al. 2018)
Hyalodendron sp.	Trichosporonaceae	Trichosporonales	Tremellomycetes	Basidiomycota	*D. candidum*	Xishuangbanna Yunan China	Chen et al. (2011)
Hypocrea viridescens	Hypocreaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. longicornu*	Yunnan Chongqing China	Cui et al. (2012)
Hypoxylon fragiforme	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. moniliforme*	Forest of Suryabinayak Nepal	Shrestha et al. (2018)
Hypoxylon spp.	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. moniliforme; D. nobile; D. officinale*	Xishuangbanna Yunan China; Center hill Nepal	Yuan et al. (2009); Zhu et al. (2016); Shah et al. (2019)
Lasiodiplodia spp.	Botryosphaeriaceae	Botryosphaeriales	Dothideomycetes	Ascomycota	*D. loddigesii*	Xingyi Guizhou Xishuangbanna Yunnan China	Chen et al. (2010); Chen et al. (2011)
Leptodontidium sp.	-	Helotiales	Leotiomycetes	Ascomycota	*Dendrobium sp. D. nobile*	Xingyi Guizhou; Xishuangbanna Yunnan China	Chen et al. (2010; Hou and Guo (2009
Leptosphaerulina chartarum	Pleosporaceae	Pleosporales	Dothideomycetes	Ascomycota	*D. moniliforme*	Forest of Suryabinayak Center hill Nepal	Shrestha et al. (2018); Shah et al. (2019)
Monographella sp.	-	Xylariales	Sordariomycetes	Ascomycota	*D. officinale*	China	Liu et al. (2017)
Mucor racemosus	Caliciaceae	Teloschistales	Lecanoromycetes	Ascomycota	*D. nobile*	Yunnan Chongqing China	Cui et al. (2012)
Muscodor albus	Xylariaceae	Xylariaceae	Sordariomycetes	Ascomycota	*D. nobile; D. chrysanthum*	Chishui Guizhou Xishuangbanna Yunnan China	Chen et al. (2012)
Mycoleptodiscus sp.	Magnaporthaceae	Magnaporthales	Sordariomycetes	Ascomycota	*D. nobile; D. chrysanthum*	Chishui Guizhou Xishuangbanna Yunnan China	Chen et al. (2012)
Nemania bipapillata	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. nobile; D. fimbriatum; D. chrysanthum; D.crystallinum*	Xishuangbanna Yunnan China	Chen et al. (2013)
Neofusicoccum parvum	Botryosphaeriaceae	Botryosphaeriales	Dothideomycetes	Ascomycota	*D. harveyanum*, *D. moschatum*	Chiang Rai, Thailand & Guizhou, China	Ma et al. (2021)
Neopestalotiopsis dendrobii	Pestalotiopsidaceae	Amphisphaeriales	Sordariomycetes	Ascomycota	*D. cariniferum*	Thailand	Ma et al. (2019)
N. primolutea	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. aphyllum*	Xishuangbanna Yunnan China	Chen et al. (2013
Nemania spp.	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. nobile. D. christyanum*	Xishuangbanna Yunnan China	Yuan et al. (2009); Chen et al. (2011)
Nigrospora spp.	-	Trichosphaeriales	Sordariomycetes	Ascomycota	*D. speciosum (Smith). D. candidum*	Southern Queensland rainforests; Redwood Park near Toowoomba Australia; Xingyi Guizhou Xishaungbanna Yunnan China	Boddington et al. (2006); Boddington and Dearnaley (2008); Chen et al. (2010); Chen et al. (2011)
Nodulisporium sp.	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. chrysotoxum; D. exile*	Herbarium Bogoriense and Bogor Botanical Garden Indonesia; Xishuangbanna Yunnan China	Mangunwardoyo et al. (2012); Meng et al. (2019)
Paraconiothyrium sp.	Montagnulaceae	Montagnulaceae	Dothideomycetes	Ascomycota	*D. loddigesii; D. nobile*	Xingyi Guizhou Xishuangbanna Yuanna China	Chen et al. (2010); Chen et al. (2011)
Penicillium brevicompactum	Trichocomaceae	Eurotiales	Eurotiomycetes	Ascomycota	*D. longicornu*	Yunnan Chongqing China	Cui et al. (2012)
P. funiculosum	Trichocomaceae	Eurotiales	Eurotiomycetes	Ascomycota	*D. nobile; D. chrysanthum*	Chishui Guizhou; Xishuangbanna Yunnan China	Chen et al. (2012)
P. griseofulvum	Trichocomaceae	Eurotiales	Eurotiomycetes	Ascomycota	*D. nobile*	Xishuangbanna Yunnan China	Yuan et al. (2009)
P. swiecickii	Trichocomaceae	Eurotiales	Eurotiomycetes	Ascomycota	*D. longicornu*	Yunnan Chongqing China	Cui et al. (2012)

Table 1.8 (continued)

Fungal endophytes name	Family	Order	Class	Phylum	Host	Location	Ref.
Penicillium spp.	Trichocomaceae	Eurotiales	Eurotiomycetes	Ascomycota	*D. nobile*	Xishuangbanna Yunan China	Yuan et al. (2009)
Periconiella sp.	Mycosphaerellaceae	Capnodiales	Dothideomycetes	Ascomycota	*D. candidum*	Xishuangbanna Yunnan China	Chen et al. (2011)
Pestalotiopsis clavispora	Pestalotiopsidaceae	Amphisphaeriales	Sordariomycetes	Ascomycota	*D. minutiflorum*	Yunnan Chongqing China	Cui et al. (2012)
P. doitungensis	Pestalotiopsidaceae	Amphisphaeriales	Sordariomycetes	Ascomycota	*Dendrobium* sp.	Chiang Rai, Thailand	Ma et al. (2019)
P. lushanensis	Pestalotiopsidaceae	Amphisphaeriales	Sordariomycetes	Ascomycota	*Dendrobium* sp.	Chiang Rai, Thailand	Ma et al. (2019)
P. microspora	Pestalotiopsidaceae	Amphisphaeriales	Sordariomycetes	Ascomycota	*D. minutiflorum; D. huoshanense; D. nobile;D. gratiosissimum*	Yunnan Chongqing China	Cui et al. (2012)
P. trachicarpicola	Pestalotiopsidaceae	Amphisphaeriales	Sordariomycetes	Ascomycota	*D. loddigesii*	Guizhou, China	Ma et al. (2019)
Pestalotiopsis spp.	Pestalotiopsis	Amphisphaeriales	Sordariomycetes	Ascomycota	*D. nobile; D. chrysanthum; D. falconeri; D. officinale*	Chishui Guizhou Xishuangbanna Yunnan China. Herbarium Bogoriense and Bogor Botanical Garden Indonesia	Chen et al. (2012). Mangunwardoyo et al. (2012); Chen et al. (2011); Zhu et al. (2016); Jin et al. (2017)
Pestalotiopsis vismiae	Pestalotiopsis	Amphisphaeriales	Sordariomycetes	Ascomycota	*D. nobile*	Xishuangbanna Yunnan China	Yuan et al. (2009)
Pezicula sp.	Cantharellaceae	Cantharellales	Leotiomycetes	Ascomycota	*D. nobile*	Xishuangbanna Yunnan China	Chen et al. (2011)
Phialophora spp.	Herpotrichiellaceae	Chaetothyriales	Eurotiomycetes	Ascomycota	*D. chrysanthum D. Speciosum*	Chishui Guizhou Xishuangbanna Yunnan China; Redwood Park near Toowoomba Australia.	Chen et al. (2012); Boddingtong and Dearnaley (2008)
Phomopsis amygdali	Diaporthaceae	Diaporthales	Sordariomycetes	Ascomycota	*D. nobile*	Xishuangbanna Yunnan China	Yuan et al. (2009)
Phomopsis spp.	Diaporthaceae	Diaporthales	Sordariomycetes	Ascomycota	*D. chrysanthum; D. officinale*	Chishui Guizhou Xishuangbanna Yunnan China	Chen et al. (2012); Liu et al. (2017)
Pleospora sp.	Pleosporaceae	Pleosporales	Dothideomycetes	Ascomycota	*D. nobile*	Xishuangbanna Yunnan China	Chen et al. (2011)
Pleurophragmium sp.	-	Helotiales	Leotiomycetes	Ascomycota	*D. pendulum*	Xishuangbanna Yunnan China	Chen et al. (2011)
Pyrenochaeta sp.	-	Pleosporales	Dothideomycetes	Ascomycota	*D. loddigesii*	Xingyi Guizhou China	Chen et al. (2010)
Schizophyllum sp.	Schizophyllaceae	Agaricales	Agaricomycetes	Basidiomycota	*D. loddigesii; D. moniliforme*	Xishuangbanna Yunnan China	Chen et al. (2011)
Sclerotium sp.	Typhulaceae	Agaricales	Agaricomycetes	Basidiomycota	*D. candidum*	Xishuangbanna Yunnan China	Chen et al. (2011)
Scolecobasidium sp.	-	-	-	Ascomycota	*D. crumenatum*	Herbarium Bogoriense and Bogor Botanical Garden Indonesia	Mangunwardoyo et al. (2012)
Sirodesmium spp.	-	Chaetothyriales	Eurotiomycetes	Ascomycota	*D. loddigesii*	Xingyi Guizhou Xishuangbanna Yunnan China	Chen et al. 2010); Chen et al. (2011)
Talaromyces sp.	Trichocomaceae	Eurotiales	Eurotiomycetes	Ascomycota	*D. officinale*	State Forestry Administration China	Zhu et al. (2016)
Thielavia californica	Chaetomiaceae	Sordariales	Sordariomycetes	Ascomycota	*D. loddigesii*	Xingyi Guizhou China	Chen et al. (2010)
Thielavia sp.	Chaetomiaceae	Sordariales	Sordariomycetes	Ascomycota	*D. loddigesii; D. hancockii*	Xishuangbanna Yunnan China	Chen et al. (2011)
Trichoderma chlorosporum	Hypocreaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. nobile*	Xishuangbanna Yunnan China	Yuan et al. (2009)

Supplementary: (continued)

Fungal endophytes name	Family	Order	Class	Phylum	Host	Location	Ref.
Trichoderma spp.	Hypocreaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. nobile; D. chrysanthum; D. moniliforme; D. speciosum*	Southern Queensland rainforests; Redwood Park near Toowoomba Australia; Chishui Guizhou; Xishuangbanna Yunnan China; Center hill Nepal	Boddington et al. (2006); Boddington and Dearnaley (2008); Chen et al. (2011); Chen et al. (2012); Shah et al. (2019)
T. harzianum	Hypocreaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. moniliforme*	Forest of Suryabinayak Nepal	Shrestha et al. (2018)
Trichoderma viride	Hypocreaceae	Hypocreales	Sordariomycetes	Ascomycota	*D. longicornu*	Yunnan Chongqing China	Cui et al. (2012)
Verticillium leptobactrum	Plectosphaerellaceae	-	Sordariomycetes	Ascomycota	*D. chrysanthum*	Chishui Guizhou Xishuangbanna Yunnan China	Chen et al. (2012)
Verticillium spp.	Plectosphaerellaceae	-	Sordariomycetes	Ascomycota	*D. nobile; D. loddigesii; D. falconeri; D. pendulum; D. moniliforme*	Chishui Guizhou Xishuangbanna Yunnan China	Chen et al. 2012; Chen et al. (2011)
Xylaria amphithele	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. nobile; D. falconeri*	Xishuangbanna Yunnan China	Chen et al. (2013)
X. apoda	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. fimbriatum;*	Xishuangbanna Yunnan China	Chen et al. (2013)
X. arbuscula	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. nobile; D. chrysanthum; D. falconeri; D. aphyllum;*	Xishuangbanna Yunnan China	Chen et al. (2013)
X. badia	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. chrysotoxum; D. falconeri*	Xishuangbanna Yunnan China	Chen et al. (2013)
X. cubensis	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. officinale*	Yunnan China	Jin et al. (2017)
X. curta	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. chrysanthum; D. aphyllum;*	Xishuangbanna Yunnan China	Chen et al. (2013)
X. castorea	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. nobile; D. chrysanthum*	Chishui Guizhou Xishuangbanna Yunnan China	Chen et al. (2012)
X. coccophora	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. nobile; D. chrysanthum*	Chishui Guizhou Xishuangbanna Yunnan China	Chen et al. (2012)
X. feejeensis	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. fimbriatum; D. chrysanthum; D.crystallinum*	Xishuangbanna Yunnan China	Chen et al. (2013
X. grammica	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. nobile; D. chrysanthum. D. fimbriatum; D. chrysotoxum; D. aphyllum*	Chishui Guizhou Xishuangbanna Yunnan China	Chen et al. (2012)
X. multiplex	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. falconeri*	Xishuangbanna Yunnan China	Chen et al. (2013)
X. papulis	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. fimbriatum; D. chrysotoxum; D. aphyllum*	Xishuangbanna Yunnan China	Chen et al. (2013)
X. plebeja	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. exile*	Xishuangbanna Yunnan China	Meng et al. (2019)
Xylaria spp.	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. nobile; D. chrysanthum; D. crumenatum; D. loddigesii. D. primulinum; D. gratiosissimum*	Chishui Guizhou Xishuangbanna Yunnan China; Herbarium Bogoriense and Bogor Botanical Garden Indonesia	Chen et al. (2012); Yuan et al. (2009); Chen et al. (2010); Chen et al. (2011) Mangunwardoyo et al. (2012)
X. venosula	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. nobile; D. chrysanthum;D. falconeri*	Chishui Guizhou Xishuangbanna Yunnan China	Chen et al. (2012); Chen et al. (2013)
X. venustula	Xylariaceae	Xylariales	Sordariomycetes	Ascomycota	*D. chrysotoxum;*	Xishuangbanna Yunnan China	Chen et al. (2013)
Zasmidium	Mycosphaerellaceae	Capnodiales	Dothideomycetes	Ascomycota	*D. huoshanense*	Anhui China	Chen et al. (2019)
Zymoseptoria sp.	Mycosphaerellaceae	Capnodiales	Dothideomycetes	Ascomycota	*D. huoshanense*	Anhui China	Chen et al. (2019)

CHAPTER 2

NON-MYCORRHIZAL ENDOPHYTIC FUNGI FROM ORCHIDS[1]

Abstract

Orchidaceae is one of the largest flowering plant familiesof the plant kingdom. The habitats of orchids arehighly diverse, ranging from tree bark and damp forestfloors to rock crevices, sandy dunes and semi-ariddeserts. The diversity of endophytes (internal symbioticfungi) associated with orchids is enormous. Most studies of endophytic fungi from orchids in the past have focused on mycorrhizal endophytes (internal symbiotic fungi associated with plant roots). Therehas, however, been an increasing trend to study nonmycorrhizal endophytes from orchids because of their physiological roles and their potential as sources of novel bioactive compounds. This review discusses the methods used in the isolation and identification of endophytic fungi from orchids, their diversity and host-specificity, their significance in orchid conservation and cultivation, and their potential application in the discovery of bioactive compounds.

Keywords: Bioactive compounds, diversity, nonmycorrhizal, endophytic fungi, orchids

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2.1 Introduction

Orchidaceae is one of the largest flowering plant families of the plant kingdom, which comprises more than 899 genera and 27,801 species (The Plant List, 2013). Of these, over 200 genera have been investigated for their endophytic fungal diversity (see Table 2.4), which is less than 30% of total orchid genera. Orchids with horticultural, ornamental, medical and commercial importance have been researched for endophytes. Rare or endangered orchids including species in *Cypripedium, Holcoglossum* and *Paphiopedilum* have also received attention (Shefferson et al., 2005; Nontachaiyapoom et al., 2010; Tan et al., 2012). Terrestrial orchids which make up nearly one third of all orchid species occupy approximately half of the endangered orchid list (The world Conservation Union, 1999)(Swarts & Dixon, 2009). Many of them have also been subjected to endophyte research (Table 2.2). Orchid species in the genera *Aa, Hadrolaelia, Gavilea* and *Satyrium* have been poorly studied and can be regarded as new topics for research (Fracchia et al., 2009; Jyothsna & Purushothama, 2014; Oliveira et al., 2014; Sebastian et al., 2014).

Research on endophytic fungi in orchids has been carried out in all trophic groups (i.e. photosynthetic, mixotrophic and mycoheterotrophic) of all growth habits (i.e. terrestrial, epiphytic and lithophytic), from highly diverse habitats (e.g. rainforest, evergreen forests, coniferous forests, bamboo forests, ectomycorrhizal forests, wetlands, swamps, calcareous coastal plains, botanical gardens, and greenhouses) in all continents except Antarctica (Table 2.2). Some orchids occur in a wide range of habitats, while others are endemic to certain regions. For example, *Platanthera minor* grows in forests on slopes and alpine meadows at elevations of 90 – 3000 m in China, Japan and Korea (Yagame et al., 2012). *Satyrium nepalense* was reported to distribute from grassy hill slopes at varying altitudes (600 – 4600 m) in India (Jyothsna & Purushothama, 2014). *Ophrys benacensis* occurs only in northern Italy (Pierce et al., 2010) and *Piperia yadonii* only in North America (Pandey et al., 2013). The epiphytic orchid *Sarcochilus parviflorus* survives only with its main host *Backhousia myrtifolia* (Gowland et al., 2013).

The purpose of this study is to review the studies on non-mycorrhizal endophytic fungi of orchids and present the main conclusions from the research.

2.2 Isolation and Identification of Fungal Endophytes from Orchids

2.2.1 Isolation

Orchid mycorrhizal fungi are known to be associated with roots of orchids (Bernard, 1899; Burgeff, 1909). Therefore, most endophyte studies on orchids have investigated orchid roots for mycorrhizal and endophytic diversity (Roy et al., 2009b). Other orchid parts including leaves, rhizomes, mature bulbs, tubers, stems, and stem-collars have also been studied for endophytes (Yuan et al., 2009; Sudheep and Sridhar 2012). Since endophytes are commonly defined as “all organisms inhabiting plant organs that at some time in their life, can colonize internal plant tissues without causing apparent harm to the host”(Petrini, 1991; Hyde & Soytong, 2008), only healthy organs were used in these studies (Tondello et al., 2012).

The study of endophytic fungi starts with collection of orchid samples, followed by isolation in the laboratory. Epiphytic microorganisms are removed via surface sterilization prior to isolation (Guo et al., 2001). All surface sterilization procedures in orchid endophytic research have used sterilizing reagents, including ethanol, chlorine (Cl_2), sodium chlorite ($NaClO_2$), sodium hypochlorite ($NaClO$), mercury (II) chloride ($HgCl_2$), hydrogen peroxide (H_2O_2) and calcium hypochlorite ($Ca(ClO)_2$) to disinfect tissues via sequentially immersing tissues in reagents (see Table 2.1 for details). The concentration and time for surface sterilization varies depending on the sterilizing reagents and the types of orchid tissues studied. The concentration of reagents is important. Sterilization with 0.1% or 0.2% $HgCl_2$ for 3 min did not kill *Bacillus* species, but using 0.3% $HgCl_2$ for 10 minutes successfully killed the bacteria (Ramakrishna et al., 1991). $NaClO$ has been reported to be more damaging to tissues than $Ca(ClO)_2$ (Fay, 1994). The degree of surface sterilization greatly affects the fungal endophytes recovered (Tondello et al., 2012) and therefore Schultz et al. (1999) suggested leaf imprinting to test the effectiveness of the protocol. However, in most orchid endophyte studies to date, leaf imprinting was not carried out. Sawmya et al. (2013) study was the only orchid endophyte study that tested the effectiveness of their surface sterilization protocol. No microorganisms grew on media after imprinting the surface-sterilized tissues on agar, which indicated that their surface sterilization protocol was successful.

The isolation of non-mycorrhizal endophytes has involved teasing apart or crushing surface-sterilized root pieces or rhizomes aseptically to liberate hyphae on media or sterilized water (Lee Taylor & Bruns, 1999; Bougoure & Dearnaley, 2005). Cultivation of surface-sterilized segments on media has also been widely used for all orchid tissues to isolate endophytic fungi (Table 2.2).

Antibiotics were used in culture-dependent isolation to prevent bacterial contamination. Streptomycin sulphate and potassium penicillin G respectively restrained the growth of G^- bacteria and G^+ bacteria (Zhu et al., 2008). Sometimes researchers added several kinds of antibiotics to prevent contamination. Otero et al. (2002) applied streptomycin, tetracycline and penicillin together to prevent contamination. A study in which *Colletotrichum* species were isolated from *Bletilla ochracea* used streptomycin and chloramphenicol to prevent contamination (Tao et al., 2013).

Different protocols may be tried for isolating endophytic fungi. *Epulorhiza* fungi could be isolated using either single peloton or root section protocol and they grew more quickly when bacteria were present than if excluded (Ma et al., 2003; Zhu et al., 2008). Some mycorrhizal fungi, however, were isolated using root section because they did not form massive hyphal colonization (Otero et al., 2002; Otero et al., 2007; Liu et al., 2010; Nontachaiyapoom et al., 2010). Moreover, not all studies on orchid endophytes used isolated fungi as materials for fungal identification. Direct sequencing of DNA extracted from orchid tissues containing fungi has also revealed diversity of fungal endophytes. However it is necessary to emphasize that although some fungal specific primers are available, they do not necessarily amplify only fungal DNA. For example, primer ITS1F (Gardes & Bruns, 1993) is intended to be specific to fungi and it can also amplify DNA of many species of eu-dicots and some orchids (Taylor & McCormick, 2008). Therefore, analysis and interpretation of the results from such protocols must be treated with caution. Comparison of the sequences of fungal endophytes with sequences of well-characterized fungi in GenBank is necessary to name the species.

2.2.2 Identification of Fungal Endophytes

Fungi can be identified using morphology, molecular analysis or a combination of both approaches. In the past, fungal identification relied on morphological characteristics such as colony, mycelium and spore characters (Currah et al., 1987). However, molecular approaches have more recently been applied to identify orchid endophytes (Rasmussen, 2002). The use of combined morphological-molecular data is probably a better approach (Ko et al., 2011), but most studies on orchid endophytes have used either morphology or molecular analysis (Table 2.2). Only a few studies performed both (Chutima et al., 2011; Jiang et al., 2011; Tao et al., 2013).

Table 2.1 Protocols used for surface sterilization in orchid endophyte studies

Tissue	Protocol	Reference
Root	5% solution of "Domestos"(20-30 min) - sterilized water	Hadley (1970)
Root	0.1% $HgCl_2$ in 20% ethanol – sterilized distilled water (4~5 changes)	Smith et al. (1966)
Root	Several changes in sterile water	Warcup and Talbot (1967)
Root	20% "Milton" (15-20 min)	Warcup (1981)
Root	20% solution of household bleach (1 min) - sterile distilled water	Currah and Hambleton (1987)
Root	70% ethanol (30s) - 5.25% household leach (10 min)	Suarez et al. (2006)
Root	75% ethanol (35s) - 3% $NaClO_2$ (1 min) - 75% ethanol (30s)	Chen et al., (2013)
Root	75% ethanol (30s) - 0.5% $NaClO_2$ (3-5 min)	Jiang et al. (2011)
Root	70% ethanol (30s) - 95% ethanol, 5.25% $NaClO_2$, sterile H_2O_2 (1:1:1) (1 min)	Chutima et al. (2011)

Table 2.1 (continued)

Tissue	Protocol	Reference
Root	75% ethanol (1 min) - 3.4% NaClO (10 min) - 75% ethanol (30s)	Bayman et al. (1997)
Root	5.25% NaClO (1 min) - sterile water (2 times)	Zelmer and Currah (1996)
Root	70% ethanol, 2.5% NaClO (1 min) - 70% ethanol (1 min)	Otero et al. (2007)
Root	70% ethanol (30s) - 2.6% NaClO (3 min)	Alfaro et al. (2007)
Root	70% ethanol (1 min) - 2% NaClO (5 min)	Pereira et al. (2003)
Stem	75% ethanol (40s) - 4% NaClO (10 min)	Yuan et al., (2009)
Root, leaf	70% ethanol (30s) - 4% NaClO (90s)	Sawmya et al. (2013)
Root	95% ethanol (20s) - 5% NaClO (3 min)	Tondello et al. (2012)
Root	70% ethanol (2 min) - 10% NaClO (3 min)	Sebastian et al. (2012)
Root	70% ethanol (1 min) - NaClO with 1% available chlorine (1 min)	Yagame et al. (2008)
Rhizome	70% ethanol (30s) - NaClO with 1% available chlorine (30s)	Roy et al. (2009)
Root	30% H_2O_2 (1 min) - sterile water	Girlanda et al. (2006)
Root	3% H_2O_2 (10 min) - sterile distilled water (3 times)	Nontachaiyapoom et al. (2010)
Root	75% ethanol (30s) - 0.1% HgCl2 (5 min)	Zhang et al. (2012)
Root	70% ethanol (1-2 min) - 0.1% HgCl2 (7-8 min)	Zhao et al. (2014)
Root	Detergent solution (5-6 min) - 10% $Ca(ClO)_2$ (7-8 min)	Saha et al. (2006)

2.2.3 Morphological Identification

Even though endophytes can be directly visualized inside the tissues by staining (Hyde & Soytong, 2008), most studies of orchid endophytes did not use this method. Most fungal endophyte studies have adopted surface sterilized tissues which can be problematic because not all endophytic fungi grow in culture, or epiphytic fungi are not completely killed (Lee Taylor & Bruns, 1999). Orchid mycorrhizal fungi can be distinguished by hyphal coils (pelotons), however, many non-mycorrhizal fungal endophytes from orchids do not possess any specific characters or have some overlapping traits in culture (Otero et al., 2002; Selosse et al., 2004).

2.2.4 Problems with Identification of Orchid Endophytic Fungi Using Morphology

Morphological identification of orchid fungal endophytes to species or sometimes even genus level is not always possible (Ko et al., 2011). Many endophytic fungi will not sporulate, even if sporulation-inducing methods are applied (Sebastian et al., 2014). These include ectomycorrhizal fungi (ECM fungi) such as *Russula* (Paduano et al., 2011). Apart from the nature of fungi, morphological identification requires researchers to have a good understanding of basic fungal taxonomy and good skills in handling fungal cultures. Morphological identification may take more time than molecular identification, as endophytic fungi may need at least three to four weeks to sporulate (Bayman et al., 1997; Boddington & Dearnaley, 2008). However, employing morphological characterization to identify endophytes is less costly.

2.2.5 Molecular Identification

Molecular identification of orchid endophytes can be done using polymerase-chain reaction (PCR) to amplify a specific DNA region and subsequently cleave the PCR product using specific restriction endonucleases (i.e. PCR-RFLP) (Bougoure et al., 2005; Pereira et al., 2005; Girlanda et al., 2006; Boddington & Dearnaley, 2008). However, the more commonly used molecular identification is sequence-based approach by which a selected DNA region is sequenced. Then the DNA sequence can be blasted in the public database (e. g. GenBank) and/or used to construct a phylogenetic tree (Table 2.2). Selection of genes/regions for molecular identification is particularly important (Boddington & Dearnaley, 2008). The ITS region (i.e. internal transcribed spacers of the rDNA gene or ITS1-5.8S rDNA-ITS2) is the region of choice because of its high degree of variation and the fact that it is the most common sequence generated (Lee Taylor & Bruns 1999,; Schoch et al., 2012). Therefore, applying ITS sequences approaches to identify fungi increases the possibility to find out similar or homologous sequences. For example, 66 distinct OTUs were isolated from *Pseudorchis* *albida* and identified through only ITS sequencing and phylogenetic analysis (Kohout et al., 2013).

However, using ITS region alone for identification of some groups of fungi is not adequate. As a result, multiple gene loci are usually sequenced (Abadie et al., 2006; Bidartondo et al., 2004). Besides the ITS, regions of DNA that have been used in sequence-based identification of orchid fungal endophytes includes the nuclear coding regions, i.e., 28S rDNA, β-tubulin (TUB2), glycerdalehyde-3-phosphate dehydrogenase (GAPDH), actin (ACT), and the mitochondrial large subunit rDNA (mt-LSU-rDNA) (Table 2.2). For example, the ITS region alone compared with the combination of ITS, TUB2 and tef1 gave relatively poor species resolution in identification of *Pestalotiopsis* species (Maharachchikumbura et al., 2012) isolated from *Dendrobium nobile* (Chen et al., 2011) and *Pholidota pallida* , as well as *Fusarium* from *Pecteilis susannae* and *Cattlteya skinneri* (Ovando et al., 2005; Chutima et al., 2011; Hyde et al., 2014). For identification of fungal endophytes from *Pholidota pallida* to generic level, the ITS region was used, however, the ITS region combined with TEF, GPDH and ACT were used for inter-specific distinction (Sawmya et al., 2013; Hyde et al., 2014). Similarly, ITS in combination with mt-LSU-rDNA were used to identify endophytes from *Habenaria radiata, Epipactis thunbergii* and six species of *Chiloglottis* (Roche et al., 2010; Cowden & Shefferson, 2013). Huang et al. (2014) reported that sequencing multiple barcodes of fungi from *Phalaenopsis microbiome* using next-generation sequencing gave much higher fungal diversity than that sequencing nuclear-ITS alone.

2.2.6 Problems with Identification of Orchid Endophytes Using Molecular Method

However, there are several disadvantages in relying on molecular methods for identifying endophytes including low quality and misidentification of a large number of ITS sequences in GenBank. These problems are now being addressed (Ko et al., 2011). For example, Cai et al. (2009) compared ITS sequences of ex-type specimens of *Colletotrichum* with the sequences in GenBank and reported that the majority of *Colletotrichum* ITS sequences in GenBank are wrongly named. Recently, some researchers advocated applying sequences of fungal ex-type for constructing phylogenetic backbone which may avoid improper identification (Ko et al., 2011; Hyde et al., 2014). Furthermore, some fungal specific primers may fail to amplify DNA of some fungi. The fungal primers ITS1F and ITS4 cannot efficiently amplify DNA of fungal species in the family Tulasnellaceae (Pecoraro et al., 2012). Therefore, although in most of the cases morphological identification or molecular identification alone is adequate, it is best, where possible, to use both morphological and molecular methods. We recommend a combination of two methods for endophytic identification in orchids. This is because endophytes may contain some fungal taxa that grow vigorously on media but others are only revealed when molecular methods are applied.

2.3 Diversity of Orchid Non-mycorrhizal Endophytes

The orchid non-mycorrhizal endophytic fungi contain over 110 genera, which are more diverse than mycorrhizal endophytes (Table 3). At least 39 genera of Sordariomycetes (i. e. *Cylindrocarpum, Hypocrea, Nigrospora, Pestalotiopsis*) (Chen et al., 2011; Sommer et al., 2012; Pecoraro et al., 2013), 25 genera of Dothideomycetes (i. e. *Alternaria, Cercospora, Lasiodiplodia, Phyllosticta*) (Tao et al., 2008; Liu et al., 2010; Sawmya et al., 2013), 12 genera of Leotiomycetes (i. e. *Chaetomella, Sclerotinia*) (Chen et al., 2011; Sommer et al., 2012) in Ascomycota and 32 genera of Agaricomycetes in Basidiomycota (i. e. *Conocybe, Gymnopus, Hydropus, Psathyrella, Resinicium*) (Gura-Tsujita & Yukawa, 2008; Martos et al., 2009) have been reported as orchid non-mycorrhizal endophytic fungi. They also involve a few species of Pezizomycetes (i. e. *Geopora*) (Shefferson et al., 2008), Eurotiomycetes (i. e. *Talaromyces*)44, Chaetothyriomycetes (i. e. *Exophiala*) (Shefferson et al., 2008), Helotiales and Xylariales of ascomycetes (i. e. *Nemania*) (Yuan et al., 2009) in Ascomycota and Tremellomycetes (i. e. *Cryptococcus*) (Tao et al., 2008) as well as Pucciniomycetes (i. e. *Tuberculina*) (Vendramin et al., 2010) in Basidiomycota. Orchid non-mycorrhizal fungi related to Chytridiomycota (i.e. *Olpidium*) (Roy et al., 2009b), Glomeromycota (Cowden & Shefferson, 2013) and Zygomycota (i. e. *Umbelopsis*) (Zhao et al., 2014) have also been reported. Among all genera observed in orchid non-mycorrhizal fungi, *Colletotrichum* and *Fusarium* frequently appeared in different orchids such as *Satyrium nepalense* and *Dendrobium nobile* (Jyothsna &

Purushothama, 2014; Yuan et al., 2009). *Aspergillus, Trichoderma* and *Verticillium* have also been repeatedly found in orchids (Bayman et al., 1997; Kasmir et al., 2011) (Table 2.3).

Since the traditional protocol of surface sterilization has a great influence on the fungal endophytes obtained, it is possible that some surface contaminants could be mistakenly identified as orchid non-mycorrhizal endophytes. This may be particularly true for species of *Aspergillus, Penicillium* and *Cladosporium* which are common surface contaminants (Hyde & Soytong, 2008) as well as *Trichoderma hamatum* and *Verticillium* sp., which are soil-dwelling fungi but reported as fungal endophytes from orchids (Kasmir et al., 2011; Salifah et al., 2011; Sommer et al., 2012).

Table 2.2 Orchid non-mycorrhizal endophytes

Orchids	**Organs**	**Orchid growth habit**	**Orchid habitat**	**Isolation method**	**Identification method**	**No. taxa or OTUs**	**Fungal trophic group**	**Reference**
Aa achalensis	Root	Terrestrial (photosynthetic)	Natural habitat, west Argentina	From surface-sterilized tissues	Morphology, ITS	1	Endophytes	Sebastian et al. (2014)
Acampe praemorsa	Root, leaf	Epiphytic (photosynthetic)	Similipal Biosphere Reserve Odisha, India	From surface-sterilized tissues	ITS	6	Endophytes	Behera et al. (2013)
Acianthus pusillus	Root	Terrestrial (photosynthetic)	Open forests, Australia	Crush peloton from surface-sterilized tissues	ITS-RFLP, ITS	2	Endophytes	Bougoure et al. (2005)
Aphyllorchis caudata	Root	Terrestrial (mycoheterotrophic)	Evergreen forest, Thailand	No isolation	ITS, 28S, or mt-LSU-rDNA	11	ECM fungi endophyte	Roy et al. (2009a)
Aphyllorchis montana	Root	Terrestrial (mycoheterotrophic)	Forests in Thailand	No isolation	ITS, 28S, or mt-LSU-rDNA	4	ECM fungi, endophytes and saprobes	Roy et al., (2009a)
Bletilla ochracea	Leaf	Terrestrial (photosynthetoc)	Mountains (1-1.6 km high), China	From surface-sterilized tissues	Morphology, ITS, TUB2, ACT, GAPDH	17	Endophytes	Tao et al. (2013)
Bletilla ochracea	Root, leaf	Terrestrial (photosynthetic)	Mountain (1310 m high), China	No isolation	ITS, cloning	17	Endophytes	Tao et al. (2008)
Bulbophyllum kaitiense	Root	Epiphytic or lithophytic (photosynthetic)	Kolli hills (80 – 869 m high), India	From surface-sterilized tissues	Morphology, ITS	2	Endophyte	Kasmir et al. (2011)

Table 2.2 (continued)

Orchids	Organs	Orchid growth habit	Orchid habitat	Isolation method	Identification method	No. taxa or OTUs	Fungal trophic group	Reference
Bulbophyllum neilgherense	Mature bulb and aerial root	Epiphytic (photosynthetic)	River Kali, Kaiga forest, India	From surface-sterilized tissues	Morphology	17	Endophytes	Sudheep and Sridhar (2012)
Bulbophyllum neilgherrense	Root, leaf	Epiphytic (photosynthetic)	Natural forest and greenhouse, India	From surface-sterilized tissues	ITS	14	Endophytes	Sawmya et al. (2013)
Caladenia (8 species)	Stem-collar	Terrestrial (photosynthetic)	Roadside, open forests, woodland, and heath, swamp, SWAFR	No isolation	ITS	1	Endophyte	Sommer et al. (2012)
Caladenia carnea	Root	Terrestrial (photosynthetic)	Helidon Hills, Australia	Crush peloton from surface-sterilized tissues	ITS-RFLP, ITS	1	Endophyte	Bougoure et al. (2005)
Cattlteya skinneri	Root	Terrestrial (mixotrophic)	Botanical Gardern, southern Mexico and orchidarium, Santo Domingo	From surface-sterilized tissues	Morphology	10	Endophytes	Ovando et al. (2005)
Cephalanthera (2 species)	Root	Terrestrial (photosynthetic)	ECM forests, northeast Bavaria, Germany	No isolation	ITS , 28S, mtLSU-rDNA	6	ECM fungi	Bidartondo et al. (2004)
Cephalanthera exigua	Root	Terrestrial (mycoheterotrophic)	Evergreen forest, Thailand	No isolation	ITS, 28S, or mt-LSU-rDNA	6	ECM fungi, saprobes (11%).	Roy et al. (2009b)
Cephalanthera longifolia	Root	Terrestrial (mixotrophic)	Calcareous coastal plain at Pussa, west of Estonia	No isolation	ITS, ITS-RFLP	12	ECM fungi, endophytes, saprobes	Abadie et al. (2006)
Chamaegastrodia sikokiana	Rhizome	Terrestrial (nonphotosynthetic)	Coniferous (350 m high) and evergreen broad-leaved forest (780 m high), Japan	Crush peloton from surface-sterilized tissues	ITS	1	ECM fungi	Yagame et al. (2008)
Changnienia amoena	Rhizome	Terrestrial (nonphotosynthetic)	Indian cedar forest and broad leaved forests in ZJ, HB, AH, China	From surface-sterilized tissues	Morphology, ITS	17	Endophytes	Jiang et al. (2011)
Chiloglottis (6 species)	Tuber, rhizome	Terrestrial (photosynthetic)	Moist, sheltered 5 places in NSW, 1 place in ACT, Austrilia	From surface-sterilized tissues	ITS, mt-LSU	3	Endophytes	Roche et al. (2010)
Corallorhiza maculata	Root or rhizome	Terrestrial (mycoheterotrophic)	CA, WA, OR, OH, WI in USA	Single peloton	ITS-RFLP; ITS	23	ECM fungi	Taylor and Bruns (1999)

Table 2.2 (continued)

Orchids	Organs	Orchid growth habit	Orchid habitat	Isolation method	Identification method	No. taxa or OTUs	Fungal trophic group	Reference
Corallorhiza mertensiana	Root or rhizome	Terrestrial (mycoheterotrophic)	Sonoma, WA, OR, UT, Tehama in USA	Single peloton	ITS-RFLP; ITS	3	ECM fungi	Taylor and Bruns (1999)
Corybas recurvus	Root, stem	Terrestrial (photosynthetic)	Open heath, SWAFR	No isolation	ITS	1	Endophyte	Sommer et al. (2012)
Cryptostylis ovata	Root	Terrestrial (photosynthetic)	Granite outcrop, SWAFR	No isolation	ITS	1	Endophyte	Sommer et al. (2012)
Cymbidium aloifolium	Root, leaf	Epiphytic or lithophytic (photosynthetic)	Similipal Biosphere Reserve Odisha, India	From surface-sterilized tissues	ITS	3	Endophytes	Hadley (1970)
Cymbidium spp.	Root	Terrestrial (mixotrophic)	Southwest of China	From surface-sterilized tissues	ITS	3	Endophytes	Zhao et al. (2003)
Cypripedium (7 species)	Root	Terrestrial (photosynthetic)	Baltic coast in Estonia, forests in the USA	No isolation	ITS, 28S, mt-LSU, RFLP	6	Parasites	Shefferson et al. (2008)
Dendrobium (10 species)	Root, stem, leaf	Epiphytic (photosynthetic)	580~1200 m above sea level in Guizhou, Yunnan, China	From surface-sterilized tissues	Morphology, ITS	80	Endophytes	Chen et al. (2011)
Dendrobium (7 species)	root	Epiphytic (photosynthetic)	Tropical rainforest in Xishuangbanna, China	From surface-sterilized tissues	Morphology, ITS, 28S, TUB2	18	Endophytes	Chen et al. (2013)
Dendrobium nanum	Root	Epiphytic (photosynthetic)	Kolli hills (80 – 869 m high), India	From surface-sterilized tissues	Morphology, ITS	2	Endophyte	Kasmir et al. (2011)
Dendrobium nobile	Root, stem, leaf	Epiphytic (photosynthetic)	Trunks or branches of standing trees, Yunnan, China	From surface-sterilized tissues	Morphology, ITS	33	Endophytes, saprobes	Yuan et al. (2009)
Dirus (2 species)	Root	Terrestrial (photosynthetic)	Open and closed forests, SWAFR	No isolation	ITS	1	Endophyte	Sommer et al. (2012)
Epipactis (4 species)	Root	Terrestrial (photosynthetic)	ECM forest and wetland, Germany	From surface-sterilized tissues and no isolation	ITS , 28S, mtLSU-rDNA	6	ECM fungi	Bidartondo et al. (2004)
Epipactis atrorubens	Root	Terrestrial (photosynthetic)	Meadow, ash hill, coast and forest, Estonia.	No isolation	ITS, 5.8S, mt-LSU, ITS-RFLP	4	ECM fungi, parasite	Shefferson et al. (2008)

Table 2.2 (continued)

Orchids	Organs	Orchid growth habit	Orchid habitat	Isolation method	Identification method	No. taxa or OTUs	Fungal trophic group	Reference
Epipactis microphylla	root	Terrestrial (mycoheterotrophic)	Alps (930 m), forest (90 m), Mont Maurice (910 m), France	No isolation	ITS, 28S, cloning-RFLP	7	ECM fungi	Selosse et al. (2004)
Epipactis thunbergii	Root	Terrestrial (photosynthetic)	Banks of bogs and drainage ponds,Japan	No isolation	ITS, mt-LSU	1	Endophytes	Cowden et al. (2013)
Epiplema grandiflorum	Root	Terrestrial (photosynthetic)	Open shrub, SWAFR	No isolation	ITS	1	Endophyte	Sommer et al. (2012)
Epipogium aphyllum	Rhizome	Terrestrial (mycoheterotrophic)	France (45°N, 02°~04°E), Russia (51°N, 82°~102°) and Japan (35°N, 138°E)	Crush peloton from surface-sterilized tissues	Morphology, ITS, 28S, ITS-RFLP	25	ECM fungi, parasites, endophytes, saprobes	Roy et al. (2009a)
Eulophia alta	Root	Terrestrial (mycoheterotrophic)	National Wildlife Refuge, Avon Park, Florida, USA	From surface-sterilized tissues	Morphology	3	Endophyte	Johnson et al. (2007)
Eulophia zollingeri	Root	Terrestrial (mycoheterotrophic)	Japan, Myanmar, Peninsula, Taiwan	No isolation	ITS	1	Endophyte	Tsujita et al. (2008)
Gastrochilus acaulis	Root	Terrestrial (photosynthetic)	Kolli hills (80 – 869 m high), India	From surface-sterilized tissues	Morphology, ITS	1	Endophyte	Kasmir et al. (2011)
Gastrodia confusa	Root	Terrestrial (nonphotosynthetic)	Dense bamboo forests (5–1000 km apart), Japan	No isolation	ITS, 28S	5	Saprobes	Tsujita et al. (2009)
Gastrodia similis	Root	Terrestrial (mycoheterotrophic)	Rainforests and second-growth forest, France	Single peloton	ITS, 28S	7	Saprobes and endophytes	Martos et al. (2009)
Geodorum densiflorum	Root	Terrestrial (photosynthetic)	Kolli hills (80 – 869 m high), India	From surface-sterilized tissues	Morphology, ITS	1	Endophyte	Kasmir et al. (2011)
Grammatophyllum (3 species)	Root	Terrestrial (photosynthetic)	Mount Kinabalu, Malaysia	From surface-sterilized tissues	Morphology	28	Endophytes, parasitism fungi	Salifah et al. (2011)
Habenaria radiata	Root	Terrestrial (photosynthetic)	Banks of bogs and drainage ponds, Japan	No isolation	ITS, mt-LSU	1	Endophytes	Cowden et al. (2013)
Himantoglossum adriaticum	Root	Terrestrial (photosynthetic)	Dry calcareous grassland (969–1047 m), Italy	No isolation	ITS, cloning	4	Endophytes	Pecoraro et al. (2013)

Table 2.2 (continued)

Orchids	Organs	Orchid growth habit	Orchid habitat	Isolation method	Identification method	No. taxa or OTUs	Fungal trophic group	Reference
Holcoglossum (9 species)	Root	Epiphytic (photosynthetic)	Yunnan, Guangxi and Hainan, China	From surface-sterilized tissues	ITS	15	Endophytes	Tan et al. (2012)
Lepanthes (7 species)	Root and leaf	Epiphytic and lithophytic (mixotrophic)	Rainforests, Puerto Rico	From surface-sterilized tissues	Morphology	10	Saprobes, endophytes	Bayman et al. (1997)
Limodorum abortivum	Root	Epiphytic (mixotrophic)	Woodlands (1420 m) in France and Italy	From surface-sterilized tissues	Morphology, cloning, ITS-RFLP	8	ECM fungi, mycobionts, endophytes	Girlanda et al. (2006)
Microtis (5 species)	Root	Terrestrial (photosynthetic)	Granite outcrop, swamp, open forests, SWAFR	No isolation	ITS	3	Endophytes	Sommer et al. (2012)
Neottia nidus-avis	Root	Terrestrial (mycoheterotrophic)	8 regions (90 - 1400m high) in France	No isolation	ITS, ITS-RFLP	12	ECM fungi, Endophytes	Selosse et al. (2002)
Orchis militaris	Root	Terrestrial (photosynthetic)	Flowering stage, hills, Italy	No isolation	ITS	3	ECM fungi, endophytes	Vendramin et al. (2010)
Orchis tridentata	Root, tuber	Terrestrial (photosynthetic)	Mountain, poor grasslands on calcareous soil, Italy	From surface-sterilized tissues	Morphology, ITS-cloning	9	Endophytes	Pecoraro et al. (2012)
Paracaleana nigrita	Stem-collar	Terrestrial (photosynthetic)	Open shrub, SWAFR	No isolation	ITS	2	Endophytes	Sommer et al. (2012)
Pecteilis susannae	Root	Terrestrial (photosynthetic)	Three field sites in Chiang Mai, Thailand	From surface-sterilized tissues	Morphology, ITS	1	Endophytes	Chutima et al. (2011)
Pholidota pallida	Root, leaf	Terrestrial (photosynthetic)	Natural forest and greenhouse, India	From surface-sterilized tissues	ITS	10	Endophytes	Sawmya et al. (2013
Platanthera chlorantha	Root	Terrestrial (photosynthetic)	Ectomycorrhizal forest, northeast Bavaria, Germany	No isolation	ITS , 28S, mt-LSU-rDNA	2	ECM fungi	Bidartondo et al. (2004
Platanthera minor	Root	Terrestrial (mixotrophic)	9 regions aititude from 90~810 m, Japan	Crush coils from surface-sterilized tissues	ITS, 28S	6	Endophytes, ECM fungi	Yagame et al. (2012)
Prasophyllum (3 species)	Root	Terrestrial (photosynthetic)	Open forests, SWAFR	No isolation	ITS	3	Endophytes	Sommer et al. (2012)
Pseudorchis albida	Root	Terrestrial (photosynthetic)	Mountain meadows, park, Czech	From surface-sterilized tissues	ITS	66	Endophytes, pathogenic fungi, saprobes	Kohout et al. (2013)

Table 2.2 (continued)

Orchids	Organs	Orchid growth habit	Orchid habitat	Isolation method	Identification method	No. taxa or OTUs	Fungal trophic group	Reference
Satyrium nepalense	Root, tuber	Terrestrial (autotrophic)	Grass hilly slopes (600-4600 m), India	From surface-sterilized tissues	Morphology, ITS	1	Saprobes, endophytes	Jyothsna and Purushothama (2014)
Spiranthes spiralis	Root	Terrestrial (photosynthetic)	The Euganean Hills, Italy	From surface-sterilized tissues	ITS	8	Endophytes	Tondello et al. (2012)
Thelymitra (3 species)	Root	Terrestrial (photosynthetic)	Open forests, granite outcrop, roadside, SWAFR	No isolation	ITS	3	Endophytes	Sommer et al. (2012)
Vanda testacea	Root, leaf	Epiphytic (photosynthetic)	River Kali, Kaiga forest, India	From surface-sterilized tissues	Morphology	20	Endophytes, entomopathogenic fungi	Sudheep and Sridhar (2012)
Vanda testacea	Root, leaf	Epiphytic (photosynthetic)	Similipal Biosphere Reserve Odisha, India	From surface-sterilized tissues	ITS	5	Endophytes	Behera et al. (2013)
Wullschlaegelia aphylla	Root, rhizome	Terrestrial (mycoheterotrophic)	Rainforests, Guadeloupe, France	Single peloton	ITS, 28S	11	Saprobes, endophytes	Martos et al. (2009)

Note*Abbreviation in column “Identification method”: nrDNA-Nuclear ribosomal DNA; ITS-Internal transcribed spacer; 5.8S rDNA-5.8S ribosomal DNA; 28S rDNA-28S ribosomal DNA; mt-LSU- rDNA-Mitochondrial large subunit ribosomal DNA; nr-LSU-Nuclear ribosomal large subunit; TUB2-β-tubulin; GAPDH-glyceraldehyde-3-phosphate dehydrogenase; ACT-actin; RFLP-Restriction fragment length polymorphism. Abbreviation in column “Orchid habitat”: NSW-New South Wales; ACT-Australian Capital Territory; ZJ-Zhe Jiang province; HB-Hu Bei province; AH-An Hui province; CA-California; WA-Washington; OR-Ore, OH-Ohio; WI-Wisconsin; UT-Utah; SWAFR-Southwest Australian Floristic Region. Most of No. taxa or OTUs were counted by fungal species and less by genera according to references.

2.4 Specificity and Factors Affect Fungal Diversity

Host-specificity between orchids and their non-mycorrhizal endophytic fungi has been less-well studied as compared to their biodiversity. Endophytes of certain nonphotosynthetic orchids appeared to be more specific than in green photosynthetic orchids (Shefferson et al., 2005). *Psathyrella candolleana* is specific to the mycoheterotrophic orchid *Eulophia zollingeri* (Martos et al., 2009). However, fungal specificity could be observed in some photosynthetic orchids. The photosynthetic orchids like *Dendrobium* spp. have frequent associations with fungi in Xylariaceae (Yuan et al., 2009; Chen et al., 2011; Chen et al., 2013). *Grammatophyllum speciosum* was reported to be colonized by *Fusarium* and *Trichoderma* (Salifah et al., 2011). Endophytic fungi isolated from another photosynthetic orchid - *Orchis militaris* were found to be host-specific (Vendramin et al., 2010). Specificity was also observed in mycoheterotrophic orchids. Thirteen different taxa each occurred only on a single sample in the study of endophytes from the mycoheterotrophic orchid *Aphyllorchis montana* (Roy et al., 2009a).

Orchid tissues used for the fungal endophyte study also affect the diversity of non-mycorrhizal endophytes. The diversity of non-mycorrhizal endophytic fungi in orchids is higher in leaves than in roots (Sudheep & Sridhar, 2012; Chen et al., 2013). Tao et al. (2008) found that few endophytic fungi in roots and leaves of *Bletilla ochracea* overlapped. They pointed out that orchid leaves and roots had different endophyte associations and speculated that this was probably because the organ texture provided different ecological habitats (air or below ground) with varying physiology and chemistry for the taxa (Tao et al., 2008; Barman & Devadas, 2013).

The diversity of orchid non- mycorrhizal endophytic fungi probably depends also on the localities from where the orchids were collected. Sudheep and Sridhar reported that relatively similar endophytic fungal assemblages were isolated from distantly related orchids *Vanda testace* and *Bulbophyllum neilgherrense* sampled in the same habitat, the Kaiga forest of the Western Ghats. There was no overlap in taxa of non-mycorrhizal endophytic fungi isolated from individuals of *Epipactis atrorubens* sampled respectively at a meadow in a coastal farm and at Ash Hill (Shefferson et al., 2008). Bunch et al. (2013) found that fungal endophytes in *Cypripedium acaule* were greatly influenced by geography and soil. Therefore, when studying orchid non-mycorrhizal endophytic fungi, sampling at different niches will help understand their fungal ecology. Furthermore, as climate change occurs, this may alter orchid niches by impacting their surroundings such as soil moisture and rainfall (Barman & Devadas, 2013). Endophyte diversity in plants may also be affected by insect-induced galls which can change fungal colonization and diffusion (Lawson et al., 2014).

2.5 Groups of Non-mycorrhizal Endophytes from Orchids

Orchid non-mycorrhizal endophytes can be classified into several groups according to their lifestyles, i. e. ECM fungi, saprobes, parasites and latent pathogens (Table 2.2). However, fungal lifestyles are not always stable traits. Some endophytic fungi can switch to a necrotrophic lifestyle at an ecological timescale (Delaye et al., 2013). Further studies on the evolution of endophytic fungi at the gene and ecological levels need to be carried out to explore their roles in orchids (Yagame et al., 2008).

2.5.1 Ecto-mycorrhizal Fungi

Roots of many mycoheterotrophic orchids with internal hyphal coils of saprotrophic fungi, were found to be associated with ECM Ascomycota (e.g. Terfeziaceae, Saroscyphaceae) (Selosse et al., 2002) and/or ECM Basidiomycota (e.g.Russulaceae, Thelephoraceae, Clavulinaceae, and Sebacinaceae) (Lee Taylor & Bruns, 1999; Selosse et al., 2002; Selosse et al., 2004; Roy et al., 2009a) of trees and shrubs. ECM symbiosis has long been understood as the way orchids derive carbon from the surrounding ectomycorrhizal trees. This hypothesis was later verified by the study of McKendrick et al. (2000) who used $^{14}CO_2$ to track the transfer of carbon from ectomycorrhizal tree seedlings via hyphal connections to the mycoheterotrophic orchid *Corallorhiza trifida* in the field that was later confirmed by other studies (Gebauer & Meyer, 2003; Roy et al., 2009b). Interestingly, photosynthetic orchids were also found to be associated with ECM fungi in the roots (Bidartondo et al., 2004; Bidartondo & Read, 2008; Vendramin et al., 2010; Yagame et al., 2012) and partial exploiters of fungal carbon (Bidartondo et al., 2004; Roy et al., 2009b; Yagame et al., 2012). The degree of specificity between orchids and their ectomycorrhizal partners, therefore, largely but not entirely depends on the degree of dependency of orchids on the fungal carbon. Achlorophyllous orchids and species with inefficient photosynthesis were reported to be specifically associated with narrow groups of ECM fungi including *Russula* (Girlanda et al., 2006; Selosse et al., 2002), whereas, chlorophyllous orchids were associated with wide range of ECM fungi (Bidartondo & Read, 2008). However, Roy et al. (2009a) studied orchid-fungal associations in tropical regions and revealed the absence of specificity in two and the presence of specificity in one mycoheterotrophic species.

The role of ECM fungi in orchids, however, is probably not limited to carbon transport. It has been speculated that mycorrhizal networks increase the bioactive zones of infochemicals by serving as the direct connecting superhighways for plants to communicate underground (Barto et al., 2012). Even though, arbuscular mycorrhizal fungi are presently the only group of fungi that have been proven to transport compounds between multiple plant species through common hyphal networks (Barto et al., 2011), it will be interesting to investigate if ECM fungi have this role in orchids.

2.5.2 Saprobic Fungi

Many saprobic species of Agaricomycetes (i.e. *Hydropus, Gymnopus, Marasmiellus*) (Ogura-Tsujita et al., 2009) and Sordariomycetes (i.e. *Clonostachys, Resinicium*) (Yuan et al., 2009) have been identified as orchid non-mycorrhizal endophytic fungi. Endophytes are important saprobic decomposers (Hyde and Soytong 2008). Gymnopoids and mycenoids saprobes isolated from mycoheterotrophic orchids *Gastrodia similis* have been reported to secrete laccases and peroxidases (Selosse et al., 2004; Valášková et al., 2007; Martos et al., 2009). *Resinicium* spp. living in *Gastrodia similis* are also wood-decaying fungi (Martos et al., 2009). *Lasiosphaeria* spp. found in the photosynthetic orchid *Habenaria radiataare* are important ligninolytic saprotrophs (Miller & Huhndorf, 2004; Cowden & Shefferson, 2013).

2.5.3 Latent Pathogen

Some of the non-mycorrhizal endophytes are plant pathogens. For example, *Fusarium oxysporum* can cause plant wilt and rot diseases (Baayen et al., 2000). *Alternaria, Aspergillus, Chaetophoma* and *Trichoderma* have relationships with cotton plant disease (Lutfunnessa & Shamsi, 2011). *Xylaria* is well-known pathogen from decaying plant organs (Malcolm et al., 2013). *Paeciliomyces* sp. isolated from *Vanda testacea* is also reported as an entomopathogen (Sudheep & Sridhar, 2012).

Latent pathogens in plants have been noticed from 1950s (Gäumann, 1951). They may exist as endophytes and probably become pathogens during a later period of life, especially when plants are stressed (Shefferson et al., 2008). Some *Colletotrichum* species are pathogens of orchids such as *Oncidium flexuosum*, *Bulbophylum cylindrum*, *Coelogyne cristata* (Tao et al., 2013; Yang et al., 2011), while they have also been isolated as endophytes from healthy orchids, such as species in Lepanthes, Dendrobium (Bayman et al., 1997; Chen et al., 2011). In fact, endophytes in plant stems and leaves can switch from latent pathogens to mutualistic symbionts (Carroll, 1988). Freeman and Rodriguez (Freeman & Rodriguez, 1993) found that non-pathogenic and pathogenic strains in plants can restrict the growth of each other and mutualists may also be pathogens. Orchids at different life stages perhaps carry latent pathogens to different extents because all plants have been found potentially infected by endophytes and when competition for energy occurs between plants and fungi, plants may tend to be more susceptible to the pathogens (Saikkonen et al., 2004). Furthermore, some well-known virulent taxa such as *Fusarium* species, which are often isolated from orchids, tend to be asymptomatic endophyte rather than pathogen under optimal growth conditions (Pecoraro et al., 2012). Therefore, although we speculate that latent pathogens exist in orchids, only further investigations can identify their roles in host tissues.

2.6 Roles of Non-mycorrhizal Endophytes in Orchids

The role of orchid non-mycorrhizal endophytes has rarely been addressed. In general plant, endophytes are thought to be resources for bioactive compounds. For example, a *Trichoderma* species from Cupressaceae was shown to have antimicrobial properties (Hosseyni-Moghaddam & Soltani, 2014). Screening bioactive compounds for disease treatment from higher plants has increased (Aly et al., 2010). Potential pharmaceutically important substances are abundant in orchids and this to some extent may be a result of extreme diversity of non-mycorrhizal fungal metabolites. *Alternaria* sp. and *Fusarium oxysporum* isolated from orchids in Brazil showed strong inhibition to *Escherichia coli* (Vaz et al., 2009). From the orchid *Anoectochilus setaceus*, an antibacterial nortriterpenoid-helvolic acid was extracted from the endophytic taxon - *Xylaria* sp.101. These orchid non- mycorrhizal endophytes may occur in other plants and possibly be involved in production of bioactive compounds. Golgo et al. (2014) screened bioactive metabolites from *Hypocrea* spp. isolated from *Dillenia indica*.(Gogoi et al., 2008) *Hypocrea* species have also been isolated from orchids, such as *Wullschlaegelia aphylla* and *Himantoglossum adriaticum* (Martos et al., 2009; Bidartondo & Read, 2008). Xu et al. (2014) reviewed that approximately 160 metabolites isolated from *Pestalotiopsis* species were found to have anti-tumor, anti-fungal or anti-microbial potential. This perhaps gives hope for decreasing pressure for the huge requirement for Taxol as the anti-tumor drug is restricted to yew trees (Heinig et al., 2013).

Besides highly bioactive alternatives, Hou and Guo (2009) showed that dark septate endophytes isolated from *Dendrobium* and *Leptodontidium* spp., interacted with the seedlings of *Dendrobium nobile* in a manner similar to that of orchid mycorrhizal fungus. The endophyte formed peloton-like structures in cortical cells of the orchid and greatly enhanced the growth and biomass of the orchid seedlings. Non-mycorrhizal *Fusarium* was reported to promote seed germination in *Cypripedium* and *Platanthera* orchids, even though the effect was relatively minor when compared to that of specific orchid *Rhizoctonia mycorrhiza* (Vujanovic et al., 2000). Similarly, *Umbelopsis nana* isolated from *Cymbidium* spp. has a vigorous effect on development of *Cymbidium hybridum*, enhancing K, Ca, Cu, Mn contents in symbiotic plantlets (Gebauer & Meyer, 2003). Researchers detected fuel potential in volatile organic compounds isolated from Phomopsis sp. from orchid Odontoglossum sp. (Suryanarayanan et al., 2012).

Table 2.3 Mycorrhizal and non-mycorrhizal endophyte genera from orchids

Orchid Mycorrhizal	**Non-mycorrhizal**	**Non-mycorrhizal**	**Non-mycorrhizal**	**Non-mycorrhizal**	**Non-mycorrhizal**	**Non-mycorrhizal**	**Non-mycorrhizal**
Ceratobasidium (anamorph:*Ceratorhiza*)	*Acephala*	*Cladophialophora*	*Entonaema*	*Hebelom*	*Marasmiellus*	*Paraconiothyrium*	*Schizophyllum*
Coprinus	*Acremonium*	*Cladosporium*	*Exidia*	*Helicomyces*	*Meliniomyces*	*Penicillium*	*Sclerotinia*
Lactarius	*Alternaria*	*Clonostachys*	*Exophiala*	*Humicola*	*Menispora*	*Periconiella*	*Scytalidium*
Leptodontidium	*Ampelomyces*	*Cochliobolus*	*Fusarium*	*Hyalodendron*	*Merismodes*	*Pestalotia*	*Steccherinum*
Moniliopsis	*Amphinema*	*Colletotrichum*	*Fusicoccum*	*Hydropus*	*Metarhizium*	*Pestalotiopsis*	*Stephanonectria*
Sebacina	*Annulohypoxylon*	*Conocybe*	*Galactomyces*	*Hymenogaster*	*Mortierella*	*Pezicula*	*Strumella*
Thanatephorus (anamorph*:Rhizoctonia*)	*Armillaria*	*Cortinarius*	*Geomyces*	*Hypocrea*	*Mycosphaerella*	*Phaeosphaeria*	*Talaromyces*
Trichosporiella	*Arthrinium*	*Cosmospora*	*Geopora*	*Hypoxylon*	*Myrmecridium*	*Phialophora*	*Terfezia*
Tuber	*Ascobolus*	*Cryptococcus*	*Geotrichum*	*Laccaria*	*Nectria*	*Phoma*	*Thelephora*
Tulasnella (anamorph:*Epulorhiza*)	*Aspergillus*	*Cryptosporiopsis*	*Gibberella*	*Lachnum*	*Nemania*	*Phomopsis*	*Tomentella*
	Aureobasidium	*Curvularia*	*Gliocladium*	*Lasiodiplodia*	*Neonectria*	*Phyllosticta*	*Trechispora*
	Bionectria	*Cylindrocarpon*	*Gloeophyllum*	*Lasiosphaeria*	*Nigrospora*	*Pleospora*	*Trichoderma*
	Botrytis	*Daldinia*	*Glomerularia*	*Leohumicola*	*Nodulisporium*	*Podospora*	*Umbelopsis*
	Candida	*Davidiella*	*Guignardia*	*Lepiota*	*Oidiodendron*	*Protoventuria*	*Varicosporium*
	Cercophora	*Didymella*	*Gymnomyces*	*Leptosphaeria*	*Olpidium*	*Psathyrella*	*Verticillium*
	Chaetomella	*Dioszegia*	*Gymnopus*	*Leptosphaerulina*	*Paecilomyces*	*Pseudogymnoascus*	*Wilcoxina*
	Chaetomium		*Halocyphina*	*Macowanites*	*Paneolus*	*Resinicium*	*Xylaria*

Table 2.4 Orchid genera in endophytic research

Orchid Genera	Reference	Orchid Genera	Reference	Orchid Genera	Reference
Aa	Sebastian et al. (2014)	*Dryadella*	Currah et al. (1997)	*Oerstedella*	Eaton (2005)
Acampe	Behera et al. (2013)	*Dryandra*	Yang et al. (2011)	*Oncidium*	Otero et al. (2002)
Acianthera	Vas et al. (2009)	*Dryas*	Yang et al. (2011)	*Onychium*	Zhang et al. (2011)
Acianthus	Bougoure et al. (2005)	*Elythranthera*	Sommer et al. (2012)	*Ophrys*	Agustini et al. (2009)
Aerangis	Dearnaley et al. (2012)	*Encyclia*	Zettler et al. (2013)	*Orchis*	Harvais and Hadley (1967)
Aeranthes	Martos et al. (2009)	*Epiblema*	Sommer et al. (2012)	*Oreorchis*	Tsutsumi and Tomita (1986)
Aerides	Saha and Rao (2006)	*Epidendrum*	Otero et al. (2002)	*Ornithidium*	Tempesta et al. (2011)
Amerorchis	Currah et al. (1987)	*Epipactis*	Selosse et al. (2004)	*Orthoceras*	Warcup (1981)
Anacamptis	Tondello et al. (2012)	*Epipogium*	Roy et al. (2009b)	*Paphiopedilum*	Saha and Rao (2006)
Angraecopsis	Martos et al. (2009)	*Eriochilus*	Warcup (1981)	*Paracaleana*	Ruibal et al. (2013)
Angraecum	Dearnaley et al. (2012)	*Erythrodes*	Otero et al. (2002)	*Pecteilis*	Chutima et al. (2011)
Anoectochilus	Guo et al. (1997)	*Erythrorchis*	Umata (1997)	*Pelexia*	Fracchia et al. (2009)
Aphyllorchis	Roy et al. (2009a)	*Eulophia*	Johnson et al. (2007)	*Peristeranthus*	Warcup (1981)
Aplectrum	Curtis (1939)	*Galeola*	Agustini et al. (2009)	*Phaius*	Agustini et al. (2009)
Apostasia	Yukawa et al. (2009)	*Gastrochilus*	Kasmir et al. (2011)	*Phajus*	Tsutsumi and Tomita (1986)
Appendiculata	Hadley and Williamson (1972)	*Gastrodia*	Tsujita et al. (2009)	*Phalaenopsis*	Saha and Rao (2006)

Table 2.4 (continued)

Orchid Genera	Reference	Orchid Genera	Reference	Orchid Genera	Reference
Arachnis	Hadley (1970)	*Gavilea*	Fracchia et al. (2014)	*Pholidota*	Sawmya et al. (2013)
Arachnorchis	Feuerherdt and Jusaitis (2005)	*Gennaria*	Liebel et al. (2010)	*Piperia*	Jumpponen et al. (1998)
Arthrochilus	Warcup (1981)	*Geodorum*	Kasmir et al. (2011)	*Platanthera*	Bidartondo et al. (2004)
Arundina	Hadley and Williamson (1972)	*Glossodia*	Warcup (1981)	*Platylepis*	Martos et al. (2009)
Beclardia	Martos et al. (2009)	*Gomesa*	Otero et al. (2013)	*Plectorrhiza*	Warcup (1981)
Benthamia	Martos et al. (2009)	*Gongora*	Currah et al. (1997)	*Pleione*	Tao et al. (2008)
Bipinnula	Steinfort et al. (2010)	*Goodyera*	Bidartondo et al. (2004)	*Pleurothallis*	Suarez et al. (2006)
Bletilla	Tao et al. (2013)	*Grammatophyllum*	Salifah et al. (2011)	*Plocoglottis*	Agustini et al. (2009)
Brassia	Curtis (1939)	*Graphorchis*	Martos et al. (2009)	*Pogonia*	Curtis (1939)
Bromheadia	Hadley and Williamson (1972)	*Graphorkis*	Dearnaley et al. (2012)	*Polystachya*	Dearnaley et al. (2012)
Buddleja	Fracchia et al. (2009)	*Gymnadenia*	Harvais and Hadley (1967)	*Pomatocalpa*	Warcup (1981)
Bulbophyllum	Sawmya et al. (2013)	*Habenaria*	Cowden and Shefferson (2013)	*Prasophyllum*	Sommer et al. (2012)
Caladenia	Bougoure et al. (2005)	*Hadrolaelia*	Oliveira et al. (2014)	*Pseudorchis*	Kohout et al. (2013)
Calanthe	Hadley and Williamson (1972)	*Hetaeria*	Currah et al. (1997)	*Psychilis*	Otero et al. (2002)
Caleana	Bidartondo et al., (2004)	*Hexalectris*	Taylor et al. (2003)	*Pteroceras*	Warcup (1981)
Calochilus	Warcup (1981)	*Hexisea*	Currah et al. (1997)	*Pterostylis*	Bougoure et al. (2005)
Calopogon	Curtis (1939)	*Himantoglossum*	Pecoraro et al. (2013)	*Pterygodium*	Waterman et al. (2011)
Calypso	Currah et al. (1987)	*Hoffmannseggella*	Oliveira et al. (2014)	*Pyrorchis*	Sommer et al. (2012)
Camaridium	Zelmer et al. (1996)	*Holcoglossum*	Tan et al. (2012)	*Renanthera*	Saha and Rao (2006)
Campylocentrum	Otero et al., 2002	*Holothrix*	Martos et al., 2009	*Rhinerrhiza*	Warcup (1981)
Catasetum	Currah et al., 1997	*Hymenocallis*	Yang et al., 2011	*Rhizanthella*	Warcup (1981)
Cattlteya	Ovando et al., 2005	*Ionopsis*	Otero et al., 2002	*Rhynchostylis*	Hossain et al. (2013）

Table 2.4 (continued)

Orchid Genera	Reference	Orchid Genera	Reference	Orchid Genera	Reference
Cephalanthera	Bidartondo et al., 2004	*Isochilus*	Pereira et al., 2005	*Robiguezia*	Currah et al. (1997)
Chamaegastrodia	Yagame et al., 2008	*Jacquinella*	Currah et al., 1997	*Robiquetia*	Warcup (1981)
Changnienia	Jiang et al., 2011	*Jumellea*	Martos et al., 2009	*Rodriguezia*	Currah et al. (1997)
Chiloglottis	Roche et al., 2010	*Laeliocattleya*	Hadley 1970	*Rossioglossum*	Currah et al. (1997)
Clivia	Yang et al., 2011	*Lecanorchis*	Okayama et al., 2012	*Saccolabiopsis*	Warcup (1981)
Coeloglossum	Zelmer et al., 1996	*Lepanthes*	Boddington et al., 2008	*Sacoila*	Fracchia et al. (2009)
Coelogyne	Tao et al., 2008	*Leporella*	Warcup 1981	*Sarcochilus*	Warcup and Talbot (1967)
Coppensia	Valadares et al., 2012	*Leucorchis*	Harvais and Hadley 1967	*Sarcoglottis*	Nogueira et al. (2005)
Corallorhiza	McKendrick et al., 2000	*Limodorum*	Girlanda et al., 2006	*Satyrium*	Jyothsna and Purushothama (2014)
Corybas	Sommer et al., 2012	*Liparis*	McKendrick et al., 2004	*Scaphyglottis*	Currah et al. (1997)
Corycium	Tedersoo et al., 2013	*Lirope*	Chen et al., 2013	*Serapias*	Liebel et al. (2010)
Corymborkis	Martos et al., 2009	*Listera*	Harvais and Hadley 1967	*Sobralia*	Currah et al. (1997)
Cranichis	Espinosa et al., 2013	*Loroglossum*	Gao et al., 2010	*Sophronitis*	Vas et al. (2009)
Cremastra	Tsutsumi and Tomita 1986	*Ludisia*	Athipunyakom et al., 2004	*Spathoglottis*	Hadley and Williamson (1972)
Cryptopus	Martos et al., 2009	*Luisia*	Sathiyadash et al., 2012	*Spiculaea*	Sommer et al. (2012)
Cryptostylis	Sommer et al., 2012	*Lycaste*	Curtis 1939	*Spiranthes*	Tondello et al. (2012)
Cymbidium	Tsutsumi and Tomita 1986	*Lyperanthus*	Sommer et al., 2012	*Stanhopea*	Esnault et al. (2009)

Table 2.4 (continued)

Orchid Genera	Reference	Orchid Genera	Reference	Orchid Genera	Reference
Cynorkis	Martos et al., 2009	*Macodes*	Agustini et al., 2009	*Stelis*	Suarez et al. (2006)
Cypripedium	Shefferson et al. 2005	*Maxillaria*	Espinosa et al. 2013	*Taeniophyllum*	Warcup (1981)
Cyrtosia	Umata 1997	*Microtis*	Sommer et al., 2012	*Thelymitra*	Sommer et al. (2012)
Cyrtostylis	Warcup 1988	*Miltonia*	Currah et al., 1997	*Thrixspermum*	Hadley (1970)
Cystopus	Currah et al., 1997	*Myoxanthus*	Currah et al., 1997	*Tipularia*	McKendrick et al. (2004)
Dactylorchis	Harvais and Hadley 1967	*Myrmechis*	Curtis 1939	*Tolumnia*	Otero et al. (2002)
Dactylorhiza	Bidartondo et al., 2004	*Neottia*	Selosse et al., 2002	*Trichoglottis*	Warcup (1981)
Dendrobium	Chen et al., 2011	*Neottianthe*	Kulikov et al., 2001	*Trichopilia*	Currah et al. (1997)
Dendrochilum	Hadley and Williamson1972	*Nervilia*	Nomura et al., 2013	*Trichosalpinx*	Currah et al., (1997)
Dichaea	Currah et al., 1997	*Neuwiedia*	Kristiansena et al., 2004	*Trigonidium*	Currah et al. (1997)
Dichromanthus	Beltran-Nambo et al., 2012	*Nidema*	Currah et al., 1997	*Trizeuxis*	Espinosa et al. (2013)
Didymoplexis	Currah et al., 1997	*Nigritella*	Haselwandter et al., 2006	*Vanda*	Hadley (1970)
Dimerandra	Currah et al., 1997	*Notylia*	Espinosa et al. 2013	*Vanilla*	Espinosa et al. (2013)
Diplocaulobium	Ma et al., 2003	*Oberonia*	Sathiyadash et al., 2012	*Vrydagzynea*	Currah et al., (1997)
Dipodium	Bougoure et al., 2005	*Octomeria*	Currah et al., 1997	*Wullschlaegelia*	Martos et al. (2009)
Disa	Sommer et al., 2012	*Odontoglossum*	Nair et al., 2014	*Yoania*	Shan et al. (2002)
Diuris	Sommer et al., 2012	*Oeceoclades*	Otero et al., 2002	*Zeuxine*	Shan et al. (2002)
Dryadella	Currah et al., 1997	*Oeonia*	Martos et al., 2009		

Applications of endophytes of other plants have been shown to have industrial potential which may be worth exploring in orchid endophytes. For example, endophytic antioxidant activities have been reported in many plants (Hamilton et al., 2012). *Phoma*, *Alternaria* and *Aspergillus* species are metal-resistant and play roles in phytoremediation (Hamilton et al., 2012). *Phomopsis* isolates can secrete enzymes including cellulases, lipases, pectinases, pectate, lyases and proteases (Suryanarayanan et al., 2012). *Cladosporium*, *Alternaria* and *Fusarium* species that are major groups of endophytic fungi in grasses have close relationships with allergen exposure, which may help us understanding the evolution of immune reaction to respiratory allergens (De Aldana et al., 2013).

CHAPTER 3

ENDOPHYTIC *COLLETOTRICHUM* SPECIES FROM *DENDROBIUM* SPP. IN SOUTHWESTERN CHINA AND NORTHERN THAILAND[2]

Abstract

Species of *Colletotrichum* are commonly found in many plant hosts as pathogens, endophytes and occasionally saprobes. Twenty-two *Colletotrichum* strains were isolated from three *Dendrobium* species – *D. cariniferum*, *D. catenatum* and *D. harveyanum,* as well as three unidentified species. The taxa were identified using morphological characterisation and phylogenetic analyses of ITS, GAPDH, ACT and ß–tubulin sequence data. This is the first time to identify endophytic fungi from *Dendrobium* orchids using the above method. The known species, *Colletotrichum boninense*, *C. citricola*, *C. fructicola, C. jiangxiense* and *C. orchidophilum* were identified as fungal endophytes of *Dendrobium* spp., along with the new species, *C. cariniferi*, *C. chiangraiense*, *C. doitungense*, *C. parallelophorum* and *C. watphraense*, which are introduced in this paper. One strain is recorded as an unidentified species. Corn meal agar is recommended as a good sporulation medium for *Colletotrichum* species. This is the first report of fungal endophytes associated with *Dendrobium cariniferum* and *D. harveyanum*. *Colletotrichum citricola*, *C. jiangxiense*, and *C. orchidophilum* are new host records for Thailand.

Keywords: *Colletotrichum*, *Dendrobium*, Endophytic Fungi, multi-loci, New Species

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3.1 Introduction

Colletotrichum is the sole genus in family Glomerellaceae (Glomerellales) (Maharachchikumbura et al., 2015; 2016; Jayawardena et al., 2016a; Hongsanan et al., 2017). Presently, there are 193 accepted *Colletotrichum* species in eleven species complexes and 23 accepted singleton species (Hyde et al., 2014; Jayawardena et al., 2016b). *Colletotrichum* species has been listed as one of the top ten fungal pathogenic genera in molecular plant pathology based on scientific/economic importance (Dean et al., 2012). Anthracnose caused by *Colletotrichum* species can be a devastating disease in many economically important crops, including fruit crops, vegetables, cassava, sorghum, as well as ornamental plant such as orchids (Prusky & Plumbley, 1992; Hyde et al., 2009a; 2009b; Cannon et al., 2012; Dean et al., 2012; Jadrane et al., 2012; Jayawardena et al., 2016a; Diao et al., 2017). Many pathogenic *Colletotrichum* species that adopt biotrophic life strategies are present as symptomless endophytes in living plant tissues (Photita et al., 2004), although a large number of non-pathogenic species also occur as endophytes (Mendgen & Hahn, 2002; Lu et al., 2004; Rojas et al., 2010; Cannon et al., 2012; Kleemann et al., 2012). Interestingly, experiments of Redman et al. (2001) showed that pathogenic *Colletotrichum* species could express mutualistic lifestyles in plants not known to be hosts and conferred disease resistance, drought tolerance, and/or growth enhancement to the host plants. Even though the diversity of *Colletotrichum* species associated with cultivated plant hosts have extensively been studied (Yang et al., 2009), a very limited number of studies has been conducted on *Colletotrichum* species from non-cultivated plants in natural and semi-natural habitats (Cannon et al., 2012).

Dendrobium SW. is the second largest genus in Orchidaceae (The Plant List 2013). Most *Dendrobium* species/hybrids are important ornamental/floricultural crops, but some species within this genus also possess medicinal values (Xu et al., 1995; Ng et al., 2012). Many *Dendrobium* orchids have been listed as Chinese medicinal herbs and are used for the treatments of atrophic gastritis, diabetes, faucitis, fever, red tongue, and/or thirst (Lin 2003; Bulpitt et al., 2007; Xing et al., 2011; Xia et al., 2012; Xu et al., 2014). Moreover, some *Dendrobium* orchids including *D. catenatum* Lindl. (widely known as *D. officinale* Kimura & Migo) have been listed as critically endangered species by the International Union for Conservation of Nature (IUCN) (www.iucnredlist.org). Due to their significance, *Dendrobium* orchids have been the subject of many studies including the diversity of endophytic fungi (Ma et al., 2015). However, only a limited number of studies on endophytic *Colletotrichum* in *Dendrobium* species have been reported and the number of *Dendrobium* species included in these studies are very few (Yuan et al., 2009; Yang et al., 2011; Chen et al., 2012; Mangunwardoyo, 2012; Noireung et al., 2012; Tao et al., 2013). In the present study, we investigated the diversity of endophytic *Colletotrichum* in five *Dendrobium* orchid species collected from a mountain (at an elevation of 1,300-1,400 m) close to the Thailand-Myanmar border and *D. catenatum* collected from Guizhou Province in China. A total of 22 endophytic *Colletotrichum* strains were isolated and identified based on both morphological and molecular characteristics. Five *Colletotrichum* strains, *C. cariniferi*, *C. chiangraiense*, *C. doitungense, C. parallelophorum* and *C. watphraense* are introduced as new species. The results of this study will contribute to the knowledge on diversity and phylogeny of *Colletotrichum*.

3.2 Materials and Methods

3.2.1 Sample Collection

Healthy roots, stems and leaves of *D. cariniferum*, *D. harveyanum* and three other *Dendrobium* taxa (referred to as Dendrobium sp. 1, 2 and 3) were collected from Wat Phra That Doi Tung (Temple of Doi Tung Pagoda), Mae Fah Luang District, Chiang Rai, Thailand. Healthy roots, stems and leaves of *D. catenatum* were collected from Guizhou Province in China. Materials were packed in zip-lock bags or tubes containing silica gel on ice. Fungal isolation was carried out within 48 hours following collection.

3.2.2 Fungal Isolation And Cultivation

Surface sterilization and endophyte isolation were carried out as described by Nontachaiyapoom et al. (2010) with some modifications. First, materials were washed with running water. Roots, stems and leaves were immersed in a solution containing 3%

(v/v) H_2O_2 and 70% (v/v) ethanol for 5 minutes, and then rinsed with sterile distilled water for three times. Sterilized materials were cut into 2 mm2 and placed on potato dextrose agar (PDA) containing 50 µg/ml oxytetracycline, 50 µg/ml penicillin and 50 µg/ml streptomycin (Otero et al., 2002). Samples were incubated at 28 °C under natural light. Single spores were transferred to fresh PDA to obtain pure cultures. The pure cultures were deposited at Mae Fah Luang University Culture Collection (MFLUCC) and International Collection of Micro–organisms from Plants (ICMP).

Table 3.1 PCR thermal cycling process

		PCR amplification				
Region/gene	**Initial denaturation**	**Cycle number**	**Denaturation**	**Annealing**	**Elongation**	**Final elongation**
ITS	95°C 3 min	30	95°C 1 min	53°C 1 min	72°C 1 min	72°C 10 min
GAPDH	95°C 4 min	35	95°C 1 min	60°C 30 s	72°C 45 s	
ACT	95°C 4 min	40	94°C 45 s	52°C 30 s	72°C 90 s	
ß-tubulin	95°C 5 min	35	94°C 1 min	55°C 55 s	72°C 1 min	

3.2.3 DNA Extraction and Amplification

DNA samples were prepared from mycelium of pure fungal culture using EZgeneTM Fungal gDNA Kit (GD2416, Biomiga, USA) as described by the manufacturer. Amplification reactions were performed using reagents purchased from BIOMIGA (San Diego, USA). Each 20-µl amplification reaction contained 10 µl of 2*Bench TopTM Taq Master Mix (0.05 units/µl Taq DNA polymerase, 0.4mM dNTPs and 4mM $MgCl_2$); 2µl forward and reverse primers; 1µl of DNA template and 7µl of water. The primers used in this study were ITS1/ITS4 (White et al., 1990), GDF/GDR (Templeton et al., 1992), 512F/783R (Carbone & Kohn, 1999) and BT2A/BT2B (Glass & Donaldson, 1995; Maharachchikumbura et al., 2012). The thermal cycling programs are presented in Table 3.1 PCR products were sent to Invitrogen (USA), Sangon Biotech and Sino GenoMax (China) for purification and sequencing.

3.2.4 Sequence Analysis

Either single-directional sequencing results (for ITS and GAPDH) or bi - directional sequencing results (for ACT and TUB2) were manually trimmed and/or assembled into contigs using CodonCode aligner software (CodonCode Corporation, Dedham, Massachusetts). Through the latest publications and the observation for ML tree topology, a selected set of ITS, GAPDH, ACT and TUB2 sequences especially those of ex-type/ex-epitype materials used in the phylogenetic analysis were downloaded from GenBank (Table 3.4). Five datasets of *Colletotrichum* spp. ITS (134nt), GAPDH (113nt), ACT (119nt), ß–tubulin (125nt) and a concatenated dataset were constructed. Sequences were aligned using MAFFT version 6 (Katoh & Toh, 2008). Aligned datasets were visually inspected and misaligned regions were manually edited where necessary using Bio–Edit version 7.2.5 (Hall, 1999). Ambiguous regions were trimmed using trimAL version 1.3 (Capella-Gutierrez, Silla-Martinez & Gabaldon, 2009) available online through Phylemon 2.0 (http://phylemon.bioinfo.cipf.es/). After trimming, the final alignments contained 578 sites for ITS, 298 sites for GAPDH, 290 sites for ACT and 480 sites for ß–tubulin. The concatenated dataset contained a total of 134 taxa and 1646 sites that were used for all subsequent analyses and submitted to TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S22431). Gaps were treated as missing data in maximum likelihood (ML), Bayesian inference (BI) and parsimony trees. Parsimony trees were constructed with PAUP (Phylogenetic Analysis Using Parsimony) version 4.0 beta 10 (Swofford, 2001). Heuristic searches were conducted as follows: 1000 starting trees were generated using stepwise addition and random addition sequence replicates, followed by branch swapping using the tree–bisection–reconnection (TBR) algorithm. The inferences for MP tree were length = 6732 steps, CI = 0.294, RI = 0.760, RC = 0.223, HI = 0.706. Maximum likelihood analyse was conducted with RAxMLGui 1.31 (Silvestro & Michalak, 2012). The general time reversible (GTR) model of nucleotide substitution was used and the inverse gamma distribution option was implemented. The topology of the resulting tree was similar to that of the maximum parsimony tree. Bootstrap support was calculated from 1000 replicates, which were subsequently mapped onto the best-scoring ML tree. Bayesian inference trees were computed using MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). The concatenated dataset was partitioned and the ultrafast bootstrap (Minh et al., 2013) implemented in the IQ-TREE software (Nguyen et al., 2014) as well as Mrmodeltest 2.3 (Nylander, 2004) were used to estimate the best fitting models according to the Bayesian information criterion (BIC). The GTR model with inverse gamma distribution and HKY model with gamma distribution were used as the most appropriate for the ITS and GAPDH respectively. The Hasegawa, Kishino & Yano (HKY) model with inverse gamma distribution and GTR model with gamma distribution were selected for the ACT and ß-tubulin datasets. Two sets of four simultaneous independent chains of Markov chains Monte Carlo (MCMC) simulations were run for 6,000,000 generations, 25% of trees were discarded as burn-in and the remaining trees were used to calculate the posterior probabilities. Convergence was assumed when the standard deviation of split sequences was less than 0.01. The fungal isolates and sequences of region/genes used in Colletotrichum phylogenetic analysis are listed in Appendix A.

3.2.5 Morphological Analysis

Sporulation of studied fungi was induced on thin pieces of Corn malt agar medium (CMA). The strains that did not sporulate on CMA were cultured on PDA or Sabouraud dextrose agar (SDA) with sterilized orchid tissues in order to induce sporulation. An autoclaved toothpick was placed on CMA for one strain C. cariniferi to induce sporulation. Cultures were grown in a dark cabinet at room temperature (28°C) and observed for every seven days or less. The growth rate was evaluated when mycelia nearly covered the whole medium surface. Once an acervuli or ascomata were observed, photos were taken with a stereomicroscope (SteREO Discovery. V8, Carl Zeiss Microscopy GmBH, Germany). Cross–sections and conidiomata crushed in water were observed under a compound microscope (EOS 600D, Nikon, Japan). Ascomata and conidiomata were observed under a Motic SMZ–140 microscope (China). Conidiophore, conidia, appressoria, ascomata, asci, ascospores and other visible structures such as chlamydospore were used for evaluating morphological characteristics in this study (Damm et al., 2014). The recommendations of Jeewon & Hyde (2016) were followed in establishing new species.

3.3 Results

3.3.1 Fungal Isolation and Identification

Twenty-two endophytic *Colletotrichum* strains were isolated from six *Dendrobium* species (Table 3.2). The highest number of *Colletotrichum* strains and species were isolated from *Dendrobium* sp.1 followed by *Dendrobium* sp.2. All three tissue types of the two orchids hosted at least one strains of *Colletotrichum*. Among the three tissue types, the highest number of *Colletotrichum* strains and species were isolated from leaves. *Colletotrichum boninense* and C. *fructicola* were respectively the most frequently isolated *Colletotrichum* species. Interestingly, *C. boninense* was isolated from *Dendrobium* species collected from both geographical areas studied (i.e. Chiang Rai, Thailand and Guizhou, China).

Table 3.2 *Colletotrichum* strains and species isolated from *Dendrobium* orchids

Orchid sample	Tissue	Number of fungal strains	***Colletotrichum* species**	Code
D. cariniferum	Root	0	0	–
	Stem	1	*C. cariniferi* sp.nov.	MFLUCC 14-0100
	Leaf	0	0	–
D. harveyanum	Root	0	0	–
	Stem	0	0	–
	Leaf	2	*C. orchidophilum*	MFLUCC 14-0161 MFLUCC 14-0162
Dendrobium sp.1	Root	2	*C. parallelophorum* sp.nov.	MFLUCC 14-0077 MFLUCC 14-0079
	Stem	3	*C.parallelophorum* sp.nov.	MFLUCC 14-0082 MFLUCC 14-0083 MFLUCC 14-0085
	Leaf	4	*C. boninense, C. jiangxiense, C. fructicola*	MFLUCC 14-0086 MFLUCC 14-0087 MFLUCC 14-0091 MFLUCC 14-0092

Table 3.2 (continued)

Orchid sample	Tissue	Number of fungal strains	*Colletotrichum* species	Code
Dendrobium sp.2	Root	2	*C. chiangraiense* sp nov.; *C. fructicola*	MFLUCC 14-0119 MFLUCC 15-0262
	Stem	3	*C. boninense*, *C. watphraense* sp.nov., *C.* sp.indet.	MFLUCC 15-0120 MFLUCC 15-0123 MFLUCC 15-0124
	Leaf	3	*C. citricola*, *C. doitungense* sp.nov.	MFLUCC 15-0128 MFLUCC 15-0129 MFLUCC 15-0131
Dendrobium sp.3	Root	0	0	–
	Stem	0	0	–
	Leaf	1	*C. boninense*	MFLUCC 15-0148
	Root	0	0	–
D. catenatum	Stem	0	0	–
`	Leaf	1	*C. boninense*	MFLUCC 15-0261

Note '–' means that no strain was isolated.

3.3.2 Sporulation results

All *Colletotrichum* strains could grow on three kinds of media. *Colletotrichum doitungense*, *C. citricola*, *C. fructicola* and *C. parallelophorum* produced both sexual and asexual morphs in culture. *Colletotrichum cariniferi*, *C. boninense*, *C. orchidophilum* and *C. watphraense* produced only the asexual morph and *C. chiangraiense* produced only sexual morph in culture. Measurements of important vegetative and reproductive characteristics of isolated strains are given in Table 3.3.

3.3.3 Phylogenetic Results

All the sequences of ITS, GAPDH, ACT and β-tubulin of 22 strains of *Colletotrichum* obtained in this study were deposited in GenBank (List in Appendix A). The three selected outgroup species (i.e. *Australiasca queenslandica* BRIP 24607; *Monilochaetes infuscans* CBS 869.96 and *Monilochaetes guadalcanalensis* CBS 346.76) formed a strongly supported cluster (100ML/1.00BI/99MP). The ingroup consisted of all *Colletotrichum* sequences and was fully supported by all three methods of analysis (100ML/1.00BI/100MP). Five strains grouped within the gloeosporioides complex: MFLUCC 14-0087, MFLCCC 14-0091, MFLUCC 14-0092, MFLUCC 14-0148 and MFLUCC 14-0262. The sequences of MFLCCC 14-0091 and MFLUCC 14-0092 were nearly identical and close to *C. jiangxiense* with strong support (99ML/0.95BI/96MP). MFLUCC 14-0087, MFLUCC 14-0148 and MFLUCC 15-0262 clustered with *C. fructicola* (ICMP 181873) (91ML/0.72BI/83MP).

Nine of the newly sequenced strains clustered within the boninense species complex: MFLUC 14-0086, MFLUCC 14-0119, MFLUCC 14-0120, MFLUCC 14-0123, MFLUCC 14-0124, MFLUCC 14-0128, MFLUCC 14-0129, MFLUCC 14-0131, MFLUCC 15-0261. MFLUCC 14-0086, MFLUCC 14-0124 and MFLUCC 14-0261 shared very similar sequences. MFLUCC 14-0128 grouped as sister to the three above-mentioned strains (66ML/1.00BI/73MP). MFLUCC 14-0123 formed a separated clade from other species by only Bayesian analysis (1.00BI). MFLUCC 14-0120, MFLUCC 14-0129 and MFLUCC 14-0131 formed a cluster with *C. fructicola* and *C. camelliae-japonicae* (76ML/1.00BI/62MP). MFLUCC 14-0120 and MFLUCC 14-0129 differed by only three base pairs in trimmed concatenated alignment. MFULCC 14-0119 was placed basally to the boninense species complex with strong support (100ML/0.96BI/90MP).

MFLUCC 14-0161 and MFLUCC 14-0162 grouped outside the currently accepted species complexes. The two had a close relationship and formed a clade with *C. orchidophilum*, which is a singleton and a sister taxon to the acutatum species complex. They hold the maximum support with all three methods of analysis. *Colletotrichum orchidophilum* differed 1.5% and 1.3% with MFLUCC 14-0161 and MFLUCC 14-0162 respectively. MFLUCC 14-0077, MFLUCC 14-0079, MFLUCC 14-0082, MFLUCC 14–0083 and MFLUCC 14-0085 formed a novel clade (100ML/1.00BI/100MP), which grouped as sister clade to the *C. excelsum-altitudum*/*C. tropicicola* clade and MFLUCC 14-0100 (88ML/1.00BI/59MP). MFLUCC 14-0100 took a solo branch in the basal position among them (99ML/1.0BI/98MP).

3.3.4 Taxonomy

The 22 strains isolated as endophytes were assigned to eleven species, five known species, five new species and one undetermined species. We obtained the sexual and asexual morphs for four strains. The sexual morph was only obtained in the case of *C. chiangraiense.* The descriptions of the fungal endophytes identified in this study are as follows.

Table 3.3 Synopsis of size (µm) of structures of *Colletotrichum* species identified in this study

		Sexual morph				**Asexual morph**		
***Colletotrichum* species**	**Vegetative hyphae diam (µm)**	**Setae (µm)**	**Ascomata (µm)**	**Size of asci (µm)**	**Size of ascospores (µm)**	**Size of conidiomata (µm)**	**Size of conidiophore (µm)**	**Size of conidia (µm)**
C. cariniferi sp. nov.	3.5–8.2	–	–	–	–	50 ×50	(37.5–) 42.3–65 (–71.6) × (3.1–) 3.8–5.9 (–6)	(24.1–) 26.8–33.0 (–36.1) × (7.9–) 8.3–9.6 (–10.2)
C. chiangraiense sp. nov.	4.6.± 1.8	–	(14.4–) 15.3–19.6 (–20.5) × (7.4–) 7.3–7.9 (–8)	(30.7–) 33.4–52.7 (–72.2) × (5.7–) 6.5–8.2 (–9.4)	(11–) 11.9–15.4 (–16.7) × (2.2–) 2.8–3.8 (–4.4)	–	–	–
C. citricola	3.1 ± 1.1	(51.8–) 54.1–67.8 (–68.5) × (2.3–) 2.4–5.8 (–7.2)	(34.5–) 46.4–84.9 (–87.1) × (31.7–) 33.8–46.5 (–50.9)	(41.3–) 49.4–65.0 (–71.6) × (8.3–) 9.5–12.9 (–14.3)	(14.4–) 14.8–17.5 (–19.3) × (5.4–) 5.7–7.1 (–7.6)	–	(10.8–) 16.7–25.6 (–30.6) × (3.1–) 4–5.3 (–5.6)	(12.5–) 13.4–15 (16.5–) × (5–) 5.9–6.9 (–7.2)
C. doitungense sp.nov.	1.1–3.5	–	(125.5–) 126.9–133.7 (–135.1) × (101.3–) 101.8–104.3 (–104.8)	(51.1–) 53.7–70.6 (–71.6) × (8.5–) 8.8–10.1 (–10.4)	(16.1–) 17.5–21.5 (–23.4) × (4.5–) 5.1–7(–7.5)	–	(9.1–) 14.3–26.8 (–29.4) × (3–) 3.1–4.5 (–5)	(6.6–) 8.6–13.8 (–15) × (2.6–) 3.8–8.9 (–13.8)
C. fructicola	2.6–5	(53–) 57.2–73.1 (–83.3) × (3.4–) 3.5–4 (–4.1)	(131.9–) 138.4–163.6 (–171.5) × (120.9–) 123.6–142.1 (–143.2)	(57.6–) 61.2–82.6 (–94.3) × (8.7–) 9.3–13.3 (–15.8)	(10–) 12.0–20.0 (–20.9) × (3.6–) 4.1–5.2 (–5.3)	500×400	–	(12.8–) 13.8–16.6 (–18.6) × (2.7–) 3.5–7.8 (–16)

Table 3.3 (continued)

				Sexual morph			Asexual morph	
***Colletotrichum* species**	**Vegetative hyphae diam (µm)**	**Setae (µm)**	**Ascomata (µm)**	**Size of asci (µm)**	**Size of ascospores (µm)**	**Size of conidiomata (µm)**	**Size of conidiophore (µm)**	**Size of conidia (µm)**
C. jiangxiense	1.3–2.1	–	–	–	–	–	(12.7–) 13.5–21.4 (–23.4) × (1.9–) 2–3 (–3.2)	(8.6–) 9–12.4 (–13.2) × (3.5–) 3.6–4.4 (–4.5)
C. orchidophilum	1.9–5.4	–	–	–	–	200×300	–	(9.6–) 11.7–14.1 (–14.7) × (2.9–) 3.5–4.4 (–4.8)
C. paralleophorum sp.nov.	2–4.3	(56.7–) 60.2–79.2 (–81.2) × (2.8–) 2.9–3.7 (–3.9)	(267–) 261.4–342.3 (–346.2) × (190.4–) 173–272.5 (–280)	(43.3–) 44.1–63.3 (–66.5) × (7.6–) 8–9.8 (–10)	(13.9–) 14.1–18 (–20.9) × (3.1–) 3.9–5.4 (–5.8)	200×200	(18.3–) 20.82–34 (–41.2) × (2.6–) 2.8–4.3 (–5.4)	(12.1–) 13.8–16.8 (–18.9) × (3.3–) 4.4–7.5 (–7.9)
C. watphraense sp. nov.	1.6–4.3	–	–	–	–	200×300	(15.8–) 18.5–26.8 (–29.1) × (3.4–) 3.8–5.1 (–5.7)	(12.4–) 12.5–14.6 (–15.2) × (4.4–) 4.5–5.8 (–6.1)
C. cariniferi sp. nov.	3.5–8.2	–	–	–	–	50 ×50	(37.5–) 42.3–65 (–71.6) × (3.1–) 3.8–5.9 (–6)	(24.1–) 26.8–33.0 (–36.1) × (7.9–) 8.3–9.6 (–10.2)
C. chiangraiense sp. nov.	4.6±1.8	–	(14.4–) 15.3–19.6 (–20.5) × (7.4–) 7.3–7.9 (–8)	(30.7–) 33.4–52.7 (–72.2) × (5.7–) 6.5–8.2 (–9.4)	(11–) 11.9–15.4 (–16.7) × (2.2–) 2.8–3.8 (–4.4)	–	–	–

Table 3.3 (continued)

		Sexual morph				Asexual morph		
***Colletotrichum* species**	**Vegetative hyphae diam (µm)**	**Setae (µm)**	**Ascomata (µm)**	**Size of asci (µm)**	**Size of ascospores (µm)**	**Size of conidiomata (µm)**	**Size of conidiophore (µm)**	**Size of conidia (µm)**
C. citricola	3.1 ± 1.1	(51.8–) 54.1–67.8 (–68.5) × (2.3–) 2.4–5.8 (–7.2)	(34.5–) 46.4–84.9 (–87.1) × (31.7–) 33.8–46.5 (–50.9)	(41.3–) 49.4–65.0 (–71.6) × (8.3–) 9.5–12.9 (–14.3)	(14.4–) 14.8–17.5 (–19.3) × (5.4–) 5.7–7.1 (–7.6)	–	(10.8–) 16.7–25.6 (–30.6) × (3.1–) 4–5.3 (–5.6)	(12.5–) 13.4–15 (16.5–) × (5–) 5.9–6.9 (–7.2)
C. doitungense sp.nov.	1.1–3.5	–	(125.5–) 126.9–133.7 (–135.1) × (101.3–) 101.8–104.3 (–104.8)	(51.1–) 53.7–70.6 (–71.6) × (8.5–) 8.8–10.1 (–10.4)	(16.1–) 17.5–21.5 (–23.4) × (4.5–) 5.1–7(–7.5)	–	(9.1–) 14.3–26.8 (–29.4) × (3–) 3.1–4.5 (–5)	(6.6–) 8.6–13.8 (–15) × (2.6–) 3.8–8.9 (–13.8)
C. fructicola	2.6–5	(53–) 57.2–73.1 (–83.3) × (3.4–) 3.5–4 (–4.1)	(131.9–) 138.4–163.6 (–171.5) × (120.9–) 123.6–142.1 (–143.2)	(57.6–) 61.2–82.6 (–94.3) × (8.7–) 9.3–13.3 (–15.8)	(10–) 12.0–20.0 (–20.9) × (3.6–) 4.1–5.2 (–5.3)	500×400	–	(12.8–) 13.8–16.6 (–18.6) × (2.7–) 3.5–7.8 (–16)
C. jiangxiense	1.3–2.1	–	–	–	–	–	(12.7–) 13.5–21.4 (–23.4) × (1.9–) 2–3 (–3.2)	(8.6–) 9–12.4 (–13.2) × (3.5–) 3.6–4.4 (–4.5)
C. orchidophilum	1.9–5.4	–	–	–	–	200×300	–	(9.6–) 11.7–14.1 (–14.7) × (2.9–) 3.5–4.4 (–4.8)
C. paralleophorum sp.nov.	2–4.3	(56.7–) 60.2–79.2 (–81.2) × (2.8–) 2.9–3.7 (–3.9)	(267–) 261.4–342.3 (–346.2) × (190.4–) 173–272.5 (–280)	(43.3–) 44.1–63.3 (–66.5) × (7.6–) 8–9.8 (–10)	(13.9–) 14.1–18 (–20.9) × (3.1–) 3.9–5.4 (–5.8)	200×200	(18.3–) 20.82–34 (–41.2) × (2.6–) 2.8–4.3 (–5.4)	(12.1–) 13.8–16.8 (–18.9) × (3.3–) 4.4–7.5 (–7.9)
C. watphraense sp. nov.	1.6–4.3	–	–	–	–	200×300	(15.8–) 18.5–26.8 (–29.1) × (3.4–) 3.8–5.1 (–5.7)	(12.4–) 12.5–14.6 (–15.2) × (4.4–) 4.5–5.8 (–6.1)

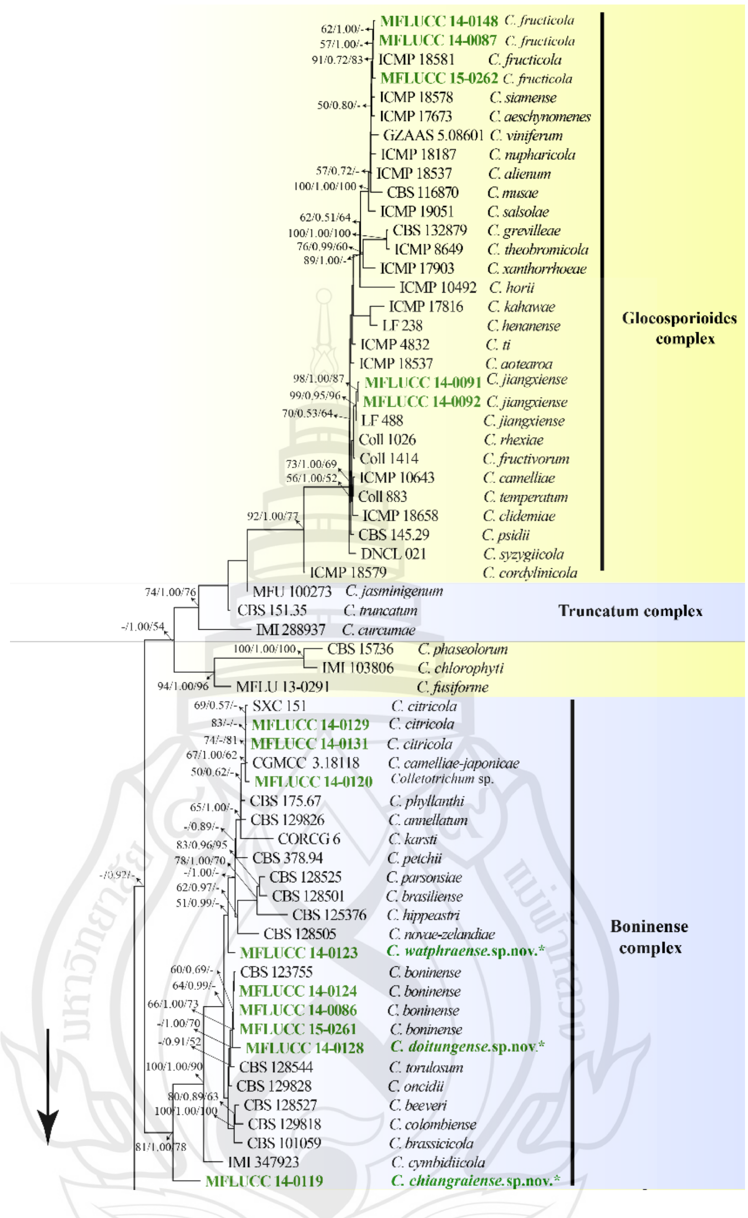


Figure 3.1 Maximum likelihood (ML) tree of *Colletotrichum* inferred from 134 taxa and 1646 sites from a concatenated dataset containing ITS, GAPDH, ACT and ß-tubulin sequence data. Values at nodes indicate bootstrap percentages (BP) for ML, Bayesian posterior probabilities (PP) and BP for maximum parsimony (MP) in this order. Only BP over 50%, PP over 0.50 and MP over 50 are shown. Dashes correspond to lower than the above-mentioned values. The isolated fungal endophytes in this study are shown in green **bold** text. Scale bar corresponds to 0.08 substitutions per site

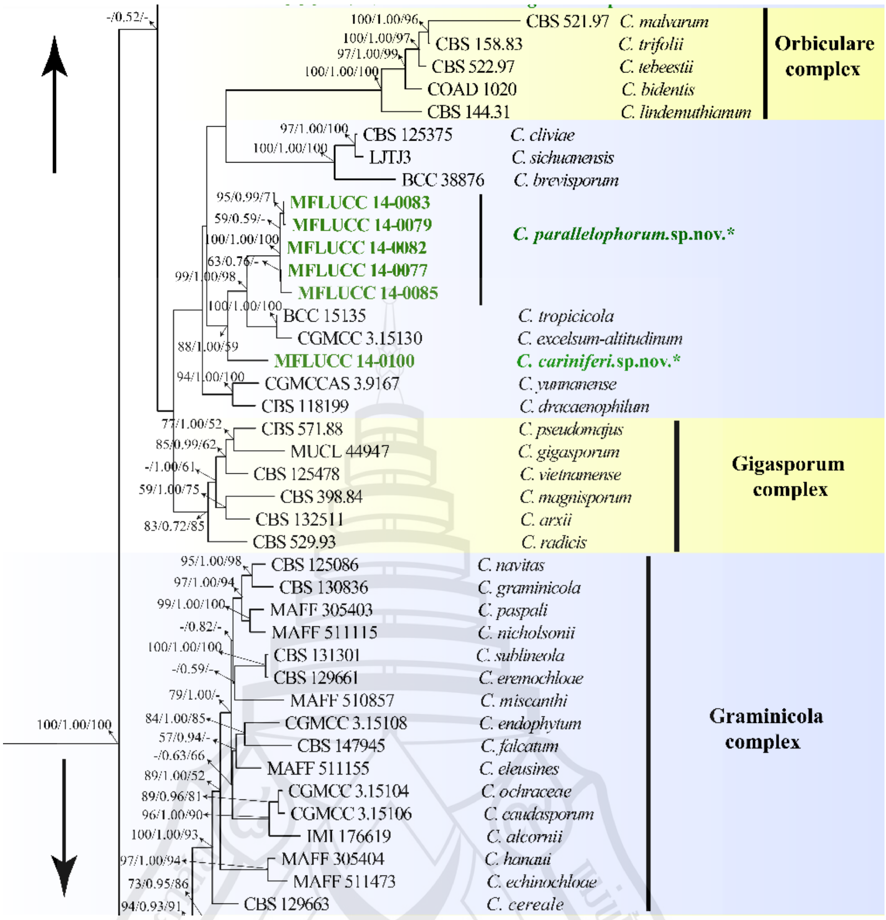


Figure 3.1(continued)

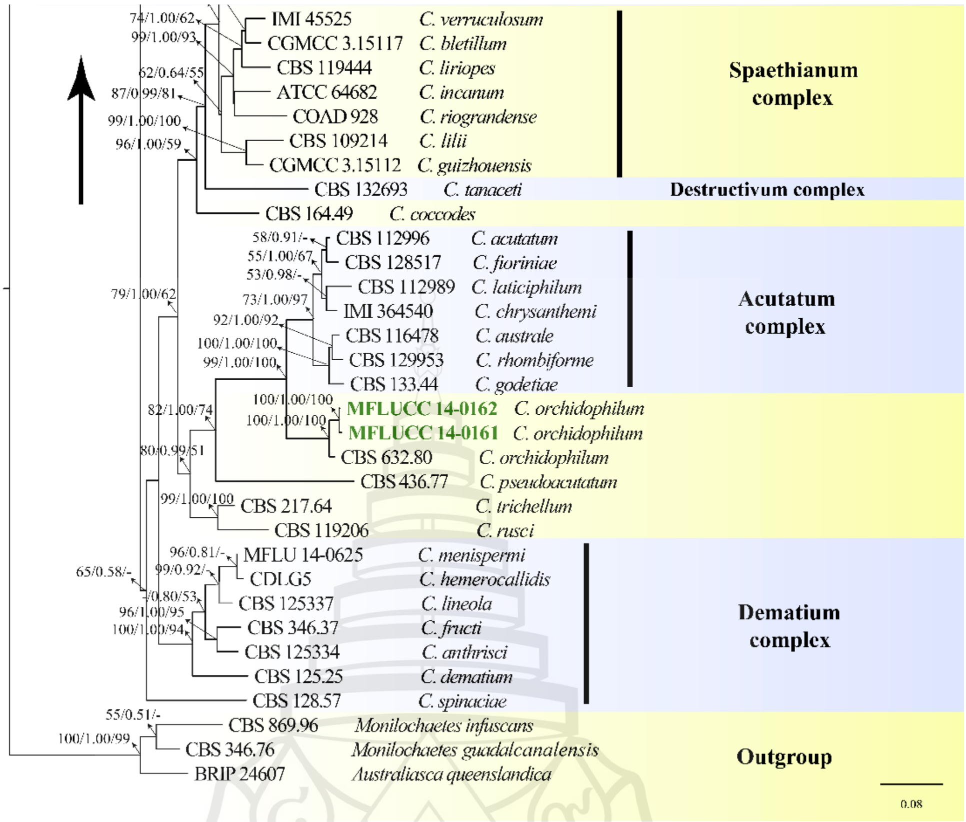


Figure 3.1(continued)

Colletotrichum cariniferi X.Y. Ma, K. D. Hyde & Jayawardena, sp.nov.

Fungal Name Number: FN570511

Etymology: In reference to the host epithet cariniferum.

Holotype: MFLC 17-1199.

Description: *Sexual morph* not observed.

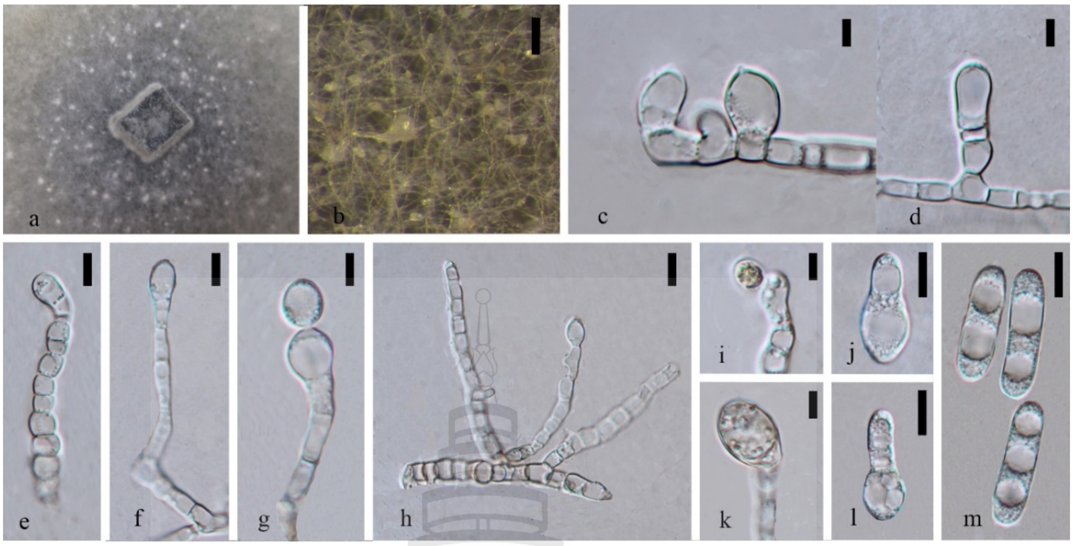
Asexual morph on CMA. Vegetative hyphae 3.5–8.2 µm diam (N=20), hyaline to brown, smooth-walled, septate, branched. Appressoria (9.7–) 10.4–17 (–20.5) × (6.5–) 7.1–11.3 (–13.6) µm (N=6), globose to sub-globose, light brown. Conidiomata 50 × 50 µm (N=10), developing with mycelia, globose to irregular, milk orange to orange, in mass brown. Conidiophores (37.5–) 42.3–65 (–71.6) × (3.1–) 3.8–5.9 (–6) µm (N=6), smooth-walled, unbranched, hyaline. The part connected with conidia of conidiogenous cell inflated and some with large guttules. Conidia (24.1–) 26.8–33.0

(–36.1) × (7.9–) 8.3–9.6 (–10.2) µm (N=30), L/W ratio = 3.4, ellipsoidal to cylindrical, with one end inflated when immature state, both ends rounded when mature, with 2 to 3 guttules, hyaline.

Cultures on CMA flat with entire margin. Growth rate: 0.23cm/day, with 50-days for sporulation. Cottony, pale cinnamon to light brown, scattered pale mycelia in spots around the middle inoculum clump, sometimes covered short, floccose-felty, white, aerial mycelium, reverse buff brown.

Material examined. Thailand, Chiang Rai, Wat Phra That Doi Tung (Temple of Doi Tung Pagodas), the host *Dendrobium cariniferum* was sampled 19 December 2013, Collector: Sureeporn Nontachaiyapoom, Natdanai Aewsakul, Xiaoya Ma. Holotype: MFLC 17–1199.

Notes: *Colletotrichum cariniferi* (MFLUCC 14-0100) clusters in a separate branch with a good support (88ML/1.00BI/59MP). The species is most phylogenetically close to *Colletotrichum excelsum–altitudum* and *C. tropicicola,* but they are morphologically different. *C. cariniferi* holds different 77 and 91 base pairs compared with *C. tropicicola* and *C. excelsum-altitudum* respectively. *Colletotrichum cariniferi* has much larger conidia than that of closely related strains in the tree (conidia (24.1–) 26.8–33 (–36.1) × (7.9–) 8.3–9.6 (–10.2) µm (N=30), L/W ratio = 3.4 vs. conidia of *C. tropicicola* 13–16.5×5–7 µm and *C. excelsum-altitudum* 14.8 ± 0.8 × 5.8 ± 0.4 µm) (Noireung 2012; Tao et al., 2013). Blastn searches with sequence of MFLUCC 14-0100 resulted in 100% matches with ITS sequence of endophytic *Colletotrichum* sp. strain S4 isolated from *Dendrobuim nobile* in China (GenBank FJ042517, Yuan et al., 2009) and 96% identity with TUB2 sequences of *C. arxii* strain CBS 169.59 isolated from *Oncidium excavatum* (GenBank KF687868, Liu et al., 2014) in Netherlands and another *C. arxii* strain CBS 132511 isolated from *Paphlopedilum* sp. in Germany (GenBank KF687881, Liu et al., 2014) respectively. *Colletotrichum cariniferi* from stems of *D. cariniferum* is introduced as a new species.



Note a Surface view of center of colony. b Conidiomata. c. i–j Appressoria. d–h Conidiophores. k–m Conidia. *Scale bars*: b = 100 µm, c–d = 5 µm, e–h = 10 µm, i–m = 5 µm

Figure 3.2 *Colletotrichum cariniferi* (holotype)

Colletotrichum chiangraiense X.Y. Ma, K. D. Hyde & Jayawardena, sp.nov.

Fungal Name Number: FN570512

Etymology: In reference to the host sample site Chiang Rai, Thailand.

Holotype: MFLU 17-1201.

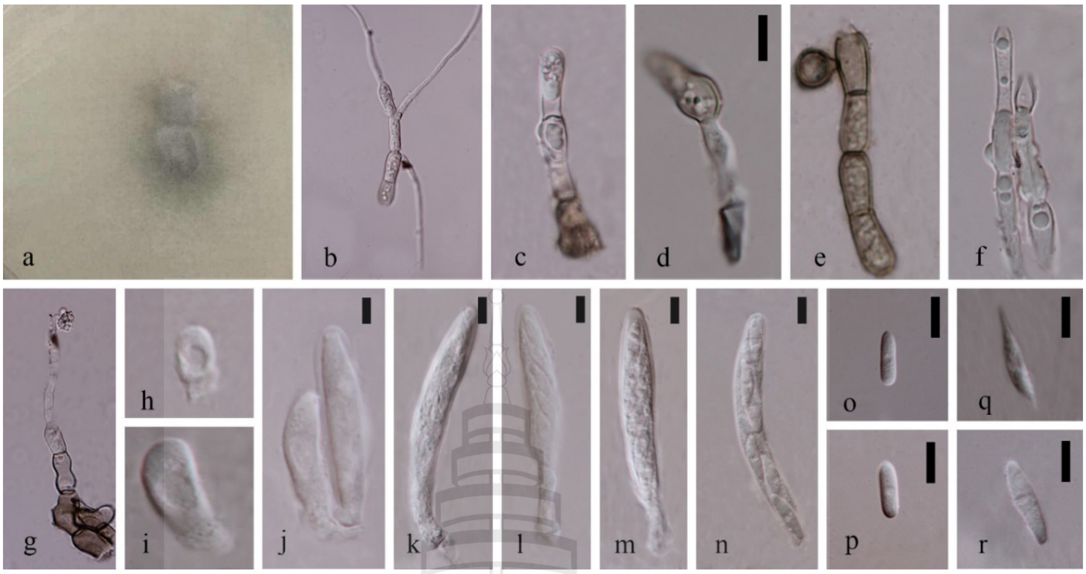
Description: *Asexual morph* not observed.

Sexual morph on CMA. Vegetative hyphae 4.6 ± 1.8 µm diam (N=20), hyaline to pale brown, smooth-walled, septate, branched. Chlamydospore globose, brown. Hyphae fusion and crozier observed. Ascomata rare, covered by mycelia, black. Appressoria (14.4–) 15.3–19.6 (–20.5) × (7.4–) 7.3–7.9 (–8) µm (N=2), single, outline ampulliform or ovate, pale brown. Asci (30.7–) 33.4–52.7 (–72.18) × (5.7–) 6.5–8.2 (–9.4) µm (N=15), cylindrical, straight to curved, unitunicate, 8–spored. Ascospores (11–) 11.9–15.4 (–16.7) × (2.2–) 2.8–3.8 (–4.4) µm (N=20), L/W ratio = 4.2, bi-seriately, smooth-walled, cylindrical or fusiform, one guttule in the middle, hyaline.

Cultures on CMA flat with entire margin. Growth rate: 0.6cm/day, with 20-days for sporulation. Fluffy, dark green in the middle and white margin, reverse black in the middle.

Material examined. Thailand, Chiang Rai, Wat Phra That Doi Tung (Temple of Doi Tung Pagodas), the host *Dendrobium* sp.2 was collected on 19 December 2013, Collector: Sureeporn Nontachaiyapoom, Natdanai Aewsakul, Xiaoya Ma. Holotype: MFLU 17–1201.

Notes: *Colletotrichum chiangraiense* (MFLUCC 14-0119) formed a single branch with the support of 81ML/1.00BI/78MP in boninense species complex. It has 125 different base pairs (mainly in ITS and ACT) from the closest strain *C. cymbidiicola.* Blastn searches with sequences of MFLUCC 14-0119 resulted in 99% identity with ITS of the endophytic *C. crassipes* strain DO93 (GenBank KP050648) isolated from *Dendrobium officinale* in China (Unpublished), 99% identity with ACT of the endophytic *Colletotrichum* sp. strain COAD 2105 (GenBank KY407893) isolated from *Cattleya jongheana* in Brazil (Unpublished), 98% identity with TUB2 of the endophytic *C. boninense* strain CBS 125502(GenBank KJ955336) isolated from *Camellia sinensis* in China (Liu et al., 2015) and 98% identity with TUB2 of the endophytic *C. boninense* strain CGMCC 3.15165 (GenBank KC244156) isolated from *Bletilla ochracea* in China (Tao et al., 2013). This species differ from *C. cymbidiicola* in having rare antheridium, mycelia fusion and crozier, which show that this species could be homothallic. Here we introduce the strain isolated from root of *Dendrobium* sp.2 as a new species.



Note a Surface view of center of colony. b Spore germination. c Conidiophore. d Appressoria. e Chlamydospore. f Mycelia fusion. g Crozier. h–n Asci. o–r Ascospores. *Scale bars*: d = 20 µm, g = 20 µm, j–n = 5 µm, o–r = 10 µm.

Figure 3.3 *Colletotrichum chiangraiense* (holotype)

Colletotrichum watphraense X.Y. Ma, K. D. Hyde & Jayawardena sp. nov.

Fungal Name Number: FN570513

Etymology: In reference to the host sample site – Wat Phra temple in Chiang Rai, Thailand.

Holotype: MFLU 17-1202.

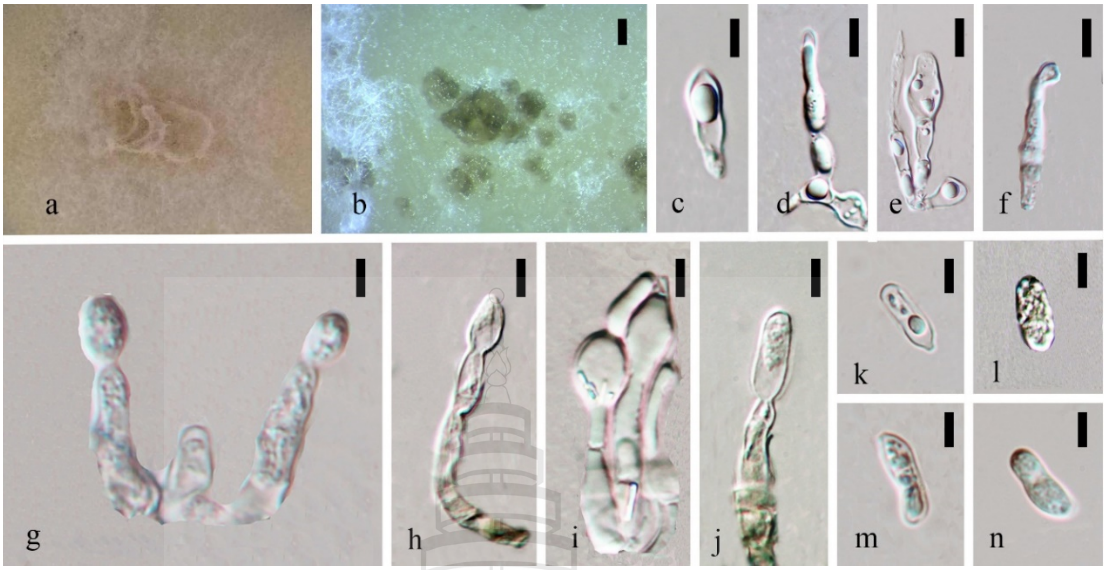
Description: *Sexual morph* not observed.

Asexual morph on CMA. Vegetative hyphae 1.6–4.3 µm diam (N=20), smooth-walled, septate, branched, hyaline. Chlamydospores and appressoria not observed. Conidiomata 200 × 300 µm, brown, Conidiophores (15.8–) 18.5–26.8 (–29.1) × (3.4–) 3.8–5.1 (–5.7) µm (N=16), smooth-walled, septate, branched or single, periclinal thickening, hyaline. Conidia (12.4–) 12.5–14.6 (–15.2) × (4.4–) 4.5–5.8 (–6.1) µm (N=5), L/W ratio = 2.3, aseptate, ellipsoidal, single guttules in the middle, the one part inflated, hyaline.

Cultures on CMA flat with entire margin. Growth rate: 0.45cm/day, with 30-days for sporulation. Fluffy, white to light buff orange. Perithecia isolated. Acervuli under white cotton-like mycelia, irregular, asymmetrical surface, light brown to brown.

Material examined. Thailand, Chiang Rai, Wat Phra That Doi Tung (Temple of Doi Tung Pagodas), the host *Dendrobium* sp.2 was collected on 19 December 2013, Collector: Sureeporn Nontachaiyapoom, Natdanai Aewsakul, Xiaoya Ma. Holotype: MFLU 17–1202.

Notes: MFLUCC 14-0123 presented a singular branch with other species and only supported by 1.00BI in boninense species complex. There were 42bp (2.6%) and 85bp (5.2%) differences in GAPDH between *Colletotrichum watphraense* and its close strains *Colletotrichum boninense*/*C. novae-zelandiae* respectively. The closest matches in a blastn search with ITS sequences of the strain MFLUCC 14-0123 are *C. cymbidiicola* strain FS21 (GenBank KP689224) iaolated from a rare medical plant *Huperzia serrata* with 99% identity in China (Wang et al., 2016), *C. gloeosporioides* strain Trtsf02 (GenBank GU479899) isolated from *Trillum tschonoskii* with 99% identity in China (Unpublished) and pathogenic *C. boninense* strain CO5016 (GenBank GU935883) isolated from ginseng with 99% identity in Korea (Unpublished). GAPDH and ACT sequences blastn results showed its closest matches are pathogenic *C. citricola* strain SXC 151 (GenBank KC293736) isolated from Proteaceae with 99% identity in Netherlands (Liu et al., 2012) and *C. boninense* strain CBS 125502 (GenBank KJ954462) isolated from *Camellia* sp. with 99% identity in unknown locality (Liu et al., 2015). Blastn search with TUB2 sequence results in 99% identity with two *C. boninense* strains CBS 125502 (GenBank KJ955336) and the strain CGMCC 3.15165 (GenBank KC244156) as mentioned above. The conidiophores were much longer (40 µm long) in *C. boninense*. Conidia of the strain CBS 123755 have straight, cylindrical to clavate, conidia with a rounded apex; and base with a prominent hilum, sometimes with two large polar guttules, which is different from *Colletotrichum watphraense*. Here we assigned the strain isolated from stem of *Dendrobium* sp.2 as a new species.



Note a Surface view of center of colony. b Fruiting body. c–j Conidiophores. k–n Conidia. *Scale bars*: b = 200 µm, c–n = 5 µm.

Figure 3.4 *Colletotrichum watphraense* (holotype)

Colletotrichum doitungense X.Y. Ma, K. D. Hyde & Jayawardena sp.nov.

Fungal Name Number: FN570514

Etymology: In reference to the host sample site Doi tung, Chiang Rai, Thailand.

Holotype: MFLU 17-1200.

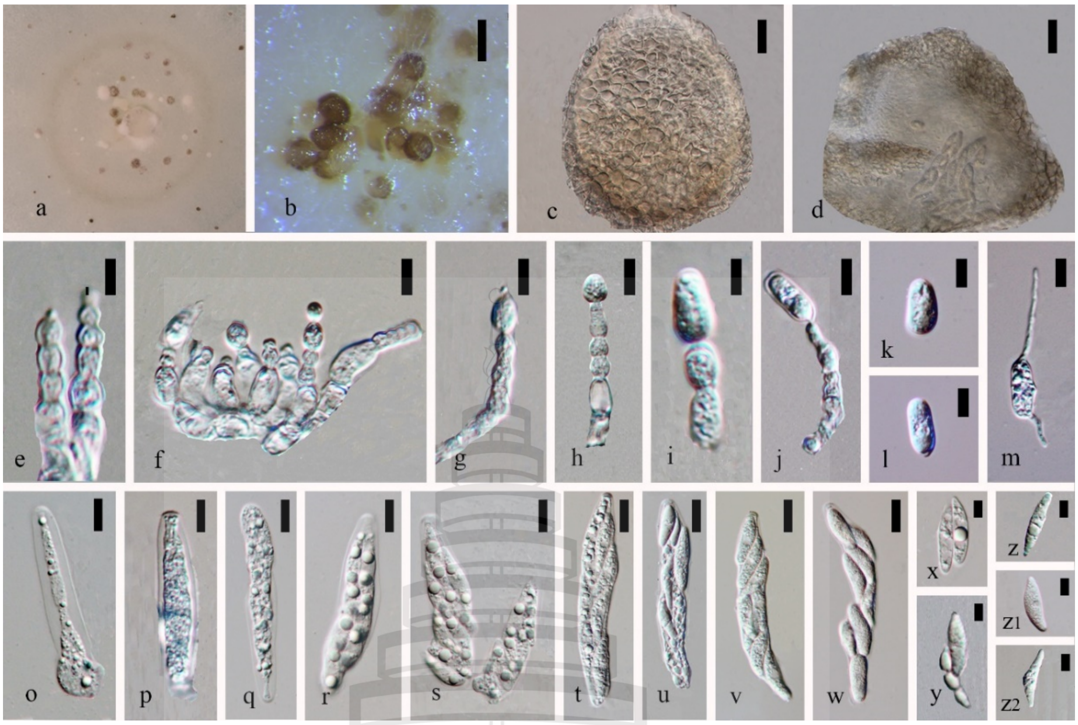
Description: Asexual morph on CMA. Vegetative hyphae 1.1–3.5 µm diam, hyaline, smooth-walled, septate, branched. Setae and chlamydospores not observed. Conidiomata and ascomata cluster together. Conidiophores (9.1–) 14.3–26.8 (–29.4) × (3–) 3.1–4.5 (–5) µm, smooth-walled, unbranched, septate, constricted septum, hyaline. Conidiogenous (3.1–) 3.2–5.8 (–7.5) × (2.6–) 3–4 (–4.5) µm (N=14), cells globose to sub-globose, smooth-walled, hyaline. Conidia (6.6–) 8.6–13.8 (–15) × (2.6–) 3.8–8.9 (–13.8) µm (N=22), L/W ratio = 1.75, globose to ellipsoidal, both ends rounded, smooth-walled, hyaline.

Sexual morph on CMA. Ascomata (125.5–) 126.9–133.7 (–135.1) × (101.3–) 101.8–104.3 (–104.8) µm (N=10), sub-globose, closed, pale brown to brown. Peridium 3–11.5 µm thick, Asci (51.1–) 53.7–70.6 (–71.6) × (8.5–) 8.8–10.1 (–10.4) µm (N=8), cylindrical, slight curved, composed of pale to medium brown flattened angular cells, unitunicate, smooth-walled, 8–spored, hyaline. Ascospores (16.1–) 17.5–21.5 (–23.4) × (4.5–) 5.1–7.0 (–7.5) µm (N=20), L/W ratio = 3.2, fusiform, blunt to somewhat acute or acute both ends, bi–seriately, single guttule in the middle, septate when immature, smooth-walled, hyaline.

Cultures on CMA flat with entire margin. Fluffy, white, reverse same. Growth rate: 0.6cm/day, with 20-days for sporulation. Brown ring in the middle. Perithecia gregarious. Acervuli and ascomata in mass light brown to brown.

Material examined. Thailand, Chiang Rai, Wat Phra That Doi Tung (Temple of Doi Tung Pagodas), the host *Dendrobium* sp.2 was collected on 19 December 2013, Collector: Sureeporn Nontachaiyapoom, Natdanai Aewsakul, Xiaoya Ma. Holotype: MFLU 17–1200.

Notes: *Colletotrichum doitungense* form an independent lineage from other strains with good support (66ML/1.00BI/73MP) in boninense species complex. The ITS sequence of MFLUCC 14-0128 100% matches with unpublished pathogenic *C. cymbidiicola* strain OORC18 (GenBank JX902424) isolated from orchid in India and *C. karstii* strain R001 (GenBank JN715846) isolated from blackberry in Colombia (Unpublished). Blastn researches with sequences of MFLUCC 14-0128 results in 98% identity with GAPDH sequence of endophytic *C. boninense* strain CGMCC 3.15168 (GenBank KC843491) isolated from *Bletilla ochracea* in China (Tao et al., 2013), 99% identity with ACT sequence of *C. boninense* strain CBS 125502 (GenBank KJ954462) and 99% identity with TUB2 sequence of *C. citricola* strain SXC 151 (GenBank KC293656) as mentioned above. Its septate conidiophores are constricted at the septum, conidiogenus cell globose to sub-globose, which differ from that in *C. boninense.* This strain has 2 and 0 in ITS, 6 and 1 in GAPDH, 3 and 2 in ACT, 17 and 16 base pair differences from its sister taxon *C. torulosum* and MFLUCC 14-0261 respectively. Here we introduce *Colletotrichum doitungense* isolated from root of *Dendrobium* sp.2 as a new species.



Note a Surface view of center of colony. b Fruiting body. c–d Ascomata. e–j Conidiophores. k–l Conidia. m Spore germination. n–w Asci. x–z2 Ascospores. *Scale bars*: b = 100 µm, c–d = 20 µm, e–m = 5 µm, n–v = 10 µm, w–a = 5 µm.

Figure 3.5 *Colletotrichum doitungense* (holotype)

Colletotrichum parallelophorum X.Y. Ma, K. D. Hyde & Jayawardena sp. nov.

Fungal Name Number: FN570515

Etymology: In reference to the parallel conidiophores.

Holotype: MFLU 17-1198.

Description: Asexual morph on CMA. Vegetative hyphae 2–4.3 µm diam (N=30), smooth-walled, septate, branched, hyaline to pale brown. Chlamydospores not observed. Conidiomata acervular, orange. Appressoria (56.7–) 60.2–79.2 (–81.2) × (2.8–) 2.9–3.7 (–3.9) µm (N=8), single, sub-globose, brown, rare. Conidiophores and setae formed on a cushion of pale brown cells (1.9–) 2.4–4 (–4.6) µm diam. Setae medium brown, smooth-walled, 2 or 3-septate; base cylindrical, constricted at the base, apex acute. Conidiophores (18.3–) 20.8–34 (–41.2) × (2.6–) 2.8–4.3 (–5.4) µm (N=20), smooth-walled, 2 to 3-septate, branched, hyaline to pale brown. Conidiophores and setae formed on a cushion of pale brown prismatic cells, sometimes with guttules. Conidia (12.1–) 13.8–16.8 (–18.9) × (3.3–) 4.4–7.5 (–7.9) µm (N=50), L/W ratio = 2.6, hyaline, smooth-walled, with 1 to 4 guttules, cylindrical with both ends rounded.

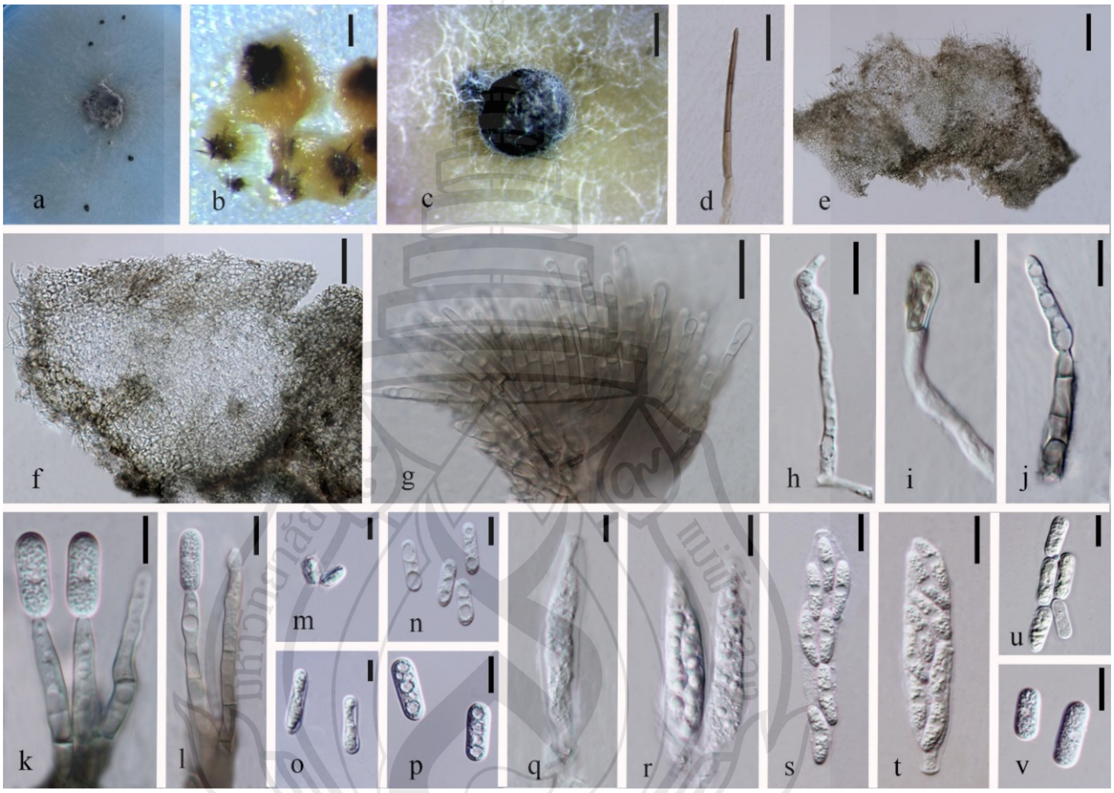
Sexual morph on CMA. Ascomata (267–) 261.4–342.3 (–346.2) × (190.4–) 173.0–272.5 (–280) µm (N=3), globose, glabrous, Ascomata isolated, scattered, irregular and asymmetrical, black. Peridium 13.6–46.4 µm thick, consist of pale to medium brown flattened angular cells. Ascogenous hyphae hyaline, smooth-walled. Asci (43.3–) 44.1–63.3 (–66.5) × (7.6–) 8.0–9.8 (–10) µm (N=7), cylindrical, straight, unitunicate, 8–spored. Ascospores (13.9–) 14.1–18 (–20.9) × (3.1–) 3.9–5.4 (–5.8) µm (N=23), L/W ratio = 3.5, uni–to bi–seriately, aseptate, smooth-walled, ellipsoidal, single guttules in the middle, both ends rounded, hyaline.

Cultures on CMA flat with entire margin. Growth rate: 0.4cm/d, with 20-days for sporulation. With fluffy, light green and white mycelia. Ascomata sometimes growing together with acervuli.

Material examined. Thailand, Chiang Rai, Wat Phra That Doi Tung (Temple of Doi Tung Pagodas), the host *Dendrobium* sp.1 was collected on 19 December 2013, Collector: Sureeporn Nontachaiyapoom, Natdanai Aewsakul, Xiaoya Ma. Holotype: MFLU 17–1198.

Notes: Strains MFLUCC 14-0077, MFLUCC 14-0079 and MFLUCC 14-0083 had identical sequence data and they formed a single clade with MFLUCC 14-0082 and MFLUCC 14-0085. They are closely related to *Colletotrichum excelsum-altitudum* and *C. tropicicola*. MFLUCC 14-0077, MFLUCC 14-0079, MFLUCC 14-0082, MFLUCC 14-0083 and MFLUCC 14-0085 have similar morphological characteristics. Therefore, the five strains are regarded as the same species. There were totally 103bp and 101bp differences between MFLUCC 14-0083 and *C. excelsum–altitudum*/*C. tropicicola* respectively (mainly in GAPDH). Blastn researches with four-gene sequences of five strains presented 99% identity with ITS sequence of *C. cordylinicola* strain LC0886, 80% identity with GAPDH (GenBank JN050229), 90% identity with ACT (GenBank JN050218) and 93% identity with TUB2 (GenBank JN050246) sequences of *C. tropicola* strain LC0598 respectively as mentioned above. Conidia size and shape were very similar among MFLUCC 14-0083, *C. excelsum–*

altitudum and *C. tropicicola*. Appressoria were rare and in strain MFLUCC–14–0083 appressoria were not variable like that in *C. excelsum–altitudum* and *C. tropicicola*. Here we introduced strains MFLUCC 14-0077, MFLUCC 14-0079, MFLUCC 14-0082 and MFLUCC 14-0083 and MFLUCC–14–0085 isolated from stems and roots of *Dendrobium* sp.1 as *Colletotrichum parallelophorum* sp.nov.



Note a Surface view of center of colony. b, c Fruiting body. d Setae. e–f Ascomata. g, j–l Conidiophores. i Appressoria. m–p Conidia. q–t Asci. u–v Ascospore. Scale bars: b = 50 µm, c = 500 µm, d = 20 µm, e = 100 µm, f = 50 µm, g = 20 µm, h–l = 10 µm, m–n = 5 µm, o–v = 10 µm.

Figure 3.6 *Colletotrichum parallelophorum* (holotype)

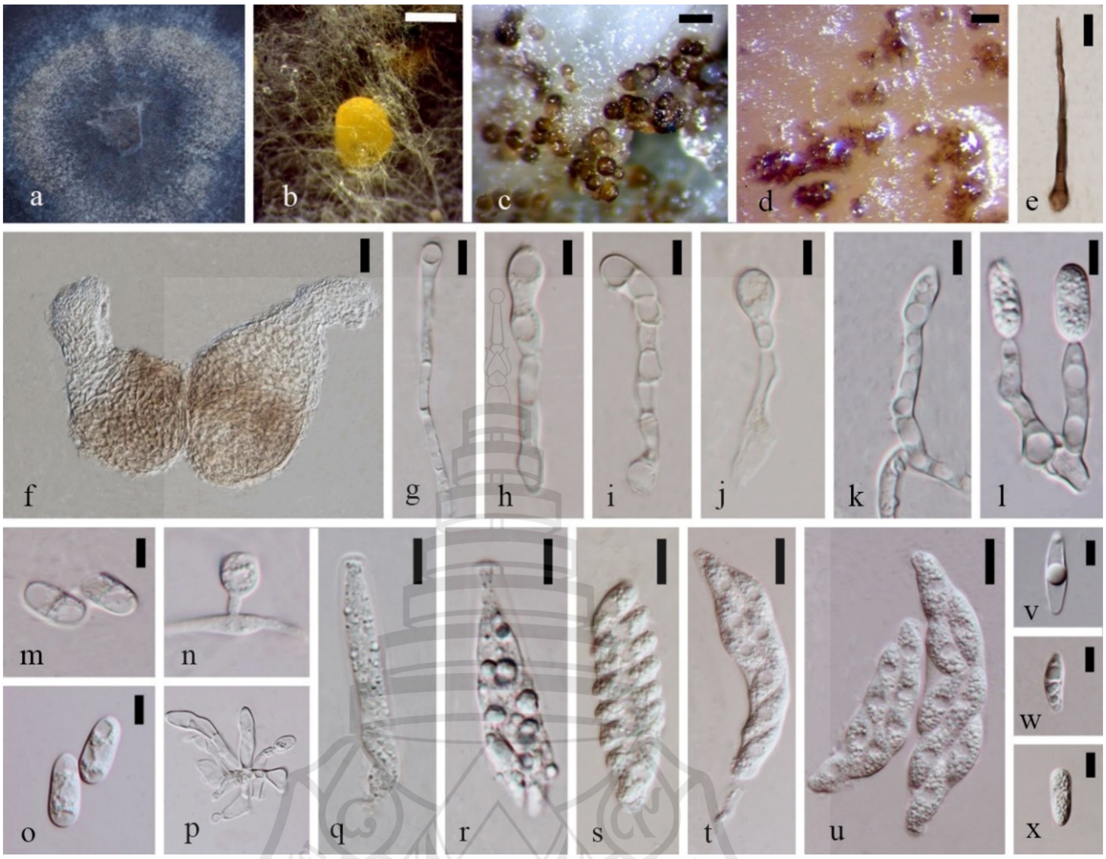
Colletotrichum citricola F. Huang, L. Cai, K.D. Hyde & H.Y. Li

Description: Asexual morph on CMA. Vegetative hyphae 3.1 ± 1.1 µm diam (N=20), smooth-walled, septate, branched, hyaline. Chlamydospores globose, hyaline. Conidiomata ovoid, orange. Setae (51.8–) 54.1–67.8 (–68.5) × (2.3–) 2.4–5.8 (–7.2) µm (N=6), smooth-walled, 1 or 3–septate, contracted to slightly inflated base, tapering to the apex, apex acute, pale brown to brown. Conidiophores (10.8–) 16.7–25.6 (–30.6) × (3.1–) 4–5.3 (–5.6) µm (N=27), smooth-walled, septate, hyaline. Conidia (12.5–) 13.4–15 (16.5–) × (5–) 5.9–6.9 (–7.2) µm (N=40), L/W ration = 2.2, ellipsoidal, smooth-walled, hyaline.

Sexual morph on CMA. Ascomata (34.5–) 46.4–84.9 (–87.1) × (31.7–) 33.8–46.5 (–50.9) µm (N=5), globose, ostiolate, clustered, pale brown to dark brown. Peridium 1.7–5.8 µm thick, composed of pale to medium brown, flattened, angular cells. Ascogenous hyphae hyaline, smooth-walled. Asci (41.3–) 49.4–65 (–71.6) × (8.3–) 9.5–12.9 (–14.3) µm (N=36), cylindrical, unitunicate, straight or curved, 8–spored. Ascospores (14.4–) 14.8–17.5 (–19.3) × (5.4–) 5.7–7.1 (–7.6) µm (N=25), L/W = 2.5, uni–or bi–seriately, smooth-walled, hyaline, fusiform or one end slightly rounded, with a single guttule in the middle.

Cultures on CMA flat with entire margin. Growth rate: 0.6cm/day, with18-days for sporulation. Fluffy, pale mycelia float on the dark scarlet pigment medium, reverse dark brown. Perithecia gregarious. Orange acervuli and ascomata in mass form thick globules.

Notes: Strains MFLUCC 14-0129 and MFLUCC 14-0131 had similar sequence data, cultures and conidia. There were 5bp and 7bp difference between the strains and *Colletotrichum camelliae–japonicae* and *C. citricola* respectively. ITS sequence is 99% identity with unpublished *C. boninense* strain LD3-8-1 isolated from strawberry in China (Unpublished). Blastn searches sequences results in GAPDH (GenBank KC293736) and TUB2 (GenBank KC293656) sequences of *C. citricola* strain SXC 151 as mention above. ACT sequence is closest to *C. karstii* strain 42a (GenBank KT122921) isolated from *Coffea arabica* in Mexico (Cristobal-Martinez et al., 2016). All morphological characteristics of the two strains were nearly the same as the protologue of *C. citricola.* Therefore, we name strains MFLUCC 14-0129 and MFLUCC 14-0131 as *C. citricola*. When compared with *C. camelliae–japonicae* (conidia: 11–14.5 ×5–6.5µm, mean ±SD = 12.5 ±0.8 ×5.5 ±0.3µm, L/W=1.5; ascospores: 13.5–18.5 ×4–5.5 µm, mean ± SD = 16.5 ±1.1×5±0.4µm, L/W = 3.3), strains MFLUCC 14-0129 and MFLUCC 14-0131 have shorter conidia and wider ascospores.



Note a Surface view of center of colony. b Conidiomata. c Fruiting bodies. d Fruiting body with setae. e Setae. f Ascomata. g–l Conidiophores. m–n Conidia. o Chlamydospore. p–u Young asci v–x Ascospores. *Scale bars*: b = 500 µm, c = 200 µm, e–f = 10 µm, g = 5 µm, m–n = 5 µm, q–u = 10 µm, v–x = 5 µm.

Figure 3.7 *Colletotrichum citricola* (MFLUCC 14-0129 and MFLUCC 14-0131)

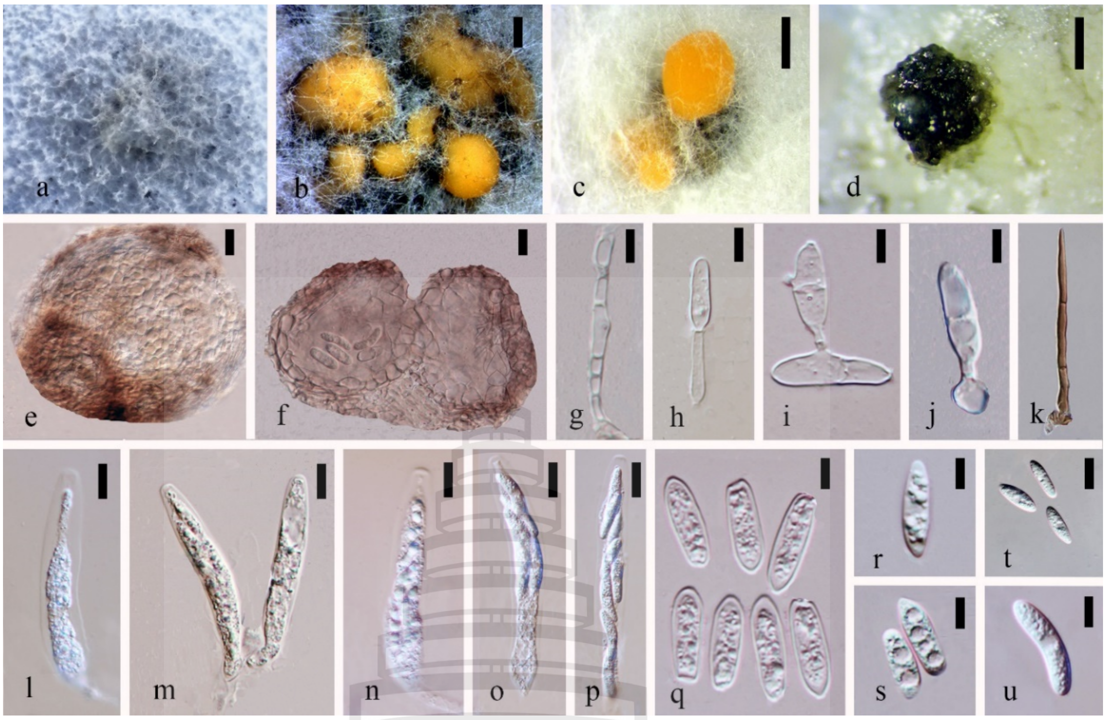
Colletotrichum fructicola Prihastuti, L. Cai & K.D. Hyde

Description: Asexual morph formed on CMA. Vegetative hyphae 2.6–5 µm diam (N=20), smooth-walled, septate, branched, hyaline. Appressoria and chlamydospores not observed. Conidiomata 500 × 400 µm (N=3), clustered, sub-globose, smooth-walled, orange. Conidiophores rare, septate, hyaline. Conidia (12.8–) 13.8–16.6 (–18.6) × (2.7–) 3.5–7.8 (–16) µm (N=21), L/W = 2.9, ellipsoidal, smooth-walled, septate, hyaline.

Sexual morph forming on CMA. Ascomata globose, pale brown to dark brown. Peridium (131.9–) 138.4–163.6 (–171.5) × (120.9–) 123.6–142.1 (–143.2) µm (N=4), composed of medium brown, flattened, angular cells. Setae (53–) 57.2–73.1 (–83.3) × (3.4–) 3.5–4(–4.1) µm (N=6), grow on the fruiting body, 2-septate, smooth-walled, contracted at the base, apex slightly rounded, brown to dark brown. Asci (57.6–) 61.2–82.6 (–94.3) × (8.7–) 9.3–13.3 (–15.8) µm (N=12), cylindrical, unitunicate, 8–spored. Ascospores (10–) 12–20 (–20.9) × (3.6–) 4.1–5.2 (–5.3) µm (N=10), L/W = 3.4, ellipsoidal to reniform, somewhat fusiform or acute both ends, 1 to 4 guttules, uni-to bi-seriate, smooth-walled, hyaline.

Cultures on CMA flat with slight serrated margin. Growth rate: 0.9cm/day, with 14-days for sporulation. Cottony, light brown to white from middle to the margin, reverse white to light brown with black spots. Ascomata gregarious and/or isolated. Acervuli and ascomata sometimes gregarious.

Notes: Strains MFLUCC 14-0087, MFLUCC 14-0148 and MFLUCC 15-0262 had the identical sequences to *Colletotrichum fructicola*. The ITS and GAPDH sequences of them 100% match with many different unpublished species. Blastn searches with ACT sequence of them results in 99% identity with the ex-holotype culture of *C. fructicola* strain ICMP 18581 (GenBank JX009501) isolated from *Coffea arabica* in Thailand (Weir et al., 2012), which we involved it in phylogenetic analysis. TUB2 sequences of them are 99% identity with *C. boninense* strain CBS 125502 (GenBank KJ955336) as mentioned above. Their ascomata, conidia, asci and ascospores were also similar. Conidia were the same size as the ex-type strain of the pathogen *Colletotrichum fructicola* (9.7–14 × 3–4.3µm) found in coffee berries (Prihastuti et al., 2009). However, ascomata were much smaller and asci as well as ascospores were much larger than the ex-type from coffee berries. In the protologue, *C. fructicola* was introduced with ascomata as 345.67 ± 36.83 × 431.33 ± 69.89 µm, asci as 41.22 ± 7.02 × 7.61 ± 0.58 µm and ascospores as 11.91 ± 1.38 × 3.32 ± 0.35 µm. Here we name strains MFLUCC 14-0087, MFLUCC 14-0148 and MFLUCC 15-0262 isolated from leaves of *Dendrobium* sp.1 and *Dendrobium* sp.3*,* root of *Dendrobium* sp.2 as *Colletotrichum fructicola*.



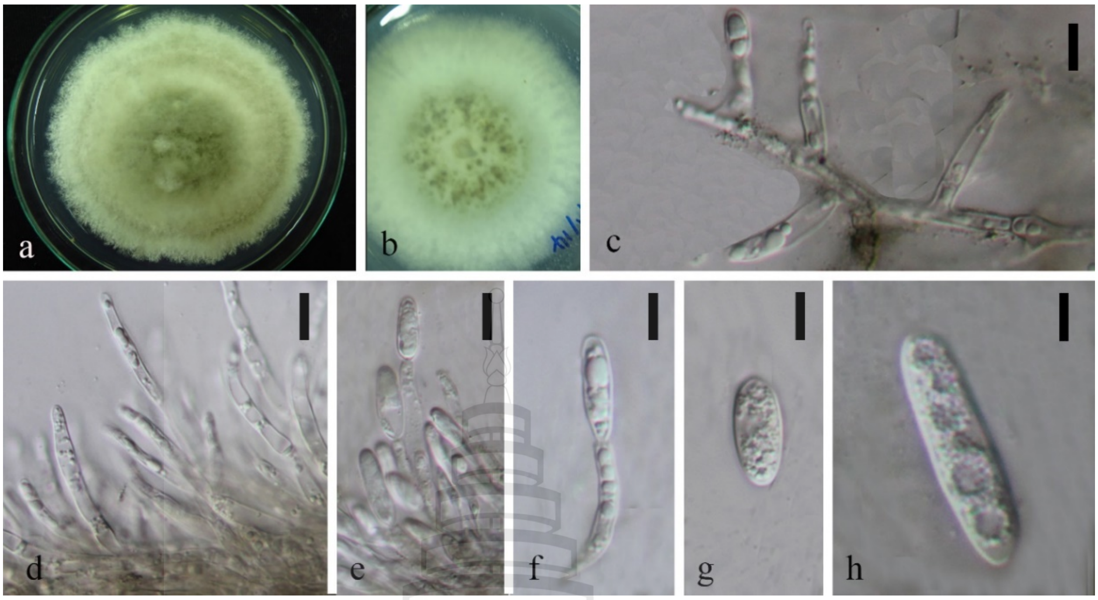
Note a Surface view of center of colony. b Conidiomata and ascomata. c–d Conidiomata. e–f Ascomata. g–j Conidiophores. l Setae. m–q Asci. r–v Ascospores. *Scale bars*: b–d = 500 µm, e–f = 20 µm, g–j = 5 µm, l = 10 µm, m–q = 10 µm, r–u = 5 µm.

Figure 3.8 *Colletotrichum fructicola* (MFLUCC 14-0087, MFLUCC 14-0148 and MFLUCC 15-0262)

Colletotrichum jiangxiense F. Liu & L. Cai

Description: *Sexual morph* not observed.

Sexual morph not observed. Asexual morph on PDA. Vegetative hyphae 1.3–2.1 µm diam (N=20), smooth-walled, septate, branched, hyaline. Setae and chlamydospores not observed. Conidiophores (12.7–) 13.5–21.4 (–23.4) × (1.9–) 2–3 (–3.2) µm (N=8), branched, hyaline. Conidia (8.6–) 9–12.4 (–13.2) × (3.5–) 3.6–4.4 (–4.5) µm (N=4), L/W = 2.6, ellipsoidal to cylindrical, smooth-walled, aseptate, one end more blunter than the other end, hyaline.



Note a Surface view of center of colony. b Colony from below. c–f Conidiophores. g–h Conidia. Scale bars: c–f = 5 µm, g–h = 2.5 µm.

Figure 3.9 *Colletotrichum jiangxiense* (MFLUCC 14-0091 and MFLUCC 14-0092)

Cultures on PDA flat with entire margin. Growth rate: 0.4cm/day, with 18-days for sporulation. Aerial mycelia dense, cottony, pale to light brown, with brown outline ring close to the edge, mycelia in the middle dark brown, reverse white to light brown.

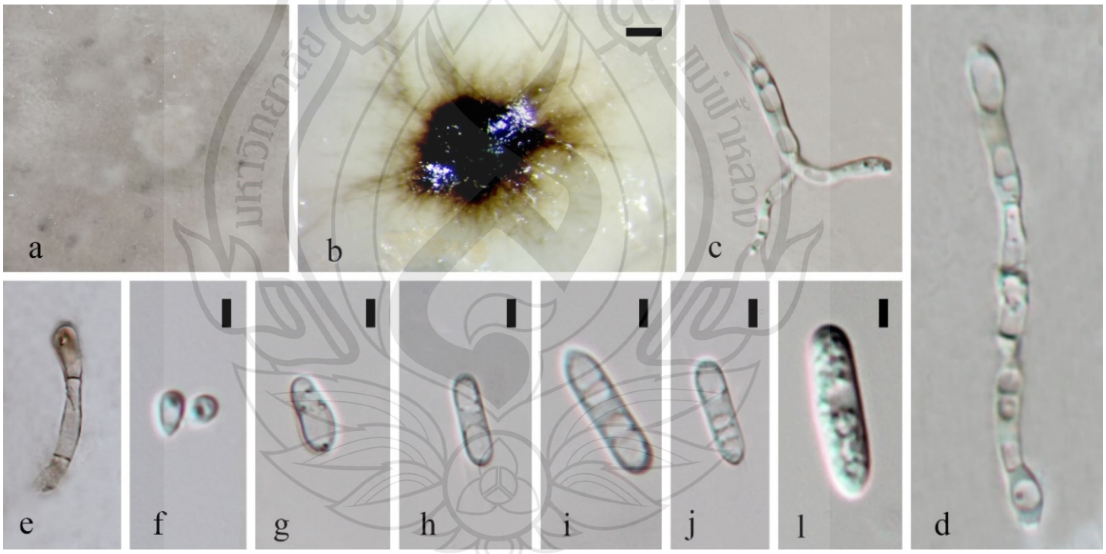
Notes: Strains MFLUCC 14-0091 and MFLUCC 14-0092 were the same species as they grouped with high support (98ML/1.0BI/87MP). They formed a very close clade with the pathogen *C. jiangxiense* isolated from *Camellia.* However, different media were used in these two studies. Blastn researches with ITS sequences results in 100% identity with *C. gloeosporioides* strain SS1-MS1 (GenBank KP900279) isolated from *Huperzia serrate* in China (Wang et al., 2016). GAPDH, ACT and TUB2 sequences of MFLUCC 14-0091 and MFLUCC 14-0092 are closest to *C. kahawae* subsp.*ciggaro* strain ICMP 18534 (GenBank JX009904) with 98% identity isolated from *Kunzea ericoides* in New Zealand, 99% identity with strain ICMP 12952 (GenBank JX009431) isolated from *Persea Americana* in New Zealand, and 99% identity with strain CO22-1 (GenBank KJ001124) isolated from *Rubus glaucus* in Colombia respectively (Weir et al., 2012; Afanador-Kafuri et al., 2014).

Conidia size reported for *C. jiangxiense* was 15.2 ± 1 × 5.2 ± 0.4 µm, which was larger and faster growing than the strains isolated in this study. There were 5bp differences between strain MFLUCC 14-0091 and *C. jiangxiense*. Here we name both of isolates from leaves of *Dendrobium* sp.1 as *C. jiangxiense*.

Colletotrichum orchidophilum Damm, P.F. Cannon & Crous

Description: *Sexual morph* not observed.

Sexual morph not observed. Asexual morph on SDA. Vegetative hyphae 1.9–5.4 µm diam, smooth-walled, septate, branched, hyaline to pale brown. Chlamydospores not observed. Appressoria brown, smooth-walled. Conidiomata superficial or under mycelia, smooth-walled, 200 × 300 µm, black. Conidiophores smooth-walled, branched or unbranched, hyaline. Conidiophores and appressoria rare. Conidia (9.6–) 11.7–14.1 (–14.7) × (2.9–) 3.5–4.4 (–4.8) µm, L/W = 3.3, cylindrical, straight, with 1 to 4 guttules, one end somewhat acute, hyaline.



Note a Surface view of center of colony. b Fruiting body. c–d Conidiophores. e Appressoria. f–k Conidia. *Scale bars*: b = 200 µm, f–k = 5 µm.

Figure 3.10 *Colletotrichum orchidophilum* (MFLUCC–14–0161 and MFLUCC–14–0162)

Cultures on SDA flat with entire margin. Growth rate: 0.44cm/day, with nearly 20-days for sporulation. White with dark green mycelia around the middle, white edge, reverse white. Cultures on PDA flat with entire margin. Growth rate: 0.45cm/day, with 30-days for sporulation. Fluffy, white, reverse light brown. Acervuli in mass black, irregular, asymmetrical, merging in media.

Notes: Strains MFLUCC–14–0161 and MFLUCC–14–0162 belong to a single species as they have similar conidia, cultures and the nearly identical sequence data. The support values of 100/1.00/100 totally grouped them with *C. orchidophilum* and their branch lengths are slightly different. Blastn researches sequences of MFLUCC 14-0161 and MFLUCC 14-0162 results in 99% identity with ITS (GenBank NR111729), GAPDH (GenBank JQ948481) and ACT (GenBank JQ949472) sequences of ex-holotype culture of *C. orchidophilum* strain CBS 632.80 isolated form *Dendrobium* sp. in USA (Damm et al., 2012). TUB2 sequence is 99% identity with pathogenic *C. fructicola* strain AV24 (GenBank KX786459) isolated from grapevine shoots in Brazil (Santos et al., 2018) and *C. gloeosporioides* strain TL-2 (GenBank KC913205) isolated from *Camellia sinensis* in China (Guo et al., 2014). Because no conidiophores were detected in culture, no measurement for the conidiophores could be given. In this study, strains MFLUCC 14-0161 and MFLUCC 14-0162 of *C. orchidophilum* were isolated from leaves of *D. harveyanum.*

Colletotrichum boninense Moriwaki, Toy. Sato & Tsukib.

For an illustrated description please refer Damm et al. (2012a).

Notes: Strains MFLUCC 14-0086, MFLUCC 14-0124 and MFLUCC 15-0261 grouped with *C. boninense* and MFLUCC 14-0128. All have very similar sequences as those as the ex-type of with *C. boninense* (only 2bp difference), while there was 11 base pair deviations between these strains and *Colletotrichum doitungense* sp. nov. Blastn researches with ITS sequences of them result in 100% identity with ITS sequence of endophytic *C. boninense* strain SL-ML18 (GenBank KP900269) isolated from *Huperzia serrate* in China (Wang et al., 2016) and strain CGMCC 3.15168 (GenBank KC244158) as mentioned above. GAPDH and ACT sequences of them are 97% identity with *C. boninense* CGMCC 3.15168 (GenBank KC843491) and 100% identity with *C. fructicola* strain 1104-7 (GenBank KX885159) isoalted from *Malus* *domestica* in China (Liang et al., 2017). TUB2 blastn result are 99% identity with *C. fructicola* strain AV24 (GenBank KX786459) and *C. gloeosporioides* strain TL-2 (GenBank KC913205) as mentioned above. Here we identify these three strains isolated from leaves of *D. catenatum* and *Dendrobium sp.*1, stem of *D.* sp.2 respectively as *Colletotrichum boninense*.

***Colletotrichum* sp.indet.**

Notes: Strain MFLUCC 14-0120 failed to sporulate and lacks a complete morphological description. It formed a single branch close to *C. camelliae-japonicae,* MFLUCC 14-0129 / MFLUCC 14-0131 with 67ML/1.00BI/62MP support. There were 15bp and 11bp differences mainly in the ACT gene region among MFLUCC 14-0120 and *C. camelliae–japonicae,* MFLUCC 14-0129/MFLUCC 14-0131 respectively. ITS sequence blastn of MFLUCC 14-0120 showed many different kinds of species with 99% identity. Blastn searches with GAPDH (GenBank KC293736) and TUB2 (GenBank KC293656) sequences result in 99% identity with *C. citricola* strain SCX 151 as mentioned above. The ACT of MFLUCC–14–0120 is 98% identity with *C. boninense* strain CBS 125502 (GenBank KJ954462) as mentioned above. Here we listed it as an unidentified species.

3.4 Discussion

3.4.1 *Colletotrichum* Species Associated with Orchid Species

Many *Colletotrichum* species have been isolated from Orchidaceae plants sampled in China in previous studies (e.g. Yang et al., 2011; Chen et al., 2012; Tao et al., 2008, 2013). Eighteen *Colletotrichum* species have been reported from these studies. For example, *Colletotrichum beeveri* isolated from *Pleione bulbocodioides; C. bletillum* and *C. caudasporum* isolated from *Bletilla ochracea*; *C. oncidii* isolated from *Oncidium sp.* (Yang et al., 2011; Damm et al., 2012a; Tao et al., 2013). The present study is the first to report endophytic fungi from *Dendrobium* spp. in Thailand combining both multi-loci sequence data and morphological characteristics. *Colletotrichum* species in this study were diverse and present in every *Dendrobium* sample collected from all sites. Therefore, we conclude that *Orchidaceae* plants are rich source of endophytic *Colletotrichum* species.

3.4.2 Methods Affecting the Identification

Hyde and Zhang (2008) and Hyde et al. (2009b) suggested that nucleotide sequence data of holotypes or epitypes is essential for analysing phylogenetic relationships among *Colletotrichum* species. A polyphasic method combining morphological characteristics and molecular phylogenetics has been applied to define and re-order species in this genus (Cai et al., 2009; Hyde et al., 2009; Damm et al., 2012a, b, c; Jayawardena et al., 2016a, b).

We found some differences in the *Colletotrichum gloeosporioides* species complex backbone tree as compared to that constructed with more genes in Weir et al. (2012), Udayanga et al. (2013) and Jayawardena et al. (2016a). *Colletotrichum jiangxiense* clusters with *C. rhexiae* rather than *C. kahawae. C. fructicola* is closer to *C. siamense* rather than *C. nupharicola.* The genes CHS-1 and HIS3 were not involved in this study and may be responsible for the differences. Actually CHS-1 and HIS3 could resolve species in several other species complexes of *Colletotrichum* (Jayawardena et al., 2016a). However, the combination of ApMat and GS turned out to be the most effective genes in species resolution in the *Colletotrichum gloeosporioides* species complex (Liu et al., 2015). Our study is the first to use multiple gene sequences to analyze fungal endophytes from *Dendrobium* orchids.

3.4.3 Relationship between *Colletotrichum* and *Dendrobium*

Few species identified in this study showed host–specificity. Nevertheless, this study provides evidence that *C. orchidophilum* colonizes a wide range of hosts in *Orchidaceae* (Damm et al., 2012b). In addition, we found that leaves contained higher numbers of *Colletotrichum* species than other parts. All *Dendrobium* leaves in this study were colonized by *Colletotrichum* strains. Our results are similar to those of Chen et al. (2011) who isolated more *Colletotrichum* species from stems and leaves of *Dendrobium* species than that from roots.

The majority of *Colletotrichum* species isolated from *Dendrobium* species in this study were fungal endophytes. This was also reported by Chen et al. (2011) and Yuan et al. (2009). The most common fungal endophytes in leaves of *Lepanthes rupestris* (*Orchidaceae*) sampled in a Puerto Rican forest was a *Colletotrichum* species which showed antagonism against other fungal taxa (Bayman et al., 2002). Most *Colletotrichum* species have been identified as plant pathogens living a hemibiotrophic life strategy, they adopt a biotrophic phase at an early stage and switch to a necrotrophic phase later (Damm et al., 2010; Cannon et al., 2012).

Here we speculate that most isolates in this study might be latent pathogens (Photita et al., 2004), since in the phylogenies, they were nested with pathogenic strains or have previously been reported to cause plant diseases (Tao et al., 2013, Hou et al., 2016). *Colletotrichum jiangxiense* was isolated as a pathogen from leaf lesions of *Camellia* sp. (Liu et al., 2015). *Colletotrichum boninense* was reported as an anthracnose causing agent from *Dendrobium kingianum* in Japan (Moriwaki et al., 2003).

Table 3.4 Fungal isolates and sequences of region/genes used in *Colletotrichum* phylogenetic analysis

Species	Isolate[a]	GenBank accession number			
		ITS	**GAPDH**	**ACT**	**ß–tubulin**
C. acutatum	CBS 128531*	JQ005776	JQ948677	JQ005839	JQ005860
C. aeschynomenes	CBS 128532*	JX010176	JX009930	JX009483	JX010392
C. alcorni	CBS 128534*	JX076858	-	-	-
C. alienum	ICMP 12071*	JX010251	JX010028	JX009572	JX010411
C. annellatum	CBS 128536*	JQ005222	JQ005309	JQ005570	JQ005656
C. anthrisci	CBS 125334*	GU227845	GU228237	GU227943	GU228139
C. aotearoa	CBS 128538*	JX010205	JX010005	JX009564	JX010420
C. arxii	CBS 132511*	NR132055	KF687843	KF687802	KF687881
C. australe	CBS 128540*	JQ948455	JQ948786	JQ949776	JQ950106
C. beeveri	CBS 128541*	JQ005171	JQ005258	JQ005519	JQ005605
C. bidentis	CBS 128542*	KF178481	KF178506	KF178578	KF178602
C. bletillum	CBS 128543*	JX625178	KC843506	KC843542	JX625207
C. boninense	CBS 128544*	JQ005153	JQ005240	JQ005501	JQ005501
C. brasiliense	CBS 128545*	JQ005235	JQ005322	JQ005583	JQ005669
C. brassicola	CBS 128546*	JQ005172	JQ005259	JQ005520	JQ005606
C. brevisporum	CBS 128547*	JQ247623	JQ247599	JQ247647	JQ247635
C. camelliae	ICMP 10643	JX010224	JX009908	JX009540	JX010436
C. caudasporum	CGMCC 3.15106*	JX625162	KC843512	KC843526	JX625190
C. camelliae–japonicae	CGMCC3.18117*	KX853165	KX893583	KX893575	KX893579
C. cereale	CBS 129663	JQ005774	-	JQ005837	JQ005858

Table 3.4 (continued)

Species	Isolate[a]	GenBank accession number			
		ITS	**GAPDH**	**ACT**	**ß–tubulin**
C. chlorophyti	IMI 103806*	GU227894	GU228286	GU227992	GU228188
C. chrysanthemi	IMI 364540	JQ948273	JQ948603	JQ949594	JQ949924
C. citricola	SXC 151*	KC293576	KC293736	KC293616	KC293656
C. clidemiae	ICMP 18658*	JX010265	JX009989	JX009537	JX010438
C. cliviae	CBS 125375*	JX519223	GQ856756	JX519240	JX519249
C. coccodes	CBS 369.75	JQ005775	HM171673	JQ005838	JQ005859
C. colombiense	CBS 129818*	JQ005174	JQ005261	JQ005522	JQ005608
C. cordylinicola	ICMP 18579*	JX010226	JX009975	HM470234	JX010440
C. curcumae	IMI 288937*	GU227893	GU228285	GU227991	GU228187
C. cymbidiicola	IMI 347923*	JQ005166	JQ005253	JQ005514	JQ005600
C. dematium	CBS 125.25*	GU227819	GU228211	GU227917	GU228113
C. dracaenophilum	CBS 118199*	JX519222	-	JX519238	JX519247
C. echinochloae	MAFF 511473*	AB439811	–	–	–
C. eleusines	MAFF 511155*	JX519218	–	JX519234	JX519243
C. endophytum	CGMCC 3.15108*	JX625177	KC843521	KC843533	JX625206
C. eremochloae	CBS 129661*	CBS 129661	–	JX519236	JX519245
C. excelsum-altitudum	CGMCC 3.15130*	HM751815	KC843502	KC843548	JX625211
C. falcatum	CBS 147945*	JQ005772	–	JQ005835	JQ005856
C. fioriniae	CBS 128517*	JQ948292	JQ948622	JQ949613	JQ949943
C. fructi	CBS 346.37*	GU227844	GU228236	GU227942	GU228138
C. fructicola	ICMP 18581*	JX010165	JX010033	FJ907426	JX010405
C. fructivorum	Coll1414*	JX145145	–	–	JX145196
C. fusiforme	MFLU 130291*	NR138010	KT290255	KT290251	KT290256
C. gigasporum	MUCL 44947*	AM982797	–	–	FN557442
C. godetiae	CBS 133.44*	JQ948402	JQ948733	JQ949723	JQ950053
C. graminicola	CBS 130836*	JQ005767		JQ005830	JQ005851
C. grevilleae	CBS 132879*	KC297078	KC297010	KC296941	KC297102
C. guizhouensis	CGMCC 3.15112*	JX625158	KC843507	KC843536	JX625185
C. hanaui	MAFF 305404*	JX519217	–	–	JX519242
C. henanense	LF238*	KJ955109	KJ954810	–	KJ955257
C. hippeastri	CBS 125376*	JQ005231	JQ005318	JQ005579	JQ005665
C. hemerocallidis	CDLG5*	JQ400005	JQ400012	JQ399991	JQ400019
C. horii	ICMP 10492	GQ329690	GQ329681	JX009438	JX010450
C. incanum	ATCC 64682*	KC110789	KC110807	KC110825	KC110816
C. jasiminigenum	MFU 10–0273*	HM131513	HM131499	HM131508	HM153770
C. jiangxiense	LF 488*	KJ955149	KJ954850	KJ954427	–
C. kahawae	ICMP 17816*	JX010231	JX010012	JX009452	JX010444
C. kartsii	CORCG 6*	HM585409	HM585391	HM581995	HM585428
C. laticiphilum	CBS 112989*	JQ948289	JQ948619	JQ949610	JQ949940

Table 3.4 (continued)

Species	**Isolate**[a]	**GenBank accession number**			
		ITS	**GAPDH**	**ACT**	**ß–tubulin**
C. lilii	CBS 109214	GU227810	GU228202	GU227908	GU228104
C. lindemuthianum	CBS 144.31*	JQ005779	JX546712	JQ005842	JQ005863
C. linicola	CBS 172.51	JQ005765	-	JQ949476	JQ949806
C. liriopes	CBS 119444*	GU227804	GU228196	GU227902	GU228098
C. magnisporum	CBS 398.84	KF687718	KF687842	KF687803	KF687882
C. malvarum	CBS 527.97*	KF178480	KF178504	KF178577	KF178601
C. menispermi	MFLU 14–0625*	KU242357	KU242356	KU242353	KU242354
C. miscanthi	MAFF 510857*	JX519221	–	JX519237	JX519246
C. musae	ICMP 19119*	JX010146	JX010050	JX009433	HQ596280
C. navitas	CBS 125086*	JQ005769	–	JQ005832	JQ005853
C. nicholsonii	MAFF 511115*	JQ005770	–	JQ005833	JQ005854
C. novae-zelandiae	CBS 128505*	JQ005228	JQ005315	JQ005576	JQ005662
C. nupharicola	ICMP 18187*	JX010187	JX009972	JX009437	JX010398
C. ochracea	CGMCC 3.15104*	JX625156	KC843513	KC843527	JX625183
C. oncidii	CBS 129828*	JQ005169	JQ005256	JQ005517	JQ005603
C. orchidophilum	CBS 632.80*	JQ948151	JQ948481	JQ949472	JQ949802
C. parsonsiae	CBS 128525*	JQ005233	JQ005320	JQ005581	JQ005667
C. paspali	MAFF 305403*	JX519219	–	JX519235	JX519244
C. petchii	CBS 378.94*	JQ005223	JQ005310	JQ005571	JQ005657
C. phaseolorum	CBS 157.36	GU227896	GU228288	GU227994	GU228190
C. phyllanthi	CBS 175.67*	JQ005221	JQ005308	JQ005569	JQ005655
C. pseudoacutatum	CBS 436.77*	JQ948480	JQ948811	JQ949801	JQ950131
C. pseudomajus	CBS 57188*	NR132059	KF687826	KF687801	KF687883
C. psidii	ICMP 19120	JX010219	JX009967	JX009515	JX010443
C. radices	CBS 52993*	NR132057	KF687825	KF687785	KF687869
C. rhexiae	Coll 1026*	JX145128	–	–	JX145179
C. rhombiforme	CBS 129953*	JQ948457	JQ948788	JQ949778	JQ950108
C. riograndense	COAD 928*	KM655299	KM655298	KM655295	KM655300
C. rusci	CBS 119206*	GU227818	GU228210	GU227916	GU228112
C. salsolae	ICMP 19051*	JX010242	JX009916	JX009562	JX010403
C. siamense	ICMP 18578*	JX010171	JX009924	FJ907423	JX010404
C. sichuanensis	LJTJ3	KP748193	KP823773	KP823738	KP823850
C. spinaceae	CBS 128.57	GU227847	GU228239	GU227945	GU228141
C. sublineola	CBS 131301*	JQ005771	–	JQ005835	JQ005855
C. syzygicola	DNCL 021*	KF242094	KF242156	KF157801	KF254880
C. tanaceti	CBS 132693*	-	JX218243	JX218238	JX218233
C. tebeestii	CBS 522.97*	KF178473	KF178505	KF178570	KF178594
C. temperatum	Coll 883*	JX145159	–	–	JX145211
C. theobromicola	ICMP 18649	JX010294	JX010006	JX009444	JX010447

Table 3.4 (continued)

Species	Isolate[a]	GenBank accession number			
		ITS	**GAPDH**	**ACT**	**ß–tubulin**
C. ti	ICMP 4832*	JX010269	JX009952	JX009520	JX010442
C. torulosum	CBS 128544*	JQ005164	JQ005251	JQ005512	JQ005598
C. trichellum	CBS 217.64*	GU227812	GU228204	GU227910	GU228106
C. trifolii	CBS 158.83*	KF178478	KF178502	KF178575	KF178599
C. tropicicola	BCC 38877*	JN050240	JN050229	JN050218	JN050246
C. trucatum	CBS 151.35	GU227862	GU228254	GU227960	GU228156
C. verruculosm	IMI 45525*	GU227806	GU228198	GU227904	GU228100
C. vietnamense	CBS 125478*	KF687721	KF687832	KF687792	KF687877
C. viniferum	GZAAS 5.08601*	JN412804	JN412798	JN412795	JN412813
C. xanthorrhoeae	ICMP 17903*	JX010261	JX009927	JX009478	JX010448
C. yunnanense	CGMCC AS3.9167*	EF369490	–	JX519239	JX519248
Australiasca queenslandica	BRIP 24607	HM237327	–	–	–
Monilochaetes guadalcanalensis	CBS 346.76	GU180625	–	–	–
Monilochaetes infuscans	CBS 869.96	JQ005780	JX546612	JQ005843	JQ005864

Note [a]Isolates marked with '*' are extype or ex-epitype strains

CHAPTER 4

ENDOPHYTIC PESTALOID TAXA IN *DENDROBIUM* ORCHIDS[3]

Abstract

Pestalotiod taxa commonly occur in plants as endophytes, pathogens or saprobes. Endophytic *Pestalotiopsis* and *Neopestalotiopsis* species were isolated from *Dendrobium cariniferum, D. loddigesii* and two unidentified orchid species sampled in southwestern China and northern Thailand. Morphological and molecular comparison identified the isolates as two new species; *Neopestalotiopsis dendrobii,* and *Pestalotiopsis doitungensis*; two existing species *P. lushanensis* and *P. trachicarpicola*. This is the first report of *Neopestalotiopsis* isolated from the orchid genus *Dendrobium* and is the first report ofpestalotiod fungi in *D. careniferum*. *Pestalotiopsis lushanensis* and *P. trachicarpicola* are new recorded fungal endophytes in *Dendrobium* orchids.

Keywords: 2 New Taxa, 2 New Records, Sporocadaceae, Multi-loci

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4.1 Introduction

Pestaloid taxa commonly occur in plants as endophytes, pathogens or saprobes. Endophytic *Pestalotiopsis* and *Neopestalotiopsis* species were isolated from *Dendrobium cariniferum, D. loddigesii* and two unidentified orchid species sampled in southwestern China and northern Thailand. Morphological and molecular comparison identified the isolates as two new species; *Neopestalotiopsis dendrobii,* and *Pestalotiopsis doitungensis*; two existing species *P. lushanensis* and *P. trachicarpicola;* and one unidentified *Neopestalotiopsis* species. This is the first report of *Neopestalotiopsis* isolated from the orchid genus *Dendrobium* and is the first report of pestaloid fungi in *D. careniferum*. *Pestalotiopsis lushanensis* and *P. trachicarpicola* are new recorded fungal endophytes in *Dendrobium* orchids.

Pestalotiopsis and *Neopestalotiopsis* (*Sporocadaceae*, Amphisphaeriales) are endophytes in various plants (Kang et al., 1998; Senanayake et al., 2015; Jaklitsch et al., 2016; Wijayawardene et al., 2017). Taxa in these genera have distinctive appendage-bearing conidia and they are known as pestaloid asexual morphs (Maharachchikumbura et al., 2011; 2012; 2014; Senanayake et al., 2015). Pestaloid species are rich producers of bioactive compounds (Strobel et al., 1996). They are widely distributed in tropical and temperature areas (Bate-Smith & Metcalfe, 1957; Maharachchikumbura et al., 2012; 2016). Pestaloid taxa have been found as endophytes in many plants, such as *Camellia*, *Fragraea*, *Podocarpus* and *Taxus* species (Strobel et al., 2000; Maharachchikumbura et al., 2012; Liu et al., 2017). *Pestalotiopsis* spp. have been reported to be associated with *Dendrobium falconeri*. and they were ubiquitous in the roots of *D. chrysanthum* and *D. nobile* (2011; Chen et al., 2012). *Neopestalotiopsis* and *Pseudopestalotiopsis* were proposed as independent pestaloid genera as they split from *Pestalotiopsis* in molecular analysis, but share similar, although differing morphological characters (Maharachchikumbura et al., 2014). *Neopestalotiopsis* species have been reported to cause fruit rot in Africa, Asia Europe and South America (Ayoubi & Soleimani, 2016; Chamorro et al., 2016; Jayawardena et al., 2016; Maharachchikumbura et al., 2016; Solarte et al., 2018). but they are rarely reported as endophytes (Chen et al., 2011; 2012). *Dendrobium* is one of the largest genera in

Orchidaceae and some species possess important medicinal and ornamental values (Ma et al., 2015). For example, moscatilin (4,4'-dihydroxy-3,3',5-trimethoxybibenzyl) isolated from many *Dendrobium* spp. possesses anti-inflammatory, anti-platelet aggregation, anti-tumor and antioxidant bioactivities (Fan et al., 2001; Hu et al., 2009; Zhang et al., 2008; Hwang et al., 2010; Sritularak et al., 2011; Zhao et al., 2016). Many *Dendrobium* orchids have been listed as endangered species due to habitat destruction and low seed germination rates (The International Union for Conservation of Nature Red List of Threatened Species, http://www.iucnredlist.org/search). Therefore, *Dendrobium* protection and related resource investigation should be undertaken. However, there have been few studies of endophytes in *Dendrobium* species (Ma et al., 2015).

This study focused on endophytic pestaloid taxa isolated from *Dendrobium cariniferum, D. loddigesii* and two unidentified *Dendrobium* species sampled in southwestern China and northern Thailand. The published *Dendrobium* related pestaloid research used ITS gene sequences to identify endophytes, but this is not satisfactory (Ko et al., 2011). Therefore, in this study we used multi-loci analysis and morphological characteristics suggested in Maharachchikumbura et al. (2012) to determine the endophytic pestaloid species in *Dendrobium*. Among the isolated endophytes, *Neopestalotiopsis dendrobii* and *Pestalotiopsis doitungensis* are introduced as new species with molecular evidence and morphological descriptions and illustrations.

4.2 Materials and Methods

4.2.1 Sample Collection

Healthy leaf, root and stems of *Dendrobium cariniferum* Rchb. f. and of two unidentified orchids (*Dendrobium* sp.1 and *Dendrobium* sp.2) were collected from Wat Phra That Doi Tung (Temple of Doi Tung Pagoda), Mae Fah Luang District, Chiang Rai, Thailand. Similar tissues of *D. loddigesii* Rolfe were sampled from an orchid nursery of Xing Yi city, Guizhou province, China. Materials were stored in zip-lock bags or tubes containing silica gel on ice. Fungal isolation was carried out within 48 hours following collection.

4.2.2 Fungal Isolation and Cultivation

Fungal isolation followed Nontachaiyapoom et al. (2010) with some modifications. Materials were washed with running tap water. They were then surface sterilized by immersing in a solution of 3% (v/v) H_2O_2 and 70% (v/v) ethanol for 5 minutes, and then rinsed with three changes of sterile distilled water. The sterilized material was cut into 2 mm^2 segments and placed on potato dextrose agar (PDA) containing 50 μg/ml oxytetracycline, 50 μg/ml penicillin and 50 μg/ml streptomycin (Otero et al., 2002). Samples were incubated at 28 °C under natural light. The plates were observed every day and mycelia on the edge of fungal colonies were transferred to fresh PDA to obtain pure cultures. The pure cultures were deposited in Mae Fah Luang University Culture Collection (MFLUCC). Dry cultures of new species were deposited in Mae Fah Luang University herbarium (MFLU).

4.2.3 Morphological Analysis

Cultures were grown on PDA media in a dark cabinet at room temperature (28 °C) and observed every five days. The growth rate was evaluated when mycelia had nearly covered the whole agar surface. Any observable structures were examined using a stereomicroscope (SteREO Discovery. V8, Carl Zeiss Microscopy GmBH, Germany). Conidiomata were placed in water on slides and observed using a compound microscope (EOS 750D, Nikon, Japan). Colony, conidiomata, conidiogenous cells, conidiophores, conidia and mycelia were examined for morphological characteristics.

4.2.4 DNA Extraction and Amplification

The whole genome of fungal isolates was extracted using EZgene Fungal gDNA Kit (GD2416, Biomiga, USA) as described by the manufacturer. The selected gene including internal transcribed spacer (ITS), translation elongation factor 1-α (EF-1α) and β-tubulin (TUB2) amplification were carried out using reagents purchased from BIOMIGA (San Diego, USA). Each 25-μl amplification reaction contained 12.5 μl of 2*Bench Top^{TM} Taq Master Mix (0.05 units/μl Taq DNA polymerase, 0.4mM dNTPs and 4mM $MgCl_2$); 2μl forward and reverse primers; 1μl of DNA template and 9.5μl of threefold-distilled water. The primers and PCR protocol followed Maharachchikumbura et al (2012). PCR products were visualized on 1 % agarose gel stained with Goldview

(Realtimes Biotech, Beijing, China). PCR products were sent to Sangon Biotech (China) for purification and sequencing.

4.2.5 Sequence Analysis

Two different datasets were used to estimate the phylogenies of *Neopestalotiopsis* and of *Pestalotiopsis* trees. Sequences assembled in this study used ContigExpress (Vector NTI suite 6.0, Informax), supplemented with additional sequences from GenBank (www.ncbi.nlm.nih.gov/BLAST) (Altschul et al., 1997) based on blast results and literature (Maharachchikumbura et al., 2012; 2014; Ariyawansa & Hyde 2018; Kumar et al., 2019; Liu et al., 2019), especially ex-type/ex-epitype strains for tree construction. The alignment was implemented via MAFFT version 6 (Katoh & Toh, 2008) (mafft. cbrc. jp/ alignment/server/). Misaligned regions in datasets were manually edited where necessary using Bio-Edit version 7.2.5 (Hall, 1999). A concatenated dataset was then constructed, containing 50 taxa and 1251 base pairs for the *Neopestalotiopsis* tree and 113 taxa and 1338 base pairs for the *Pestalotiopsis* tree. The concatenated datasets were partitioned and the ultrafast bootstrap (Minh et al., 2013) implemented in IQ-TREE software (Nguyen et al., 2014) to estimate the best fitting models according to the Bayesian information criterion (BIC). Gaps were treated as missing data in maximum likelihood (ML) and Bayesian inference (BI) trees. The ML tree was constructed using RAxML-HPC2 on XSEDE in The CIPRES Science Gateway V. 3.3 with GTR + GAMMA model and 1000 bootstrap iterations (http://www.phylo.org/index.php/). The BI tree was built by MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). Two sets of four simultaneous Markov Chain Monte Carlo (MCMC) chains were run for 5,000,000 generations and sampled every 100 generations, 25% of trees were discarded and the remaining trees were used to calculate the posterior probabilities. Convergence was assumed when the standard deviation of split sequences was less than 0.01. The maximum parsimony (MP) tree was constructed with PAUP version 4.0 beta 10 (Swofford, 2001). The 1000 starting trees were generated using stepwise addition and random addition sequence replicates. Branch swapping used the tree bisection reconnection (TBR) algorithm. In maximum parsimony trees, the references including tree length (TL), consistency index (CI), rescaled consistency index (RC) and retention index (RI) were recorded. The constructed trees were submitted to TreeBase(http://purl.org/phylo/treebase/phylows/study/TB2:S24083). The resulting trees were viewed with Figtree v.1.4.0 and the arrangement was carried out with Adobe Illustrator CS6.

4.3 Results

4.3.1 Fungal Isolation and Identification

Six endophytic pestaloid strains were isolated and identified as three *Pestalotiopsis* species and two *Neopestalotiopsis* species (Table 4.1).

4.3.2 Phylogenetic Results

The selected sequences of strains are listed in Table 4.2. In the *Neopestalotiopsis* tree (Fig. 1), the final alignments contained 505 characters for ITS, 299 characters for EF-1α and 447 characters for ß–tubulin. There were 190 parsimony-informative characteristics in parsimony trees (TL=680, CI=0.657, HI=0.343, RC=0.422, RI=0.642). In *Pestalotiopsis* tree (Fig. 2), the final alignments contained 548 characteristics for ITS, 340 characteristics for EF-1α and 448 characteristics for ß–tubulin. There were 402 parsimony-informative characteristics in parsimony trees (TL =1626, CI=0.504, HI=0.496, RC=0.380, RI=0.754). The Hasegawa, Kishino & Yano (HKY) model with gamma distribution was selected for Bayesian trees construction. The Bayesian and maximum parsimony trees had the same topology as the Maximum Likelihood tree. Maximum likelihood bootstrap values (MLB), Bayesian posterior probabilities (PP) and maximum parsimony bootstrap values (MP) are labelled at the end of nodes (50% majority rule).

Table 4.1 Endophytic pestaloid fungi isolated from *Dendrobium* spp.

Orchid host	**Sample sites**	**Tissues**	**Pestaloid species**	**Culture number**
Dendrobium sp.1	Chiang Rai, Thailand	Leaf	*Pestalotiopsis doitungensis*.sp. nov.	**MFLUCC 14-0090**
Dendrobium cariniferum	Chiang Rai, Thailand	Root	*Neopestalotiopsis* sp.	MFLUCC 14-0099
		Stem	*Neopestalotiopsis dendrobii* sp.nov.	**MFLUCC 14-0106**
Dendrobium sp.2	Chiang Rai, Thailand	Root	*Pestalotiopsis rhododendri*	MFLUCC 14-0115
		Leaf	*Neopestalotiopsis dendrobii* sp.nov.	**MFLUCC 14-0132**
Dendrobium loddigesii	Guizhou, China	Stem	*Pestalotiopsis trachicarpicola*	MFLUCC 19-0222

Note *New isolates are in bold

Neopestalotiopsis tree was rooted with *Pseudopestalotiopsis theae* MFLUCC 12-0055 and *P. cocos* CBS 272.29 supported by 100MLB/1.0PP/100MP. Two new strains (MFLUCC 14-0106 and MFLUCC 14-0132) clustered together and formed a sister clade with *Neopestalotiopsis foedans* CGMCC 3.9123 with low support values. Isolate MFLUCC 14-0099 formed a clade close to *N. foedans* with weak support values. The *Pestalotiopsis* tree (Fig. 2), was rooted with *Neopestalotiopsis cubana* CBS 600.96 and *N. saprophytica* MFLUCC 12-0282. Isolate MFLUCC 19-0222 was determined as *P. trachicarpicola* supported by 88MLB/0.98PP/63MP close to *P. kenyana.* MFLUCC 14-0090 clustered with *P. macadamiae* supported by 78MLB/0.85PP/73MP. MFLUCC 14-0115 located at the middle of *P. lushanensis* and *P. rhododendri*.

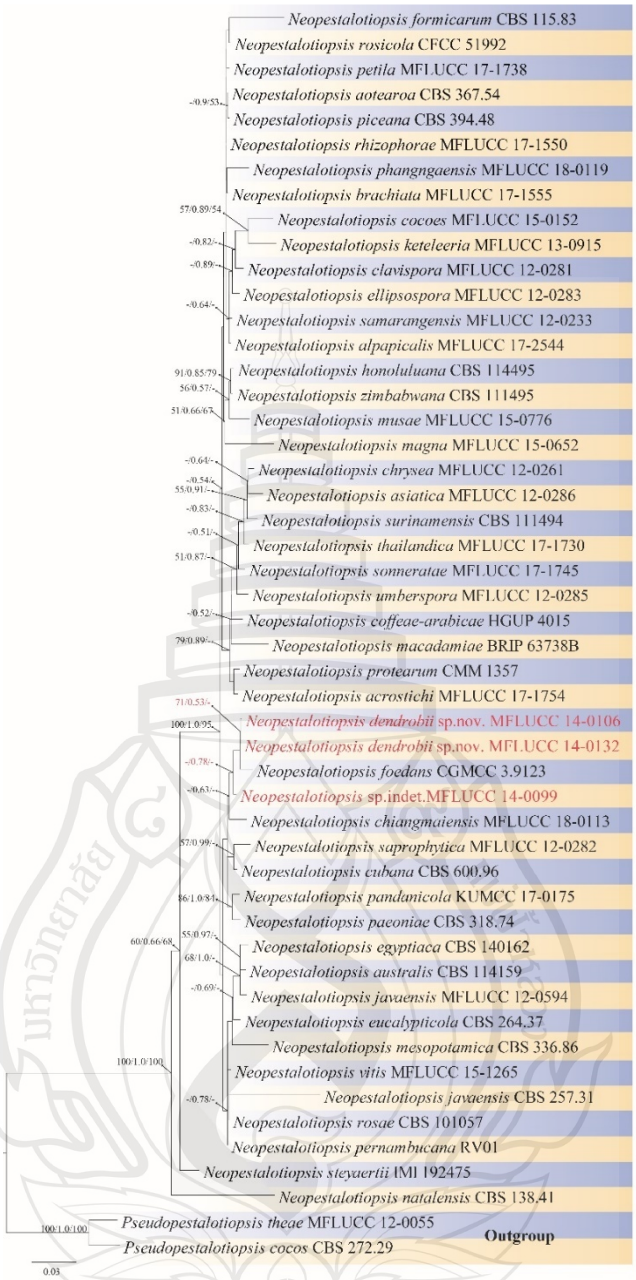


Figure 4.1 Consensus phylogram of 1,000 trees resulting from a RAxML analysis of the (ITS+TUB2+EF-1α) alignment of the analysed *Neopestalotiopsis* sequences. The isolates derived from *Dendrobium* are in red. Dashes act as lower than 50% values. Scale bar corresponds to 0.03 substitutions per sites

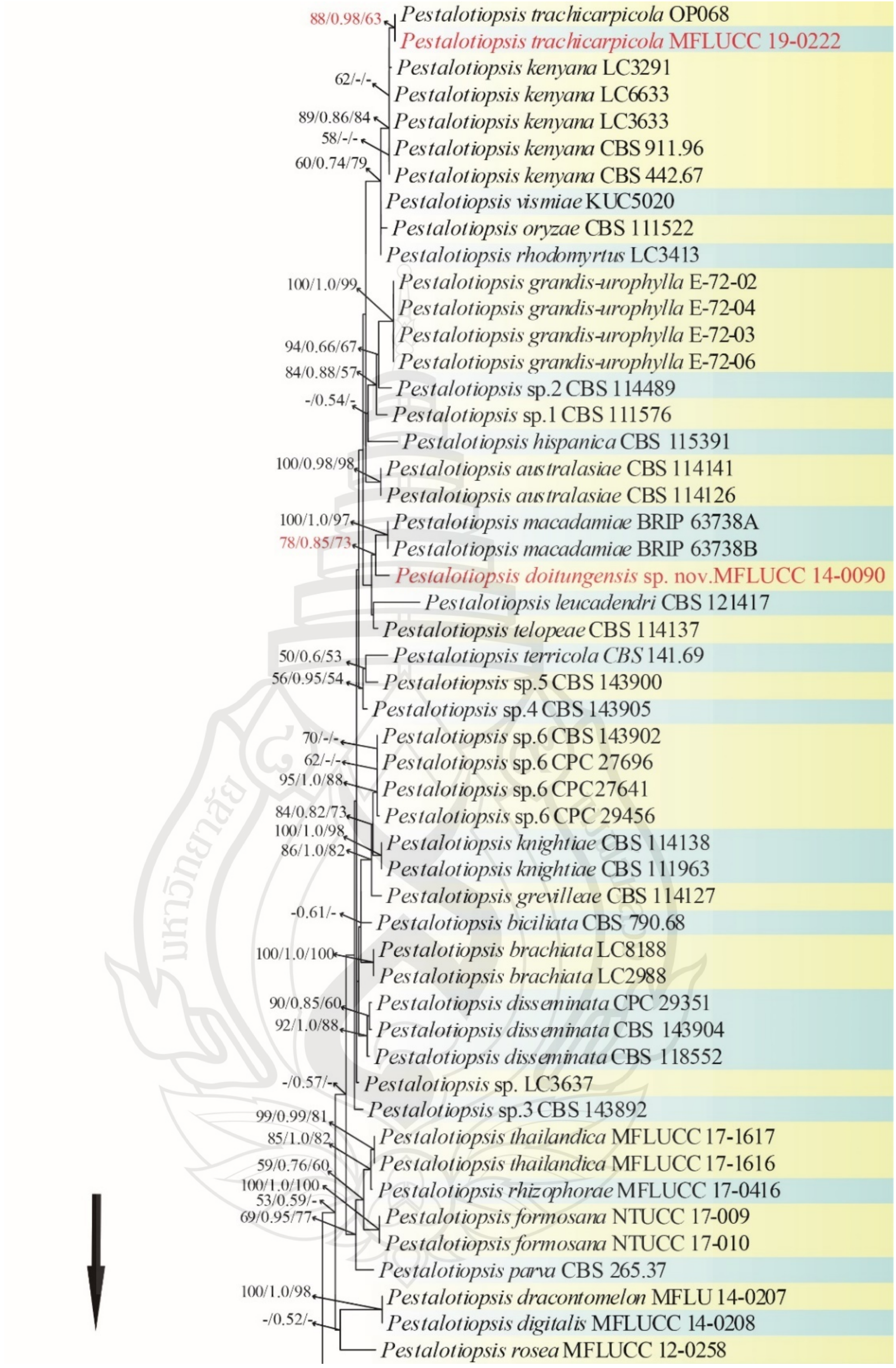


Figure 4.2 Consensus phylogram of 1,000 trees resulting from a RAxML analysis of the (ITS+TUB2+EF-1α) alignment of the analysed *Pestalotiopsis* sequences. The isolates derived from *Dendrobium* are in red. Dashes act as lower than 50% values. Scale bar corresponds to 0.05 substitutions per site

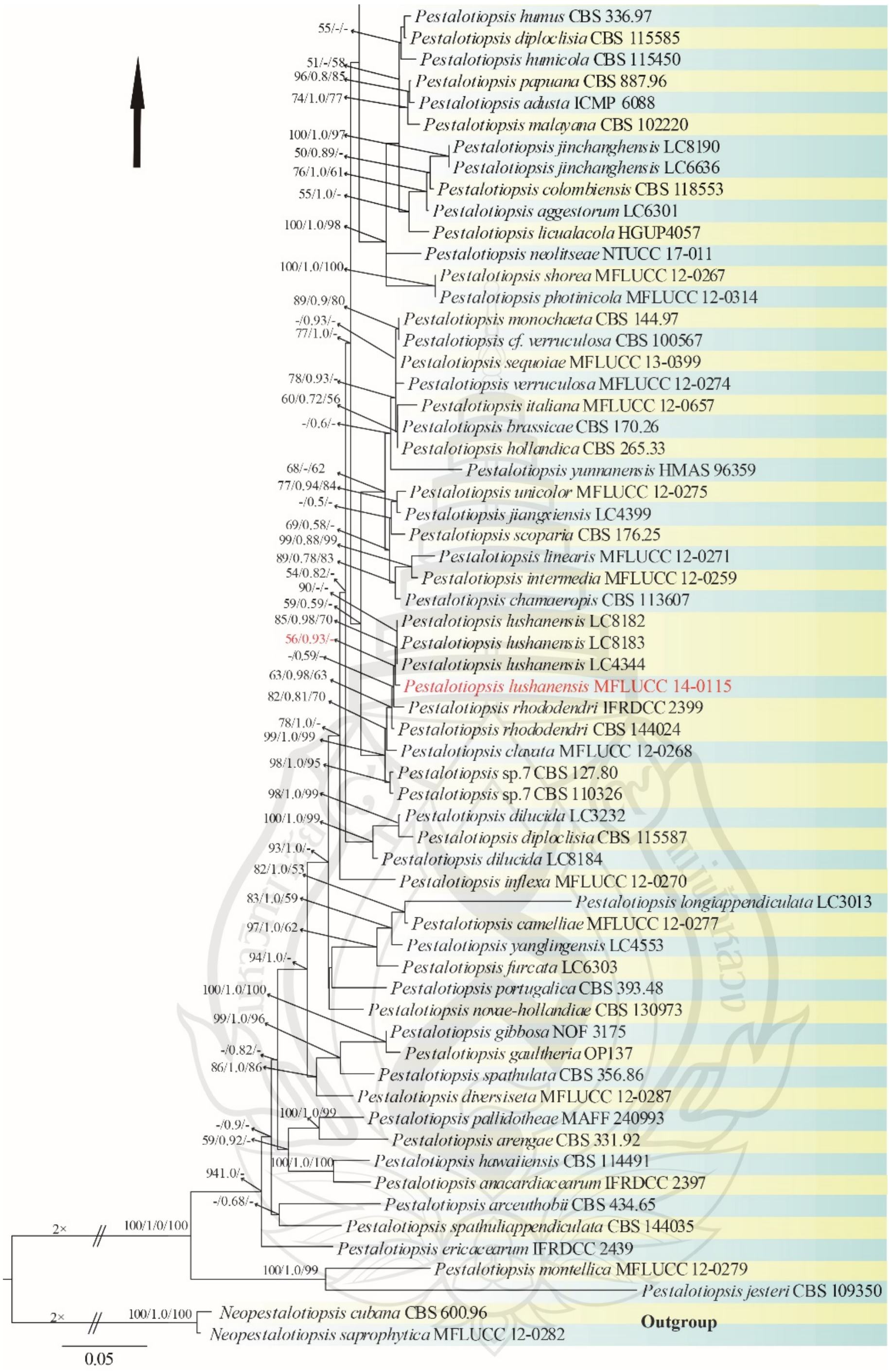


Figure 4.2 (continued)

4.3.3 Taxonomy

***Neopestalotiopsis dendrobii* X.Y. Ma, K.D. Hyde & J.C. Kang, sp. nov.**

Facesoffungi: FOF 06098

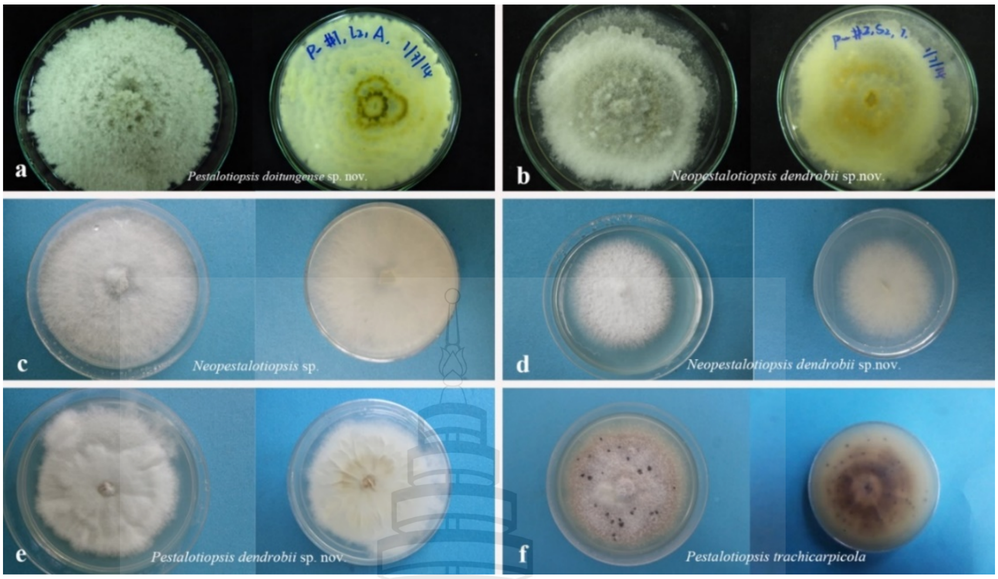
MycoBank: MB 830981

Figs 3.b

Etymology: In reference to the host genus, *Dendrobium*.

Type. Thailand, Chiang Rai, isolated from *Dendrobium cariniferum,* 20 Dec 2013, X.Y. Ma, holotype MFLU 19-0749; ex-holotype culture MFLUCC 14-0106 = MFLUCC 14-0132.

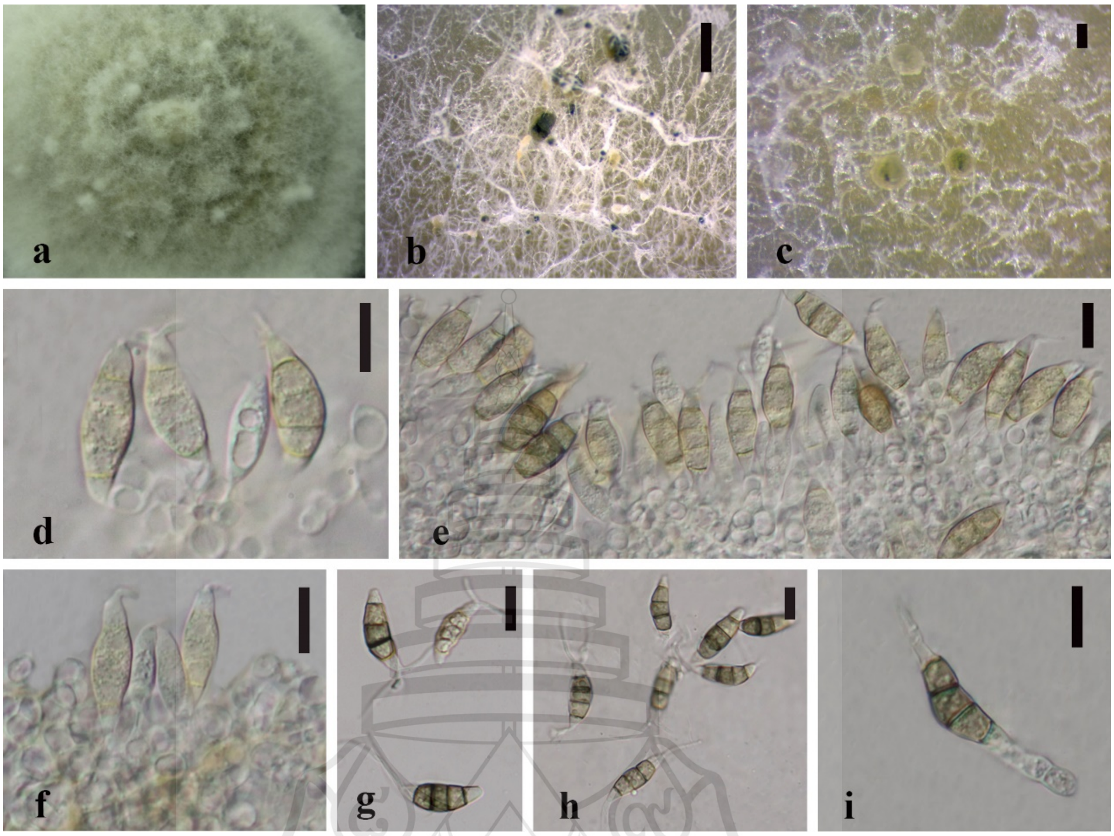
Description. *Colonies* on PDA white with grey aerial mycelia in the center, black conidiomata, irregular edge; growth rate: 2.0 mm/day at 28 °C, reverse yellowish; mycelia hyaline, sparse, septate, 1.8–4.5 µm diam. *Conidiomata* after 20 days scattered in mycelia or bulging on the surface, globose, soft, light yellow to black, 200 × 300 µm. *Conidiophores* reduced to conidiogenous cells, hyaline. *Conidiogenous cells* discrete, globose to sub-globose, hyaline, rugose-walled, proliferating 1–2 times percurrently. *Conidia* fusoid, straight or slightly curved, 4-septate, (19.3–) 20.6 – 23.3 (–24.7)× (5.9–) 6.4 – 7.4 (–7.9) µm, mean±SD= 22.0±1.3 × 6.9±0.5 µm; basal cell conic with obtuse base, hyaline, thin-walled, 3.7 – 5.1µm long, basal appendage single, tubular, unbranched, centric, 2.5–3.4 µm long; three median cells doliiform, (12.6–) 13.7 – 15.6 (–16.5) µm long, mean±SD= 14.7 ± 1.0 µm, wall rugose, versicolored; second cell (from the base) cylindrical, brown to dark brown, lighter than the third cell, 3.9 – 6.0 µm long; third cell darker brown than others, 3.9–5.7 µm long; fourth cell pale brown, 4.3–5.9 µm long, apical cell 3.9–6.4 µm long, hyaline, subcylindrical to obconic, rugose and thin-walled; with 2–3 tubular apical appendages (mostly 2), arising from the apical crest, unbranched, filiform, straight or flexuous, (4.3–) 4.9–6.5(–6.6) µm long, mean ± SD = 5.7 ± 0.9 µm.



Note Strains MFLUCC 14-0106 and MFLUCC 14-0132 were isolated, respectively, from stems of *Dendrobium cariniferum* and leaves of *Dendrobium* sp. 2 sampled in northern Thailand. They formed an independent clade adjoining

Figure 4.3 Colonies on PDA of endophytic pestaloid fungi

Neopestalotiopsis foedans CGMCC 3.9123 (epitype of *N. foedans* isolated from mangrove in China, Maharachchikumbura et al., 2012). There were 11 base pairs differences (excluding gaps) in TUB2 sequences between *N. foedans* CGMCC 3.9123 and MFLUCC 14-0106, MFLUCC 14-0132. Morphologically, the apical appendages of *N. foedans* CGMCC 3.9123 are longer than those of MFLUCC 14-0106 (13.3 µm vs. 5.7 ± 0.9 µm). The conidiogenous cells of CGMCC 3.9123 were reported to be filiform, whereas those in the present study are globose to sub-globose (Maharachchikumbura et al., 2012). Therefore, we introduce the two strains as a new species *Neopestalotiopsis dendrobii* sp. nov.



Note a Surface of colony on PDA. b-c Conidiomata. d-f Conidiogenous cells with conidia. g–i Conidia. *Scale bars*: b=50 µm, c=100 µm, d–i=10 µm,

Figure 4.4 *Neopestalotiopsis dendrobii* (Holotype)

***Neopestalotiopsis* sp.** (Figs 3.c)

Note: Isolate MFLUCC 14-0099 formed a clade with weak support values -/0.78PP/-. It has two different base pairs in ITS sequences, and nine different base pairs in TUB2 and EF-1α sequences compared with *N. foedans* CGMCC 3.9123. There were 3 and 9 distinguished base pairs in TUB2 and EF-1α sequences between MFLUCC 14-0099 and MFLUCC 14-0106. The morphological characteristics of MFLUCC 14-0099 were not determined.

***Pestalotiopsis doitungensis* X.Y. Ma, K.D. Hyde & J.C. Kang, sp. nov.**

Facesoffungi: FOF 060100

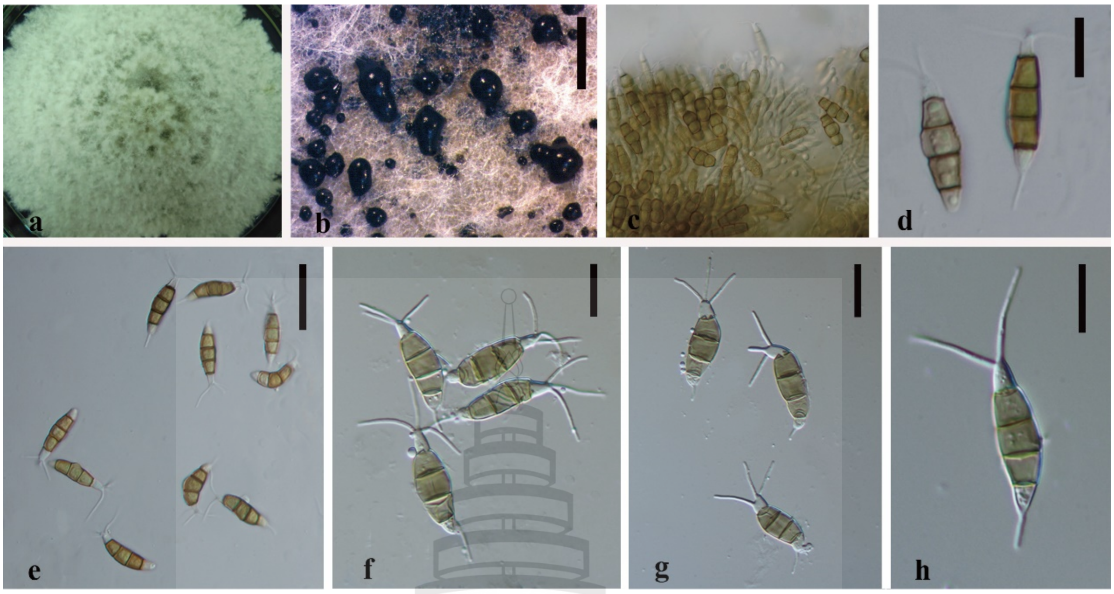
MycoBank: MB 830983

Figs 3.a

Etymology. In reference to the sample site Doitung.

Type. Thailand, Chiang Rai, isolated from *Dendrobium* sp. 2*,* 20 Dec 2013, X.Y. Ma, MFLU 19-0748; ex-holotype culture MFLUCC 14-0090.

Description: *Colonies* on PDA white with grey mycelia in the center, felty, black conidiomata, lobed edge; growth rate: 1.6 mm/day at 28 °C, reverse white with brown and yellowish concentric circles. Mycelia hyaline, branched, septate, 1.9–5.3 µm diam. *Conidiomata* producing on mycelia, globose to irregular, 100 × 100 µm. *Conidiophores* reduce to conidiogenous cell, hyaline to brown. *Conidiogenous cells* turbular, light brown to brown, smooth, thin-walled. *Conidia* fusoid, straight or slightly curved, 4- septate, (21.3–)21.7–24.9(–25.7) × (5.1–)5.3–6.5(–7.4) µm, mean±SD= 23.3±1.6 × 5.9±0.6 µm; basal cell conic with obtuse base, hyaline, thin-walled, 3.5–5.6 µm long, basal appendage single, tubular, unbranched, centric, 4.2–6.1 µm long; three median cells doliiform, with guttles, (14.4–)15.1 – 16.2(–16.5) µm long, mean±SD= 15.7 ± 0.6 µm, wall rugose, versicolored, second cell from the base brown, 3.6–6.2 µm long; third cell darker brown than other cells, 4.4–5.5 µm long; fourth cell pale brown, 4.8–6.3 µm long, apical cell 3.1–4.3µm long, hyaline, conical to subcylindrical, rugose and thin-walled; with 2–3 tubular apical appendages (mostly 3), appendages arising from the apical crest, unbranched, filiform, straight or flexuous, (2.5–) 3.8–12.2(–15.4) µm long, mean ± SD = 8.4 ± 3.9 µm.



Note a Surface of colony on PDA. b Conidiomata. c Conidiogenous cell with conidia. d–h Conidia. *Scale bars*: b=100 µm, d=5 µm, e=20 µm, f–g=10 µm, h=10 µm.

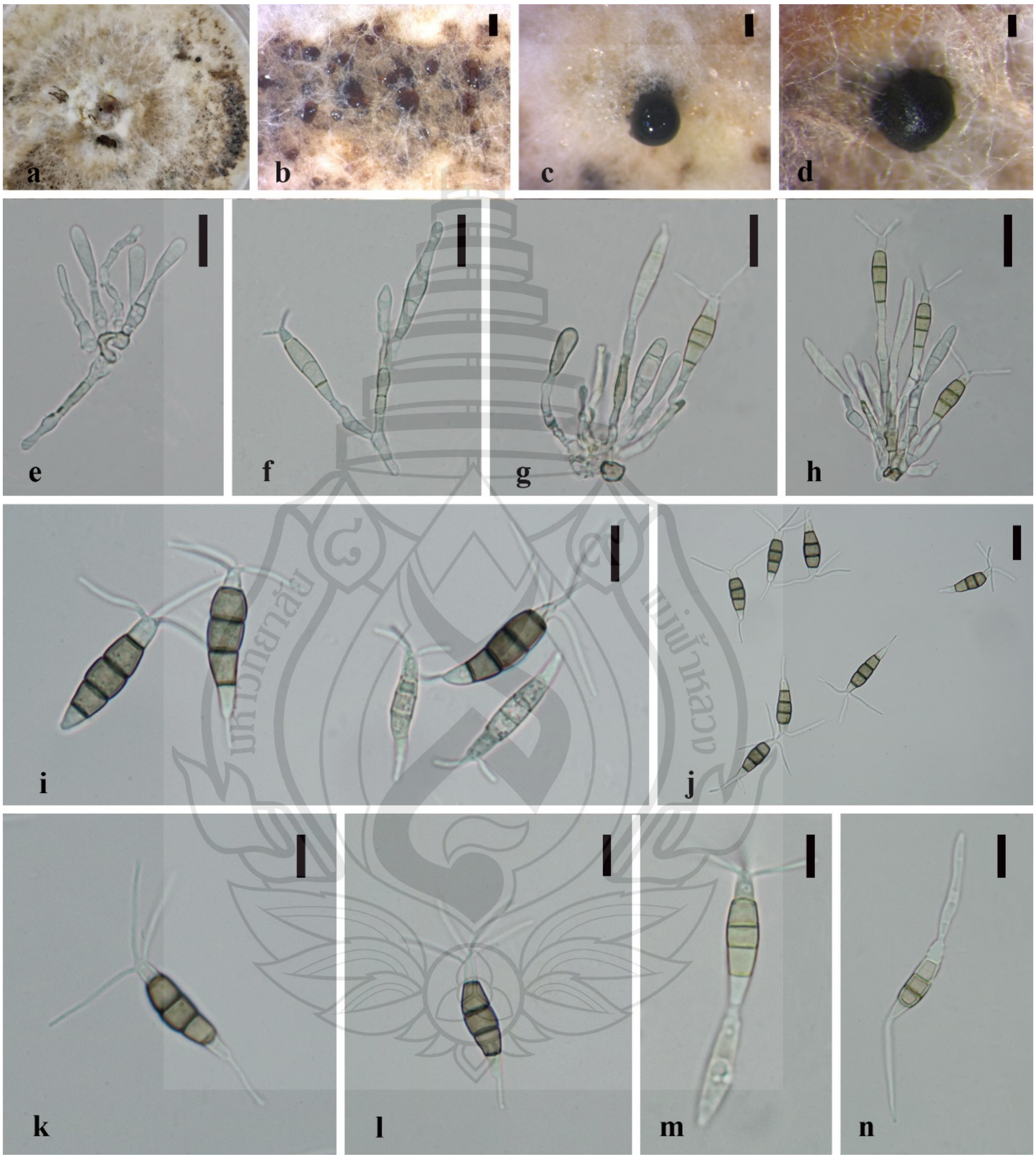
Figure 4.5 *Neopestalotiopsis doitungensis* (Holotype)

Notes. In the molecular analysis, strain MFLUCC 14-0090 clustered with *P. macadamiae* (BRIP 63738A and BRIP 63738B) isolated from *Macadamia* sp. in Australia (Akinsanmi et al., 2017) with support values 78MLB/0.85PP/73MP. They had identical TUB2 sequences. There are one and four base pairs differences in ITS and EF-1α sequences. The conidiomata were smaller than those of *P. macadamiae* (100 µm diam vs. 200–500 µm), the shape of conidiogenous cell was different (turbular vs. ampulliform to cylindrical), and the conidia of MFLUCC 14-0090 are shorter than those of *P. macadamiae* ((21.3–)21.7 – 24.9(–25.7) µm vs. (23–)24–28(–29) µm.). Here we assign MFLUCC 14-0090 as a new species *Pestalotiopsis doitungensis*.

Pestalotiopsis lushanensis F. Liu & L. Cai **(**Figs 3.e**)**

Notes. MFLUCC 14-0115 was isolated from a root of *Dendrobium* sp. 2 sampled from northern Thailand. It lied between *P. lushanensis* (LC4344, LC8182, LC8183) isolated from *Camellia* sp. in China (Liu et al., 2017) with support values 56MLB/0.93PP/- and *P. rhododendri* (IFRDCC 2399) isolated from leaf spot of *Rhododendron sinogrande* in China (Zhang et al., 2013) with support values -/0.59PP/-.

The isolate has only 3 and 9 different base pairs compared with *P. lushanensis* (LC 4344, 2 and 1 in TUB2 and EF-1α sequences, respectively) and *P. rhododendri* (3 in ITS, 4 in TUB2 and 2 in EF-1α sequences, respectively). Although the conidiomata of



Note a Surface of colony on PDA. b–d Conidiomata. e–h Conidiogenous cells and developing conidia. i–n Conidia. *Scale bars*: b–d=100 µm. e–i=10 µm. j=20 µm, k–n=10 µm.

Figure 4.6 *Pestalotiopsis lushanensis* (MFLUCC 14-0115)

MFLUCC 14-0115 are smaller than those of *P. lushanensis* (200–300 µm vs. 750 µm); conidia possess 2–4 apical appendages rather than the 2–3 in *P. lushanensis* as well as *P. rhododendri,* here we identify it as *P. lushanensis*.

Pestalotiopsis trachicarpicola Y. M. Zhang & K.D. Hyde (Figs 3.f)

Notes. Strain MFLUCC 19-0222 was isolated from the stems of *Dendrobium loddigesii* sampled in southwestern China. It clustered with *Pestalotiopsis trachicarpicola* OP068 isolated from leaf spots of *Trachycarpus fortunei* in China with the support values of 88MLB/0.98PP/63MP (Zhang et al., 2012).

4.4 Discussion

Eighteen fungal genera including *Pestalotiopsis* have been reported associated with *Dendrobium loddigesii* (Chen et al., 2010). The current study provides the first report of *Pestalotiopsis lushanensis* and *P. trachicarpicola* in *D. loddigesii*. There are some disagreements in the current phylogenetic trees compared with other studies (Liu et al., 2017, 2019). For example, *N. formicarum* and *P. ericacearum* were located in different places compared with the latest backbone trees (Liu et al., 2017, 2019).

Neopestalotiopsis and *Pestalotiopsis* are pathogens of many plants (Zhang et al., 2012; Maharachchikumbura et al., 2017). Pestaloid fungi in this study are likely to be potential pathogens because they all nested phylogenetically with species reported as pathogens such as *P. digitalis* and *P. macadamiae*. This study shows that *Dendrobium* orchids may be rich in culturable and unrecognized pestaloid fungi.

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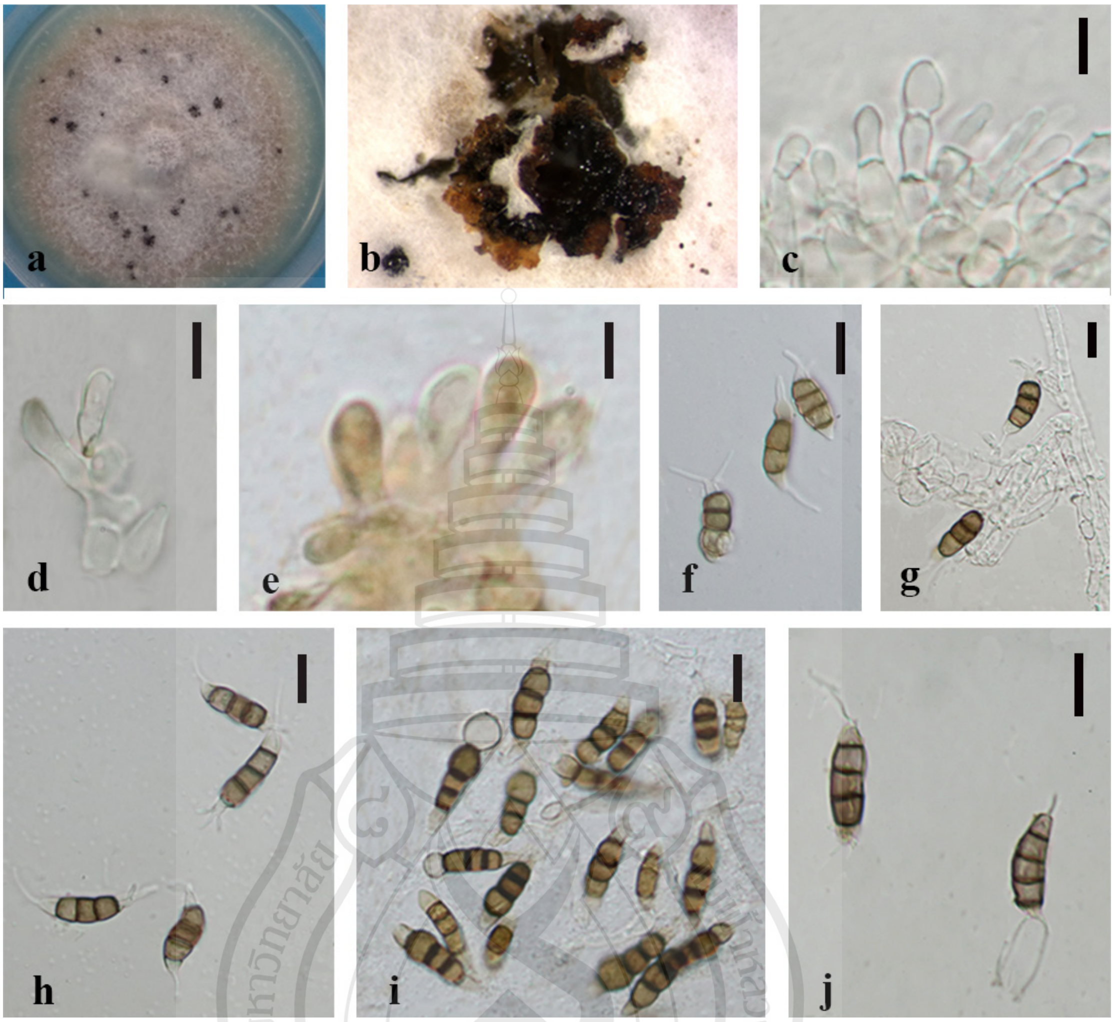

Note a Surface of colony on PDA. b Conidiomata. c–e Conidiogenous cells. f–j Conidia. *Scale bars*: c–j=10µm.

Figure 4.7 *Pestalotiopsis trachicarpicola* (MFLUCC 19-0222)

Table 4.2 Taxa used in this study and their GenBank accession numbers. New sequences are in bold

Species name	Strain number	Strain accession number			Reference
		ITS	TUB2	EF-1α	
Neopestalotiopsis acrostichi	MFLUCC 17-1754*	MK764272	MK764338	MK764316	Norphanphoun et al. (2019)
Neopestalotiopsis alpapicalis	MFLUCC 17-2544*	MK357772	MK463545	MK463547	Kumar et al. (2019)
Neopestalotiopsis aotearoa	CBS 367.54*	MH857366	KM199454	KM199526	Maharachchikumbura et al. (2014)
Neopestalotiopsis asiatica	MFLUCC 12-0286*	NR120181	JX399018	JX399049	Maharachchikumbura et al. (2014)
Neopestalotiopsis australis	CBS 114159*	KM199348	KM199432	KM199537	Maharachchikumbura et al. (2014)
Neopestalotiopsis brachiata	MFLUCC 17-1555*	MK764274	MK764340	MK764318	Norphanphoun et al. (2019)
Neopestalotiopsis dendrobii	MFLUCC 14-0106*				This study
	MFLUCC 14-0132				This study
Neopestalotiopsis chiangmaiensis	MFLUCC 18-0113*	—	MH412725	MH388404	Tibpromma et al. (2018)
Neopestalotiopsis chrysea	MFLUCC 12-0261*	JX398985	JX399020	JX399051	Maharachchikumbura et al. (2014)
Neopestalotiopsis clavispora	MFLUCC 12-0281*	JX398979	JX399014	JX399045	Maharachchikumbura et al. (2014)
Neopestalotiopsis cocoes	MFLUCC 15-0152*	KX789687	—	KX789689	Hyde et al. (2016)
Neopestalotiopsis coffee-arabicae	HGUP 4015*	KF412647	—	—	Song et al. (2013)
Neopestalotiopsis cubana	CBS 600 .96*	KM199347	KM199438	KM199521	Maharachchikumbura et al. (2014)
Neopestalotiopsis egyptiaca	CBS 140162*	KP943747	KP943746	KP943748	Crous et al. (2015)
Neopestalotiopsis ellipsospora	MFLUCC 12-0283*	JX398980	JX399016	JX399047	Maharachchikumbura et al. (2014)
Neopestalotiopsis eucalypticola	CBS 264.37*	KM199376	KM199431	KM199551	Maharachchikumbura et al. (2014)
Neopestalotiopsis foedans	CGMCC 3.9123*	JX398987	JX399022	JX399053	Maharachchikumbura et al. (2014)
Neopestalotiopsis formicarum	CBS 115.83*	KM199344	KM199444	KM199519	Maharachchikumbura et al. (2014)
Neopestalotiopsis honoluluana	CBS 114495*	KM199364	KM199457	KM199548	Maharachchikumbura et al. (2014)
Neopestalotiopsis javaensis	CBS 257.31*	KM199357	KM199437	KM199543	Maharachchikumbura et al. (2014)
	MFLUCC 12-0594	KX816905	KX816933	KX816874	Maharachchikumbura et al. (2014)
Neopestalotiopsis keteleeria	MFLUCC 13-0915*	KJ503820	KJ503821	KJ503822	Song et al. (2014)
Neopestalotiopsis macadamiae	BRIP 63737B*	KX186604	KX186654	KX186627	Akinsanmi et al. (2017)
Neopestalotiopsis magna	MFLUCC 15-0652*	KF582795	KF582793	KF582791	Maharachchikumbura et al. (2014)
Neopestalotiopsis mesopotamica	CBS 336.86*	NR145244	KM199441	KM199555	Maharachchikumbura et al. (2014)
Neopestalotiopsis musae	MFLUCC 15-0776*	KX789683	KX789686	KX789685	Hyde et al. (2016)

Table 4.2 (continued)

Species name	Strain number	Strain accession number			Reference
		ITS	**TUB2**	**EF-1α**	
Neopestalotiopsis natalensis	CBS 138.41*	KM199377	KM199466	KM199552	Maharachchikumbura et al. (2014)
Neopestalotiopsis paeoniae	CBS 318.74	MH554031	MH554707	—	Liu et al. (2019)
Neopestalotiopsis pandanicola	KUMCC 17-0175*	—	MH412720	MH388389	Tibpromma et al. (2018)
Neopestalotiopsis pernambucana	RV01*	KJ792466	—	KU306739	Silverio et al. (2016)
	RV02	KJ792467	—	KU306740	Silverio et al. (2016)
Neopestalotiopsis petila	MFLUCC 17-1738*	MK764275	MK764341	MK764319	Norphanphoun et al. (2019)
Neopestalotiopsis phangngaensis	MFLUCC 18-0119*	MH388354	MH412721	MH388390	Tibpromma et al. (2018)
Neopestalotiopsis piceana	CBS 394.48*	KM199368	KM199453	KM199527	Maharachchikumbura et al. (2014)
Neopestalotiopsis protearum	CMM 1357*	KY549597	KY549632	KY549594	Maharachchikumbura et al. (2014)
Neopestalotiopsis rhizophorae	MFLUCC 17-1550*	MK764277	MK764343	MK764321	Norphanphoun et al. (2019)
Neopestalotiopsis rosae	CBS 124745*	KM199360	KM199430	KM199524	Maharachchikumbura et al. (2014)
Neopestalotiopsis rosicola	CFCC 51992*	KY885239	KY885245	KY885243	Jiang et al. (2018)
Neopestalotiopsis samarangensis	CBS 115451*	KM199365	KM199447	KM199556	Maharachchikumbura et al. (2014)
Neopestalotiopsis saprophytica	MFLUCC 12-0282*	KM199345	KM199433	KM199538	Maharachchikumbura et al. (2014)
	CBS 115452	KM199345	KM199433	KM199538	Maharachchikumbura et al. (2014)
Neopestalotiopsis sonneratae	MFLUCC 17-1745*	MK764279	MK764345	MK764323	Norphanphoun et al. (2019)
Neopestalotiopsis sp.	MFLUCC 14-0099				This study
Neopestalotiopsis steyaertii	IMI 192475*	NR156270	KF582794	KF582792	Maharachchikumbura et al. (2014)
Neopestalotiopsis surinamensis	CBS 111494*	NR145240	KM199462	KM199530	Maharachchikumbura et al. (2014)
Neopestalotiopsis thailandica	MFLUCC 17-1730*	MK764281	MK764347	MK764325	Norphanphoun et al. (2019)
Neopestalotiopsis umberspora	MFLUCC 12-0285*	JX398984	JX399019	JX399050	Maharachchikumbura et al. (2012)
Neopestalotiopsis vitis	MFLUCC 15-1265*	KU140694	KU140685	KU140676	Jayawardena et al. (2016)
Neopestalotiopsis zimbabwana	CBS 111495*	JX556231	KM199456	KM199545	Maharachchikumbura et al. (2014)
Pestalotiopsis adusta	ICMP 6088*	AF409957	JX399037	JX399070	Jeewon et al. (2003)
Pestalotiopsis aggestorum	LC6301*	KX895015	KX895348	KX895234	Liu et al. (2017)
Pestalotiopsis anacardiacearum	IFRDCC 2397*	KC247154	KC247155	KC247156	Maharachchikumbura et al. (2013b)
Pestalotiopsis arceuthobii	CBS 434.65*	NR147561	KM199427	KM199516	Maharachchikumbura et al. (2014)
Pestalotiopsis arengae	CBS 331.92*	NR147560	KM199426	KM199515	Maharachchikumbura et al. (2014)

Table 4.2 (continued)

Species name	Strain number	Strain accession number			Reference
		ITS	**TUB2**	**EF-1α**	
Pestalotiopsis australasiae	CBS 114126*	NR147546	KM199409	KM199499	Maharachchikumbura et al. (2014)
	CBS 114141	KM199298	KM199410	KM199501	Maharachchikumbura et al. (2014)
Pestalotiopsis biciliata	CBS 790.68*	KM199305	KM199400	KM199507	Maharachchikumbura et al. (2014)
Pestalotiopsis brachiata	LC2988*	KX894933	KX895265	KX895150	Liu et al. (2017)
	LC8188	KY464142	KY464162	KY464152	Liu et al. (2017)
Pestalotiopsis brassicae	CBS 170.26*	KM199379	—	KM199558	Maharachchikumbura et al. (2014)
Pestalotiopsis camelliae	MFLUCC 12-0277*	NR120188	JX399041	JX399074	Zhang et al. (2012)
Pestalotiopsis cf.verruculosa	CBS 100567	MH553951	MH554610	MH554369	Liu et al. (2019)
Pestalotiopsis chamaeropis	CBS 113607*	KM199325	KM199390	KM199472	Maharachchikumbura et al. (2014)
Pestalotiopsis clavata	MFLUCC 12-0268*	JX398990	JX399025	JX399056	Maharachchikumbura et al. (2012)
Pestalotiopsis colombiensis	CBS 118553*	NR147551	KM199421	KM199488	Maharachchikumbura et al. (2014)
Pestalotiopsis digitalis	MFLUCC 14-0208*	KP781879	KP781883	—	Liu et al. (2015)
Pestalotiopsis dilucida	LC3232*	KX894961	KX895293	KX895178	Liu et al. (2017)
	LC8184	KY464138	KY464158	KY464148	Liu et al. (2017)
Pestalotiopsis diploclisiae	CBS 115587*	KM199320	KM199419	KM199486	Maharachchikumbura et al. (2014)
	CBS 115585	KM199315	KM199417	KM199483	Maharachchikumbura et al. (2014)
Pestalotiopsis disseminatas	CBS 118552	MH553986	MH554652	MH554410	Liu et al. (2019)
	CBS 143904	MH554152	MH554825	MH554587	Liu et al. (2019)
	CPC 29351	MH554166	MH554839	MH554601	Liu et al. (2019)
Pestalotiopsis diversiseta	MFLUCC 12-0287*	JX399009	JX399040	JX399073	Maharachchikumbura et al. (2012)
Pestalotiopsis doitungensis	MFLUCC 14-0115*				This study
Pestalotiopsis dracontomelon	MFLUCC 14-0207*	—	—	KP781880	Liu et al. (2015)
Pestalotiopsis ericacearum	IFRDCC 2439*	KC537807	KC537821	KC537814	Zhang et al. (2013)
Pestalotiopsis formosana	NTUCC 17-009*	MH809381	MH809385	MH809389	Ariyawansa et al. (2018)
	NTUCC 17-010	MH809382	MH809386	MH809390	Ariyawansa et al. (2018)
Pestalotiopsis furcata	MFLUCC 12-0054*	NR120087	—	—	Maharachchikumbura et al. (2013a)
Pestalotiopsis gaultheria	OP137*	KC537805	KC537819	KC537812	Maharachchikumbura et al. (2013a)
Pestalotiopsis gibbosa	NOF 3175*	LC311589	LC311590	LC311591	Watanabe et al. (2018)
Pestalotiopsis grandis-urophylla	E-72-02*	KU926708	KU926716	KU926712	Carvalho et al. (2019)
	E-72-03	KU926709	KU926717	KU926713	Carvalho et al. (2019)
	E-72-04	KU926710	KU926718	KU926714	Carvalho et al. (2019)

Table 4.2 (continued)

Species name	Strain number	Strain accession number			Reference
		ITS	TUB2	EF-1α	
Pestalotiopsis grandis-urophylla	E-72-06	KU926711	KU926719	KU926715	Carvalho et al. (2019)
Pestalotiopsis grevilleae	CBS 114127*	KM199300	KM199407	CBS114127	Maharachchikumbura et al. (2014)
Pestalotiopsis hawaiiensis	CBS 114491*	NR147559	KM199428	KM199514	Maharachchikumbura et al. (2014)
Pestalotiopsis hispanica	CBS 115391*	MH553981	MH554640	MH554399	Liu et al. (2019)
Pestalotiopsis hollandica	CBS 265.33*	NR147555	KM199388	KM199481	Maharachchikumbura et al. (2014)
Pestalotiopsis humicola	CBS 115450	KM199319	KM199418	KM199487	Maharachchikumbura et al. (2014)
Pestalotiopsis humus	CBS 336.97*	KM199317	KM199420	KM199484	Maharachchikumbura et al. (2014)
Pestalotiopsis inflexa	MFLUCC 12-0270*	JX399008	JX399039	JX399072	Maharachchikumbura et al. (2012)
Pestalotiopsis intermedia	MFLUCC 12-0259*	JX398993	JX399028	JX399059	Maharachchikumbura et al. (2012)
Pestalotiopsis italiana	MFLUCC12_0657*	KP781878	KP781882	KP781881	Liu et al. (2015)
Pestalotiopsis jesteri	CBS 109350*	KM199380	KM199468	KM199554	Strobel et al. (2000)
Pestalotiopsis jiangxiensis	LC4399*	KX895009	KX895341	KX895227	Liu et al. (2017)
Pestalotiopsis jinchanghensis	LC8190*	KY464144	KY464164	KY464154	Liu et al. (2017)
	LC6636	KX895028	KX895361	KX895247	Liu et al. (2017)
Pestalotiopsis kenyana	CBS 442.67*	KM199302	KM199395	KM199502	Maharachchikumbura et al. (2014)
	CBS 911.96	KM199303	KM199396	KM199503	Maharachchikumbura et al. (2014)
	LC6633	KX895027	KX895360	KX895246	Liu et al. (2017)
	LC3633	KX894992	KX895323	KX895209	Liu et al. (2017)
	LC3291	KX894962	KX895294	KX895179	Liu et al. (2017)
Pestalotiopsis knightiae	CBS 114138*	KM199310	KM199408	KM199497	Maharachchikumbura et al. (2014)
	CBS 111963	KM199311	KM199406	KM199495	Maharachchikumbura et al. (2014)
Pestalotiopsis leucadendri	CBS 121417*	MH553987	MH554654	MH554412	Liu et al. (2019)
Pestalotiopsis licualacola	HGUP 4057*	KC492509	KC481683	KC481684	Ariyawansa et al. (2018)
Pestalotiopsis linearis	MFLUCC 12-0271	JX398994	JX399027	JX399060	Maharachchikumbura et al. (2012)
Pestalotiopsis longiappendiculata	LC3013*	KX894939	KX895271	KX895156	Liu et al. (2017)
Pestalotiopsis lushanensis	LC8183	KY464137	KY464157	KY464147	Liu et al. (2017)
	LC4344*	KX895005	KX895337	KX895223	Liu et al. (2017)
	LC8182	KY464136	KY464156	KY464146	Liu et al. (2017)
	MFLUCC 14-0090*				This study
Pestalotiopsis macadamiae	BRIP 63738a	KX186678	KX18668	KX186622	Akinsanmi et al. (2017)
	BRIP 63738b*	KX186588	KX186680	KX186620	Akinsanmi et al. (2017)

Table 4.2 (continued)

Species name	Strain number	Strain accession number			Reference
		ITS	TUB2	EF-1α	
Pestalotiopsis malayana	CBS 102220*	NR147550	KM199411	KM199482	Maharachchikumbura et al. (2014)
Pestalotiopsis monochaeta	CBS 144.97*	KM199327	KM199386	KM199479	Maharachchikumbura et al. (2014)
Pestalotiopsis montellica	MFLUCC 12-0279*	JX399012	JX399043	JX399076	Maharachchikumbura et al. (2012)
Pestalotiopsis neolitseae	NTUCC 17-011*	MH809383	MH809387	MH809391	Ariyawansa et al. (2018)
Pestalotiopsis novae-hollandiae	CBS 130973*	NR147557	KM199425	KM199511	Maharachchikumbura et al. (2014)
Pestalotiopsis oryzae	CBS 111522*	KM199294	KM199394	KM199493	Maharachchikumbura et al. (2014)
Pestalotiopsis pallidotheae	MAFF 240993*	NR111022	LC311584	LC311585	Watanabe et al. (2010)
Pestalotiopsis papuana	CBS 887.96*	KM199318	KM199415	KM199492	Maharachchikumbura et al. (2014)
Pestalotiopsis parva	CBS 265.37*	KM199312	KM199404	KM199508	Maharachchikumbura et al. (2014)
Pestalotiopsis photinicola	GZcc 16-0028*	KY092404	KY047663	KY047662	Chen et al. (2017)
Pestalotiopsis portugalica	LC4337*	KX895003	KX895335	KX895221	Maharachchikumbura et al. (2014)
Pestalotiopsis rhizophorae	MFLUCC 17-0416*	MK764283	MK764349	MK764327	Norphanphoun et al. (2019)
Pestalotiopsis rhododendri	IFRDCC 2399	KC537804	KC537818	KC537811	Zhang et al. (2013)
	CBS 144024	MH554109	MH554782	MH554543	Liu et al. (2019)
Pestalotiopsis rhodomyrtus	LC3413*	KX894981	KX895313	KX895198	Song et al. (2013)
Pestalotiopsis rosea	MFLUCC 12-0258*	JX399005	JX399005	JX399005	Maharachchikumbura et al. (2012)
Pestalotiopsis scoparia	CBS 176.25*	KM199330	KM199330	KM199330	Maharachchikumbura et al. (2014)
Pestalotiopsis sequoia	MFLUCC 13-0399*	NR153271	—	—	Hyde et al. (2016)
Pestalotiopsis shorea	MFLUCC 12-0314*	KJ503811	KJ503814	KJ503817	Song et al. (2014)
Pestalotiopsis sp.	LC3637*	KX894993	KX895324	KX895210	Liu et al. (2017)
Pestalotiopsis sp.1	CBS 111576	MH553961	MH554620	MH554379	Liu et al. (2019)
Pestalotiopsis sp.2	CBS 114489	MH553978	MH554637	MH554396	Liu et al. (2019)
Pestalotiopsis sp.3	CBS 143892	MH554129	MH554802	MH554564	Liu et al. (2019)
Pestalotiopsis sp.4	CBS 143905	MH554153	MH554826	MH554588	Liu et al. (2019)
Pestalotiopsis sp.5	CBS 143900	MH554142	MH554815	MH554577	Liu et al. (2019)
Pestalotiopsis sp.6	CBS 143902	MH554146	MH554819	MH554581	Liu et al. (2019)
	CPC 27696	MH554147	MH554820	MH554582	Liu et al. (2019)
	CPC 27641	MH554145	MH554818	MH554580	Liu et al. (2019)
	CPC 29456	MH554167	MH554840	MH554602	Liu et al. (2019)
Pestalotiopsis sp.7	CBS 110326	MH553957	MH554616	MH554375	Liu et al. (2019)
	CBS 127.80	MH553995	MH554664	MH554422	Liu et al. (2019)
Pestalotiopsis spathulata	CBS 356.86*	NR147558	KM199423	KM199513	Maharachchikumbura et al. (2014)

Table 4.2 (continued)

Species name	Strain number	Strain accession number			Reference
		ITS	**TUB2**	**EF-1α**	
Pestalotiopsis spathuliappendiculata	CBS 144035	MH554172	MH554845	MH554607	Liu et al., 2019
Pestalotiopsis telopeae	CBS 114137*	KM199301	KM199469	KM199559	Maharachchikumbura et al., 2014
Pestalotiopsis terricola	CBS 141.69*	MH554004	MH554680	MH554438	Liu et al., 2019
Pestalotiopsis thailandica	MFLUCC 17-1616*	MK764285	MK764351	MK764329	Norphanphoun et al., 2019
	MFLUCC 17-1617	MK764286	MK764352	MK764330	Norphanphoun et al., 2019
Pestalotiopsis trachicarpicola	OP068*	JQ845947	JQ845945	JQ845946	Zhang et al., 2012
	MFLUCC 19-0222				This study
Pestalotiopsis unicolor	MFLUCC 12-0275*	JX398998	JX398998	JX398998	Maharachchikumbura et al., 2012
Pestalotiopsis verruculosa	CBS 100567	MH553951	MH554610	MH554369	Maharachchikumbura et al., 2012
Pestalotiopsis vismiae	KUC5020*	GQ241288	—	—	Zhang et al., 2003
Pestalotiopsis yanglingensis	LC4553*	KX895012	KX895345	KX895231	Liu et al., 2017
Pestalotiopsis yunnanensis	HMAS 96359*	AY373375	—	—	Wei et al., 2013
Pseudopestalotiopsis cocos	CBS 272.29*	MH855069	KM199467	KM199553	Maharachchikumbura et al., 2014
Pseudopestalotiopsis theae	MFLUCC 14-0055*	JQ683727	JQ683711	JQ683743	Maharachchikumbura et al., 2014

CHAPTER 5

ENDOPHYTIC BOTRYOSPHAERIALES SPECIES IN *DENDROBIUM* SPECIES[4]

Abstract

Neofusicoccum and *Phyllosticta* species are endophytes, saprobes and opportunistic pathogens of many economic and ornamental plants. There are few reports of Boytryosphaeriales species are available to be associated with orchids worldwide. In this study, five endophytic *Neofusicoccum* and two *Phyllosticta* strains were isolated from *Dendrobium* orchid leaves and stems in southwestern China and northern Thailand. Morphological characteristics and phylogenetic analysis of the combined partial nuclear rDNA internal transcribed spacer (ITS), part of the actin gene (ACT), partial RNA polymerase II second largest subunit (RPB2), part of the translation elongation factor 1 alpha (EF-1α) and beta-tubulin (TUB2) dataset revealed that these isolates belong to two species *Neofusicoccum* occulatum, *N. parvum* and *Phyllosticta capitalensis*. This is the first record of *Neofusicoccum occulatum* and *Phyllosticta capitalensis* associated with *Dendrobium chrysanthum* while *N.parvum* associated with *D. harveyanum* and *D. moschatum*.

Keywords: *Botryosphaeriaceae*, Endophytes, Multi-genes, New Host Records, Orchidaceae

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5.1 Introduction

Dendrobium Sw. is an important medicinal and ornamental genus in Orchidaceae (Bulpitt et al., 2007). Many species in this genus have been endangered according to The International Union for Conservation of Nature red list of threatened species. A considerable amount of the research on Dendrobium has focused on their cultivation and associated mycorrhizae (Cameron et al., 2007; Nontachaiyapoom et al., 2010; Shao et al., 2020).

Endophytes reside in plants asymptomatically (Hyde & Soytong, 2008; Huang et al., 2009; DeMers & May, 2020). Many species are endophytes and pathogens in the fungal order Botryosphaeriales on a wide range of woody plants (Yang et al., 2017; Jami et al., 2018). Especially, Neofusicoccum and Phyllosticta species in Botryosphaeriales are common endophytes in orchids (Yuan et al., 2009; Sawmya et al., 2013).

Certain fungal assemblages could be important plant resources that are helpful for plant cultivation and exploration of secondary products (Pant et al., 2017). Fungal endophytes can be symbionts contributing to orchid seed germination and development (Nontachaiyapoom et al., 2010; Smith & Read, 2010; Sebastian et al., 2014). They likely help in forming orchid mycorrhizae and establishment of orchid species (Brundrett, 2006; Jasinge et al., 2018). Fungal endophytes may produce the same bioactive secondary metabolites as their host plants (Strobel et al., 1996; Tanapichatsakul et al., 2019). Some secondary metabolites such as alkaloids and phenols have been found to possess several bioactivities in orchids (Vaz et al., 2009; Guo et al., 2013; Bungtongdee et al., 2019).

Neofusicoccum Crous, Slippers & A.J.L. Phillips belongs in family *Botryosphaeriaceae* (Botryosphaeriales) (Crous et al., 2006; Phillips et al., 2019). This group represents *Botryosphaeria*-like species with *Fusicoccum*-like asexual morphs and many from *Dichomera*-like synanamorph species with hyaline to brown, globose to pyriform, muriform, septate conidia with age (Crous et al., 2006). *Neofusicoccum* species can be distinguished from *Botryosphaeria* species using multi-loci analysis due to many overlapping morphological characteristics among species (Sakalidis et al., 2011; Yang et al., 2017). The combined multi-locus ITS, EF-1α and TUB2 dataset phylogeny indicates that the genus forms a monophyletic group in the Botryosphaeriales tree (Crous et al., 2006; Sakalidis et al., 2011; Yang et al., 2017; Zhang et al., 2017). The combined ITS, RPB2, EF-1α and TUB2 sequences are recommended for delineating Botryosphaeriaceae species (Schoch et al., 2009; Berraf-Tebbal et al., 2014; Hyde et al., 2014; Yang et al., 2017; Jayawardena et al., 2019). There are 46 and 44 valid *Neofusicoccum* epithets in Index Fungorum (http://www.indexfungorum.org/names/Names.asp, accessed November 2020) and MycoBank (https://www.mycobank.org/page/Simple%20names%20search). Most *Neofusicoccum* species are dieback and canker causing fungi. They are widespread in many woody and medicinal hosts, such as Aesculus hippocastanum, Malus domestica and Melia azedaracha (Ismail et al., 2013; Lopes et al., 2016; Moral et al., 2017). However, in *Orchidaceae*, only *Neofusicoccum* umdonicola was isolated from hanging velamen roots of Acampe praemorsa in southwestern India and there are no any related reports from other orchids (McGregor et al., 2016; Deepthi & Ray, 2018).

Phyllosticta was introduced by Persoon (1818) to accommodate *P. convallariae* (nom. inval., No description). *Phyllosticta cruenta* is a synonym of *P. convallariae*, which was proposed as the type species (Sutton & van der Aa, 1974). It was regarded as a genus in *Botryosphaeriaceae* (Schoch et al., 2006), confirmed in Phillips et al. (2019). Liu et al. (2012a) found that *Phyllosticta* is distinct from other genera in the *Botryosphaeriaceae* and it was widely accepted as a single genus. At present, more than 2000 species have been recorded (https://botryosphaeriales.org/botryosphaeriales-/phyllostictaceae/). Species of *Phyllosticta* have significant economic importance causing diseases on leaf and fruit, which was regarded as a quarantine pest in Europe and the USA (Baayen et al., 2002, Glienke et al., 2011, Wikee et al., 2013a). Some species are common fungal endophytes (Wikee et al., 2013a, Liu et al., 2017a). This genus is mainly characterized by hyaline and aseptate conidia that may be or not covered by a mucoid layer with a single apical appendage growing in pycnidia (asexual), and erumpent ascomata (globose to pyriform) (Wikee et al., 2013b).

In this study, we identified endophytic five *Neofusicoccum* and two *Phyllosticta* strains isolated from *Dendrobium chrysanthum*, *D. harveyanum*, *D. moschatum* and *Dendrobium* sp. collected in southwestern China and northern Thailand. This is the first record of *Neofusicoccum* strains from *Dendrobium harveyanum* and *D. moschatum*, and also first report of *Phyllosticta capitalensis* from *Dendrobium chrysanthum*.

5.2 Materials and Methods

5.2.1 Sample Collection

Healthy leaves of *Dendrobium harveyanum* and *D. moschatum* were collected from Wat Phra That Doi Tung (Temple of Doi Tung Pagoda), Mae Fah Luang District and Huaykrai Mai Temple, Huaykrai Sub-District, Mae Sai District respectively. Both sites are located in Chiang Rai Province, northern Thailand. Healthy stems of *Dendrobium chrysanthum* and *Dendrobium* sp. were sampled from the Animal Husbandry and Veterinary Institute, Guiyang City and from an orchid nursery in Xingyi, respectively. The two sites are located in Guizhou Province, southwestern China. Fresh materials were stored in zip-lock bags or tubes containing silica gel on ice to keep the material fresh.

5.2.2 Fungal Isolation and Cultivation

Fungal isolation was carried out within 48 hours after collection. The surface sterilization was done following the method by Nontachaiyapoom et al. (2010) with modifications. Materials were washed under tap water and immersed in a solution containing 3% (v/v) H_2O_2 and 70% (v/v) ethanol for 5 minutes aseptically. Then they were rinsed with sterile distilled water for three times. The sterilized materials were cut into 2 mm^2 pieces and placed on potato dextrose agar (PDA) containing 50 µg/ml oxytetracycline, 50 µg/ml penicillin and 50 µg/ml streptomycin (Otero et al., 2002). Plates were incubated at 28 °C in the dark. Cultures were observed everyday. The vegetative mycelia were transferred to fresh PDA for purification. The pure cultures were deposited at Mae Fah Luang University Culture Collection (MFLUCC). The dry cultures of new species were deposited in the herbarium of Mae Fah Luang University (MFLU).

5.2.3 Sporulation and Observation

PDA and 2% of water agar (WA) slide cultures were used to induce sporulation in Neofusicoccum isolates (McClenny 2005). Slide cultures comprised a sterilized slide with 1 mm^2 cube of water agar (WA) in aseptic moist plates. Vegetative mycelia of pure cultures were transferred to PDA and WA on the slide culture. Cultures were kept in a dark cabinet at room temperature (28 °C) and observed every day up to five days. The growth rate was measured by mycelia covering the whole plate surface. When conidiomata-like structures were observed, vertical sections were cut with a razor blade using a stereomicroscope (SteREO Discovery. V8, Carl Zeiss Microscopy GmBH, Germany). The detected other structures were picked out, crushed and mounted in water or 10% potassium hydroxide (KOH) and stained with 5% Congo red. Slide preparations were observed with a compound microscope (EOS Y-TV55, Nikon, Japan or Leica, DM 2500). All observed structures were incorporated to evaluate morphological characteristics. The morphological features were measured with Image Frame Work (IFW) v.0.9.7 and photo plates were prepared using Photoshop CS 6.0.

5.2.4 DNA Extraction and Amplification

The whole fungal genome was extracted using EZgene Fungal gDNA Kit (GD2416, Biomiga, USA) following the given protocols. There are four loci were selected for DNA amplification (Lopes et al., 2016). The nuclear rDNA internal transcribed spacer (ITS) region that includes ITS1, the 5.8S rRNA gene and ITS2 were amplified with primers ITS1 and ITS4 (White et al., 1990). The partial RNA polymerase II second largest subunit (RPB2) was amplified with RPB2-6F and RPB2-7Cr (Liu et al., 1999). The partial translation elongation factor 1 alpha (TEF1) was amplified with EF1-728F and EF1-986R (Carbone & Kohn, 1999). And the partial beta-tubulin (TUB2) was amplified with BT2A and BT2B (Glass and Donaldson 1995).

The PCR protocols followed the method in Yang et al (2017). The amplification was carried out using reagents purchased from BIOMIGA (San Diego, USA). Each 25μl amplification reaction contained 12.5 μl of 2*Bench Top^{TM} Taq Master Mix (0.05 units/μl Taq DNA polymerase, 0.4 mM dNTPs and 4 mM $MgCl_2$); 1μl forward and reverse primers respectively; 1μl of DNA template and 9.5 μl of threefold-distilled water. The PCR products were visualized on 1 % agarose gel stained with Goldview

(Realtimes Biotech, Beijing, China). PCR products were sent to TsingKe (China) for purification and sequencing.

5.2.5 Sequence Analysis

Sequences were checked and edited with DNASTAR SeqMan v.7.1.0. The corrected sequences were subjected to BLASTn searches in GenBank to select related sequences (www.ncbi.nlm.nih.gov/BLAST) (Altschul et al., 1997). Sequences of supplemental strains referred mainly from the literature of Yang et al. (2017), especially ex-type and ex-epitype sequences were retrieved from GenBank. All selected strains are listed (TABLE 5.1). Sequences were aligned with MAFFT v.7.0 (https://mafft.cbrc.jp/alignment/server/) and edited using AliView v.1.26 (Larsson 2014). The four loci were combined into a concatenated dataset with SequenceMatrix 1.7.8 (Vaidya et al., 2011).

The best fitting nucleotide substitution models for each locus were estimated by IQ-TREE software with the ultrafast bootstrap (Nguyen et al., 2014; Hoang et al., 2017). Gaps were regarded as missing data and all phylogenetic analyses were done in the CIPRES Science Gateway V. 3.3 (http://www.phylo.org/index.php/). The maximum likelihood (ML) tree was constructed using RAxML-HPC2 on XSEDE. The Generalized time - reversible (GTR) with gamma distribution was default setting model for bootstrapping phase. Maximum likelihood bootstrap values (MLB) were calculated with 1000 iterations. The Bayesian Inference (BI) tree was implemented by MrBayes on XSEDE (3.2.6). Two sets of four simultaneous independent chains of Markov chains Monte Carlo (MCMC) simulations were run for 5,000,000 generations, 25% of the trees were discarded as the burn-in and the remaining trees were used to calculate Bayesian posterior probabilities (BPP). Convergence was assumed when the standard deviation of split sequences was less than 0.01. The constructed trees were submitted to TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S26966). The resulting trees were inspected with Figtree v.1.4.0 and the layout was done with Adobe Illustrator CS6.

Table 5.1 Details of fungal strains involved in this study

Species	**Strain**	**Strain Accession No.**				**Reference**
		ITS	**TUB2**	**TEF1**	**RPB2**	
Botryosphaeria corticis	CBS 119047	DQ299245	EU673107	EU017539	–	Phillips et al. (2008)
B. dothidea	CBS 115476	AY236949	AY236927	AY236898	–	Hane et al. (2007)
Dothiorella casuarinae	**CBS 120688**	MH863089	–	–	KX463970	Vu et al. (2019)
D. longicollis	**CBS 122068**	KF766162	KF766130	EU144069	KX463972	Vu et al. (2019)
D. sarmentorum	CBS 165.33	AY573208	–	AY573225	–	Phillip et al. (2005)
D. striata	**CBS 124731**	EU673321	–	EU673288	KX463976	Yang et al. (2017)
D. vidmadera	CBS 725.79	KX464130	KX464888	KX464622	KX463979	Yang et al. (2017)
Neofusicoccum algeriense	CBS 719.85	KX464151	KX464921	KX464646	KX464000	Yang et al. (2017)
	ALG1	KJ657702	–	KJ657715	–	Berraf-Tebbal et al. (2014)
	ALG9	KJ657704	–	KJ657721	–	Berraf-Tebbal et al. (2014)
	ALG11	KJ657705	–	KJ657722	–	Berraf-Tebbal et al. (2014)
N. andinum	CBS 117921	KX464152	KX464924	KX464647	–	Yang et al. (2017)
	CBS 117453	AY693976	KX464923	AY693977	KX464002	Crous et al. (2006)
N. arbuti	CBS 116576	KX464156	KX464928	KX464651	–	Yang et al. (2017)
	CBS 116131	AY819720	KF531793	KF531792	KX464003	Crous et al. (2006)
N. australe	**CMW 6837**	AY339262	AY339254	AY339270	EU339573	Slippers et al. (2013)
	CMW 6853	AY339263	AY339255	AY339271	–	Slippers et al. (2013)
N. batangarum	CBS 124923	FJ900607	FJ900634	FJ900654	FJ900615	Schena et al. (2018)
	CBS 124924	FJ900608	FJ900635	FJ900653	FJ900616	Schena et al. (2018)
N. brasiliense	CMM 1338	JX513630	KC794031	JX513610	–	Marques et al. (2013)
	CMM 1285	JX513628	KC794030	JX513608	–	Marques et al. (2013)
N. buxi	CBS 113714	KX464164	KX464954	KX464677	KX464009	Yang et al. (2017)
N. cordaticola	CMW 13992	EU821898	EU821838	EU821868	EU821928	Pavlic et al. (2009)
	CBS 123634	EU821898	EU821838	EU821868	EU821928	Vu et al. (2019)
N. corticosae	CBS 118099	KX464168	KX464957	KX464681	KX464012	Yang et al. (2017)
	CBS 120081	DQ923533	KX464958	KX464682	KX464013	Yang et al. (2017)
N. cryptoaustrale	CMW 20738	FJ752740	FJ752754	FJ752710	–	Pavlic et al. (2017)
N. eucalypticola	**CMW 6539**	AY615141	AY615125	AY615133	–	Slippers et al. (2005)
	CBS 115791	AF283686	AY236920	AY236891	–	Smith et al. (2001)
N. eucalyptorum	CAA 511	KX505907	KX505919	KX505896	–	Lopes et al. (2017)
N. grevilleae	CPC 16999	JF951137	–	–	–	Crous et al. (2011)
N. hellenicum	CERC 1959	KP217057	KP217073	KP217065	–	Chen et al. (2015)
N. hongkongense	CERC 2973	KX278052	KX278261	KX278157	KX278283	Chen et al. (2015)
N. illicii	CGMCC 3.18313	KY350152	KY350158	KY817758	–	Zhang et al. (2017)
N. italicum	**MFLUCC 15-0900**	KY856755	–	KY856754	–	Marin-Felix et al. (2017)
N. kwambonambiense	CMW 26856	GQ471843	–	GQ471800	–	Lopes et al. (2016)
	CMW 14023	EU821900	EU821840	EU821870	EU821930	Pavlic et al. (2009)
N. lumnitzerae	CMW 41469	KP860881	KP860801	KP860724	KU587925	Osorio et al. (2017)

Table 5.1 (continued)

Species	Strain	Strain Accession No.				Reference
		ITS	**TUB2**	**TEF1**	**RPB2**	
N. luteum	CBS 562.92	KX464170	KX464968	KX464690	KX464020	Yang et al. (2017)
	CBS 110299	AY259091	DQ458848	AY573217	–	Lopes et al. (2017)
N. macroclavatum	**CBS 118223**	DQ093196	DQ093206	DQ093217	KX464022	Yang et al. (2017)
N. mangiferae	CBS 118531	AY615185	AY615172	DQ093221	–	Yang et al. (2017)
	CBS 118532	AY615186	AY615173	DQ093220	KX464023	Yang et al. (2017)
N. mangroviorum	CMW 41364	KU587959	KU587870	KU587949	KU587904	Osorio et al. (2017)
N. mediterraneum	**CBS 121718**	MH863145	–	–	KY855815	Vu et al. (2019)
	CBS 121558	GU799463	GU799461	GU799462	–	Urbez-Torres et al. (2010)
N. microconidium	CERC 3498	KX278054	KX278263	KX278159	MF410204	Chen et al. (2015)
N. nonquaesitum	**CBS 126655**	MH864187	–	–	KX464025	Vu et al. (2019)
N. occulatum	**CBS 128008**	MH864743	EU339472	EU339509	EU339558	Vu et al. (2019)
	DAR 82923	MH168676	–	MH252415	MH215468	Scarlett et al. (2019)
	CBS 256.80	KX464175	KX464973	KX464696	KX464026	Yang et al. (2017)
	BRIP64094	MH057202	–	MH102265	–	Tan et al. (2019)
	MFLUCC 20-0234	MW386873	MW435150	MW392586	–	This study
N. pandanicola	KUMCC 170184	MH275072	–	MH412778	–	Tibpromma et al. (2018)
N. parvum	**CMW 9081**	AY236943	AY236917	AY236888	EU821963	Slippers et al. (2004)
	CBS 110301	AY259098	EU673095	AY573221	–	Phillips et al. (2008)
	CBS 124491	MH863377	KX464961	KX464683	KX464016	Yang et al. (2017)
	MFLUCC 14-0163	MW386876	MW435153	MW392589	–	This study)
	MFLUCC 15-1171	MW386872	–	–	–	This study)
	MFLUCC 19-0242	MW386874	MW435151	MW392587	MW435148	This study)
	MFLUCC 19-0244	MW386875	MW435152	MW392588	MW435149	This study)
N. pennatisporum	**MUCC 510**	EF591925	EF591959	EF591976	–	Taylor et al. (2009)
N. pistaciae	CBS 595.76	MH861007	KX464953	KX464676	KX464008	Taylor et al. (2009)
N. pistaciarum	CBS 113083	KX464186	KX464998	KX464712	KX464027	Yang et al. (2017)
	CBS 113084	KX464466	KX4649	KX464713	KX464028	Yang et al. (2017)
N. pistacicola	CBS 113089	KX464199	KX465014	KX464727	KX464033	Yang et al. (2017)
N. protearum	**CBS 114176**	AF452539	KX465006	KX464720	KX464029	Yang et al. (2017)
	CBS 111199	KX464192	KX465004	KX464718	–	Yang et al. (2017)
N. pruni	CBS 121112	–	KX465016	–	KX464034	Yang et al. (2017)
N. ribis	MUCC 296	EU339525	EU339477	EU339514	EU339563	Sakalidis et al. (2011)
	CBS 115475	AY236935	AY236906	AY236877	EU339554	Schoch et al. (2009)
N. sinense	BJFUGJJ 16022010	KY350148	KY350154	–	–	Zhang et al. (2017)
N. sinoeucalypti	CERC 2005	KX278061	KX278270	KX278166	KX278290	Chen et al. (2015)
N. stellenboschiana	CBS 282.70	KX464225	KX465051	KX464758	–	Yang et al. (2017)
N. terminaliae	**CBS 125264**	GQ471805	KX465053	GQ471783	KX464046	Begoude et al. (2010)
N. umdonicola	CMW 28412	KU997418	KU997572	KU997143	–	Mehl et al. (2017)
N. ursorum	**CBS 122811**	MH863237	KX465056	FJ752709	KX464047	Vu et al. (2019)

Table 5.1 (continued)

Species	Strain	Strain Accession No.				Reference
		ITS	TUB2	TEF1	RPB2	
N. variabile	CMW 37742	MH558609	MH569154	MH576585	–	Jami et al. (2018)
N. viticlavatum	**CBS 112878**	AY343381	KX465058	–	KX464048	Yang et al. (2017)
N. vitifusiforme	**CBS 110887**	MH862869	KX465061	–	KX464049	Vu et al. (2019)

5.3 Results

5.3.1 Fungal Isolation and Sporulation

Five *Neofusicoccum* strains were isolated from *Dendrobium* species (TABLE 5.2). All isolates were sporulated after one and half months except for the isolate MFLUCC 19-0242.

Table 5.2 Endophytic *Neofusicoccum* strains collected in this study

Orchid host	Sample sites	Tissues	*Neofusicoccum* species	Strain number
Dendrobium chrysanthum	Guiyang City, China	Stem	*Neofusicoccum occulatum*	MFLUCC 20-0234
D. harveyanum	Chiang Rai Province, Thailand	Leaf	*N. parvum*	MFLUCC 14-0163
D. moschatum	Chiang Rai Province, Thailand	Leaf	*N. parvum*	MFLUCC 15-1171
Dendrobium sp.	Guiyang City, China	Stem	*N. parvum*	MFLUCC 19-0242;
Dendrobium sp.	Guiyang City, China	Stem	*N. parvum*	MFLUCC 19-0244

5.3.2 Phylogenetic Results

The combined dataset comprised 80 sequences containing 1873 nucleotides (including 537 ITS sites, 436 TUB2 sites, 310 EF-1α sites and 594 RPB2 sites). The selected nucleotide substitutional models for ITS and TUB2 dataset were Symmetrical (SYM) and Hasegawa-Kishino-Yano model (HKY) respectively with gamma distribution. The Kimura 2-parameter model (K2P) turned out to be the best-fitting model for both EF-1α (with gamma distribution) and RPB2 (with invgamma) dataset.

The tree was rooted with two *Botryosphaeria* species with robust support values (100% MLB/1.00 BPP). *Neofusicoccum* species were well-separated from the neighbouring genus *Dothiorella*. The isolate MFLUCC 20-0234 was claded with several *Neofusicoccum occulatum* species and were with weak support values. Another four strains MFLUCC 14-0163, MFLUCC 15-1171, MFLUCC 19-0242 and MFLUCC 19-0244 clustered between *N. parvum* and close to *N. algeriense* and *N. italicum* species with low support values (FIGURE 5.1).

The *Phyllosticta* phylogenetic tree generated from maximum likelihood analysis based on combined ITS, ACT and TEF1-a sequence data. Seventy-nine strains are included in the combined analyses which comprised 1076 characters (564 characters for ITS, 235 characters for ACT, 277 characters for TEF1-a) after alignment. Tree topology of the maximum likelihood analysis is similar to the Bayesian analysis. The best RaxML tree with a final likelihood value of -14943.587215 is presented. Estimated base frequencies were as follows: A = 0.185925, C = 0.309856, G = 0.273842, T = 0.230377; substitution rates AC = 1.270161, AG = 3.369060, AT = 1.523062, CG = 1.329349, CT = 5.079430, GT = 1.000000; gamma distribution shape parameter a = 0.5. Bootstrap support values for ML greater than 75% and Bayesian posterior probabilities greater than 0.95 are given near nodes respectively. The tree is rooted with *Botryosphaeria_dothidea* (MFLUCC 17-0961) and *Botryosphaeria_obtusa* (CMW 8232). Ex-type strains are in bold and black.

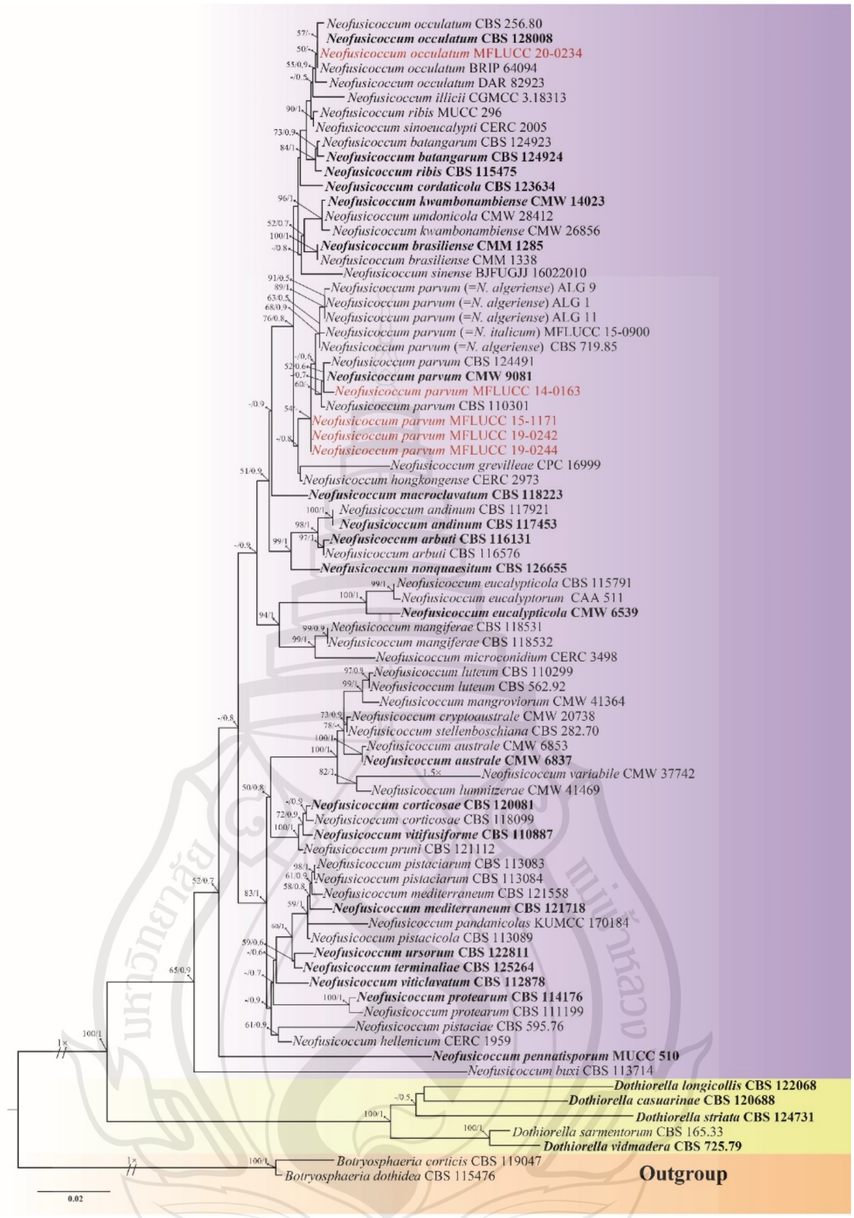


Figure 5.1 The consensus phylogram resulting from a RAxML analysis of the combined four loci alignment (ITS-RPB2-EF-1α-TUB2) of the analyzed *Neofusicoccum* and related species sequences. Strains isolated in this study are in red. Ex-epitype and ex-type isolates are in bold. Maximum likelihood (ML) and Bayesian posterior probabilities (PP) are labelled at the nodes (50% majority rule). Dashes are indicated values lower than 50%. The scale bar corresponds to 0.02 substitutions per site

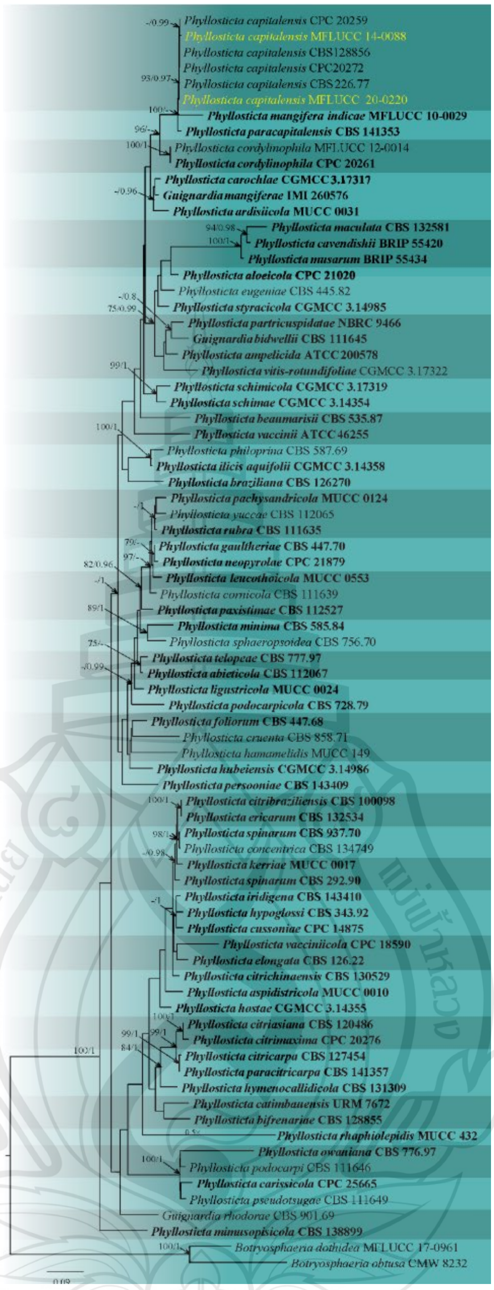


Figure 5.2 The consensus phylogram resulting from a RAxML analysis of the combined four loci alignment (ITS-EF-1α-ACT) of the analyzed *Phyllosticta* and related species sequences. Strains isolated in this study are in red. Ex-epitype and ex-type isolates are in bold. Maximum likelihood (ML) and Bayesian posterior probabilities (PP) are labelled at the nodes. Dashes are indicated values lower than 50%. The scale bar corresponds to 0.09 substitutions per site

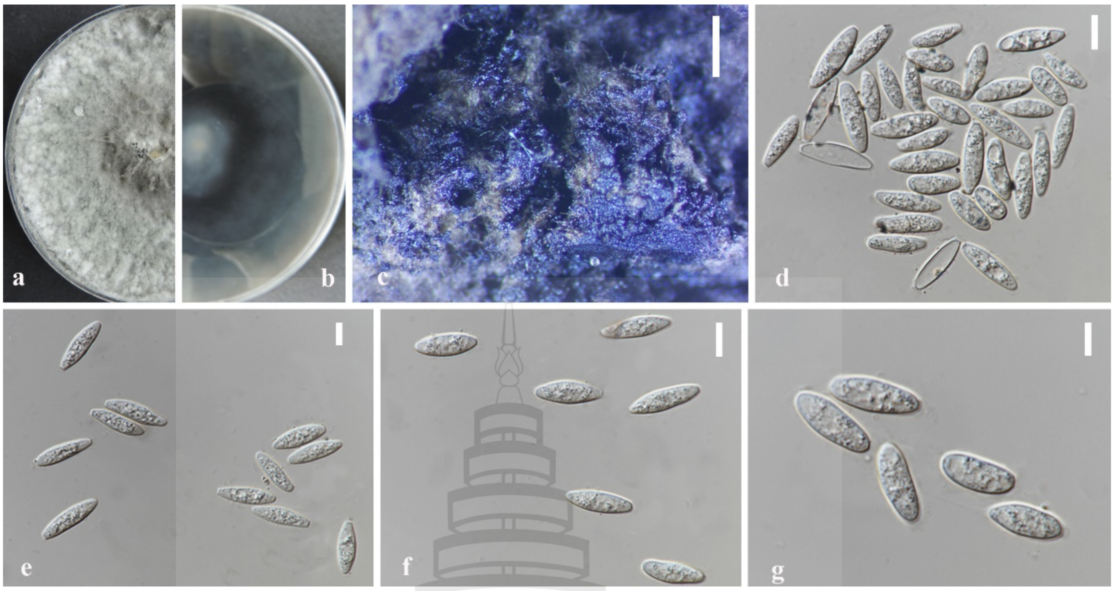
5.3.3 Taxonomy

Neofusicoccum occulatum Sakalidis & T. Burgess

Description: — *Colony* on PDA superficial, grey, fluffy, irregular edge; reverse dark brown to black, with concentric rings, growth rate: 2.5 mm/day. *Colony* on WA was similar to that on PDA. *Vegetative mycelia* 2–4.5 μm diam, hyaline to brown, septate, smooth. *Conidiomata* found under mycelia, black, aggregated, irregular. *Conidia* (15–) 17.5 – 22 (–23) × (5–) 5.5 – 6 (–6.5) μm ($\bar{x}$ = 20 × 6 μm, L:W = 3.3), fusiform to ellipsoidal with obtuse apex, hyaline, smooth, thin-walled, septate, with minute guttules.

Material examined: — CHINA, Guizhou Province, Guiyang City, Animal Husbandry and Veterinary institute, endophytic in the stem of *Dendrobium chrysanthum*, 11 April 2016, S.X. Zhou & X.Y. Ma, living culture, MFLUCC 20-0234.

Notes: — Blast results for MFLUCC 20-0234 hit *Neofusicoccum ribis* (ITS, 100% similarity), *N. parvum* (*TUB*$_2$, 100% similarity) and *Neofusicoccum* sp. (EF-1α, 99% similarity). All sequences of MFLUCC 20-0234 are identical with those of CBS 128008 (*N. occulatum*), which is the type. Morphological characteristics of MFLUCC 20-0234 are the same as the type strain CBS 128008 and those of pathogenic strains isolated from *Eucalyptus* spp. by Sakalidis et al. (2011). Therefore, we identified our isolate as *Neofusicoccum occulatum*.



Note a Colony on PDA (MFLUCC 20-0234) a–b Colony on PDA (a: surface, b: reverse). c Conidiomata masses. d–g. Conidia. Scale bars: c=500 µm, d–g=10 µm.

Figure 5.3 *Neofusicoccum occulatum* (MFLUCC 20-0234)

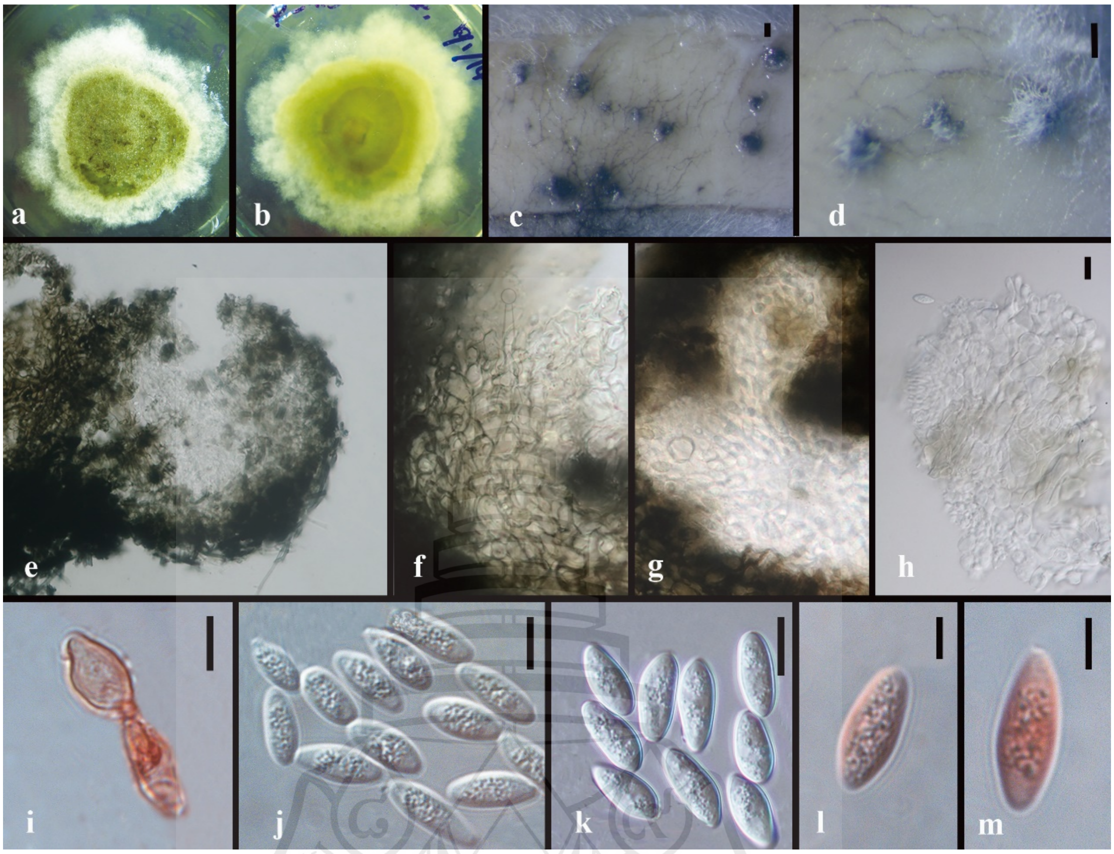
Neofusicoccum parvum (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips

= ***Neofusicoccum italicum*** Dissan. & K.D. Hyde (IF: 820799), in Marin-Felix et al., Stud. Mycol. 86: 170 (2017)

Description: — *Colony* on PDA superficial, yellow brown with white irregular and loose margin, felty, with concentric rings, water drops secretion densely on the surface; reverse flat, yellow brown with white irregular and loose margin; growth rate: 2.4 mm/day. *Vegetative mycelia* 1–4 µm diam, hyaline to brown, smooth, septate, dense. *Conidiomata* 200–500 µm, stromatic, globose to subglobose, solitary or aggregated, black, uni- to multi-locular, ostiolate. *Ostiole* circle, lateral. *Conidiomatal walls* consisting of thick-walled, brown to dark brown cells of *textura angularis* in the outer layers, becoming hyaline in the inner layers. *Conidiophores* reduced to conidiogenous cell. *Conidiogenous cell* enteroblastic, phialidic, hyaline, cylindrical, determinate, integrated, smooth. *Conidia* (14–) 15–17 (–18) × (5–) 5.5–6 (–6.5) µm ($\bar{x}$ = 16 × 6 µm, L:W = 2.8), fusiform to ellipsoid with obtuse apex and slightly truncate end, hyaline, smooth, with dense cytoplasm.

Colony on WA superficial, white with grey mycelia, irregular edge, fluffy, reverse dark grey, centre black, growth rate: 1.3 mm/day. On WA (slide culture), *vegetative mycelia* 1–3.5 µm diam, hyaline to brown, smooth, septate, dense. *Conidiomata* found on the rubber band in slide culture, dark brown to black, stromatic, pycnidial, solitary to aggregated, globose to sub-globose, multi-locular, glabrous, ostiolate. *Ostiole* circle, central. *Conidiomatal wall* consisting of thick-walled, brown to dark brown cells of textura angularis in the outer layers, becoming hyaline cells in the inner layers. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cell* 9–33 × 2–3 µm, rising from inner layers, enteroblastic, phialidic, hyaline, cylindrical to subcylindrical, deter minate, integrated, smooth and thick-walled. *Conidia* (11–) 12 – 14 (–15.5) × (5–) 6 – 6.5 (–7) µm ($\bar{x}$ = 13 × 6 µm; L:W = 2.3), hyaline, oval, fusiform to ellipsoid with obtuse apex and slightly truncate base, aseptate, smooth, thick-walled, with guttules.

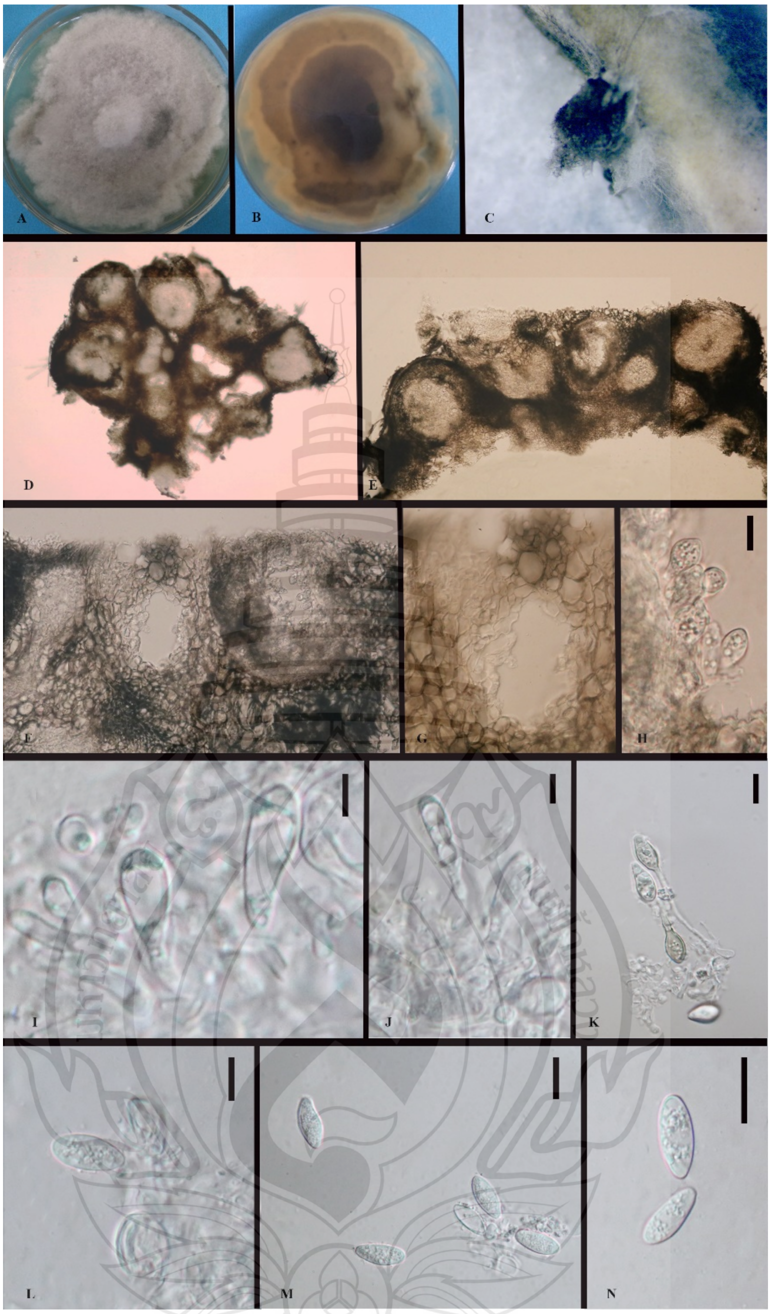
Material examined: — THAILAND, Chiang Rai Province, Wat Phra That Doi Tung (Temple of Doi Tung Pagoda), endophytic in the leaf of *Dendrobium harveyanum*, 19 December 2013, S. Nontachaiyapoom, N. Aewsakul & X.Y. Ma, living culture, MFLUCC 14-0163; Chiang Rai Province, Mae Sai district, Huaykrai Mai Temple, endophytic in the leaf of *D. moschatum*, 10 May 2015, S. Nontachaiyapoom, B. Mala & X.Y Ma, living culture, MFLUCC 15-1171; China, Guizhou Province, Xingyi City, orchid nursery, endophytic in the stem of *Dendrobium* spp., 4 October 2019, B.W. Chen, living culture, MFLUCC 19-0242 and MFLUCC 19-0244.



Note a–b Colony on PDA (A. surface, B. reverse). c–g Conidiomata on PDA. h Conidiogenous cells with conidia. i Conidiogenous cell with conidium in Congo red. j–m Conidia (i, l, m in Congo red). Scale bars: c= 200 µm, d=500 µm , h=20 µm, i–k=10 µm, l–m=5 µm.

Figure 5.4 *Neofusicoccum parvum* (MFLUCC 14-0163 on PDA)

Notes: — The sequences of isolates MFLUCC 14-0163, MFLUCC 15-1171, MFLUCC 19-0242 and MFLUCC 19-0244 have less than 1% different base pairs. The blast results showed that they were most similar to *Neofusicoccum parvum*. There were four different base pairs between MFLUCC 19-0244 and the ex-type strain of *N. parvum* CMW 9081 (1 in ITS, 2 in EF-1α and 1 in *RPB*$_2$). The characteristics, which do not separate the four strains, are very similar with the type strain CMW 9081 and that of pathogenic strains of *N. parvum* (Ismail et al., 2013). Thus, we consider our isolates to be *N. parvum*.



Note a–b Colony on PDA (A: surface, B: reverse). c Conidiomata on slide culture. d–g Pycnidia. h–l Conidiogenous cells with conidia. m–n Conidia. Scale bars: h=10 µm, i–j=5 µm, k–n=10 µm.

Figure 5.5 *Neofusicoccum parvum* (a–b from PDA, c–n from WA)

Table 5.3 Comparison among *Neofusicoccum parvum*, *N. algeriense* and *N. italicum*

Strain (Media)	**Conidiophore**	**Conidiogenous cell**	**Conidium**	**Different base pairs (compared with *N. parvum*)**
MFLUCC 15-0900 (PDA)	Conidiophores reduced to conidiogenous cells	Holoblastic, cylindrical to subobpyriform, hyaline, discrete, determinate, occasionally indeterminate and proliferating percurrently with indistinct annellations, 9–16.5 × 2.5–3.5 µm	Obovoid, fusiform, base truncate, apex obtuse to subobtuse, hyaline, guttulate, non-septate, older conidia may become brownish and septate before germination, 13–18.5 × 3.5–6 µm	3 in ITS
CBS 130995 (PDA)	–	Holoblastic, hyaline, cylindrical, 4.5–19.3 µm long, 1.5–2.7 µm wide	Hyaline, (14.3–) 15.4–17.6 (–19.3) × (5.0–) 5.4–6.2(–6.6) µm	–
ALG1 (MEA)	Cylindrical, hyaline, smooth, thin-walled, septate	Cylindrical, smooth, holoblastic, phialidic with periclinal thickening, 11.5–14(–16.5) × 3–2.5(–5) µm	Hyaline, fusiform, base subtruncate to bluntly rounded, non-septate, rarely forming a septum before germination, (14.5–)17–18(–21) × (4.5–)5.5–5.7(–6.5) µm	2 in ITS, 3 in TUB_2

Neofusicoccum italicum (MFLUCC 15-0900) clustered with the *N. algeriense* / *N. parvum* group in this study (2 base pairs differences in ITS). *Neofusicoccum algeriense* was regarded as a synonym of *N. parvum* (Lopes et al., 2017), therefore, we also regard *N. italicum* as a synonym of *N. parvum*. A comparison of characters is given in TABLE 5.3.

Phyllosticta capitalensis Henn., Hedwigia 48: 13 (1908) [1909]

Index Fungorum number: IF168326; Facesoffungi number: FoF06888, Fig. 17, 18

Endophytic in the leaves of *Dendrobium chrysanthum* Wall. ex Lindl. Sexual morph: (on WA of a slide culture) vegetative mycelia 1.7–4.4 µm diameter, brown, curved, septate, swollen at nodes. Ascomata protrude from the suface of media, clavate to cylindrical, black, independent. *Asci* (45–) 50–61 (–62) × (7.5–) 8–8.5 (–9) µm ($\bar{x}$ = 54.8±6 × 8.1±0.5 µm, n = 12), unitunicate, mostly with 8 ascospores, cylindrical to clavate with obtusely rounded or slightly square at apex, hyaline, smooth-walled. *Ascospores* (7.5–) 8–9 (–10) × (3.5–) 4–4.5 (–5) µm ($\bar{x}$ = 8.5±0.8 × 4.5±0.4 µm, n = 8), sub-globose to pyriform, two ends rounded or truncated, hyaline, widest in the middle or near the middle, guttulate, smooth-walled, listed interlaced. Asexual morph: (on PDA) vegetative hyphae 0.8–2.1 µm diameter, hyaline, septate, branched, smooth. *Conidiomata* scattered around colony surface, aggregate, globose to irregular, greenish black. *Conidiophores* 40 –52.5 µm long, cylindrical, straight to flexuous, hyaline, septate. *Conidiogenous cells* holoblastic. *Conidia* (6–) 6.5–7 (–8) × (3–) 3.5–4 (–4.5) µm ($\bar{x}$ = 6.6±0.6 × 3.6±0.3 µm, n = 20) , ellipsoidal, hyaline, guttulate, sometimes slightly curved, solitary, smooth-walled.

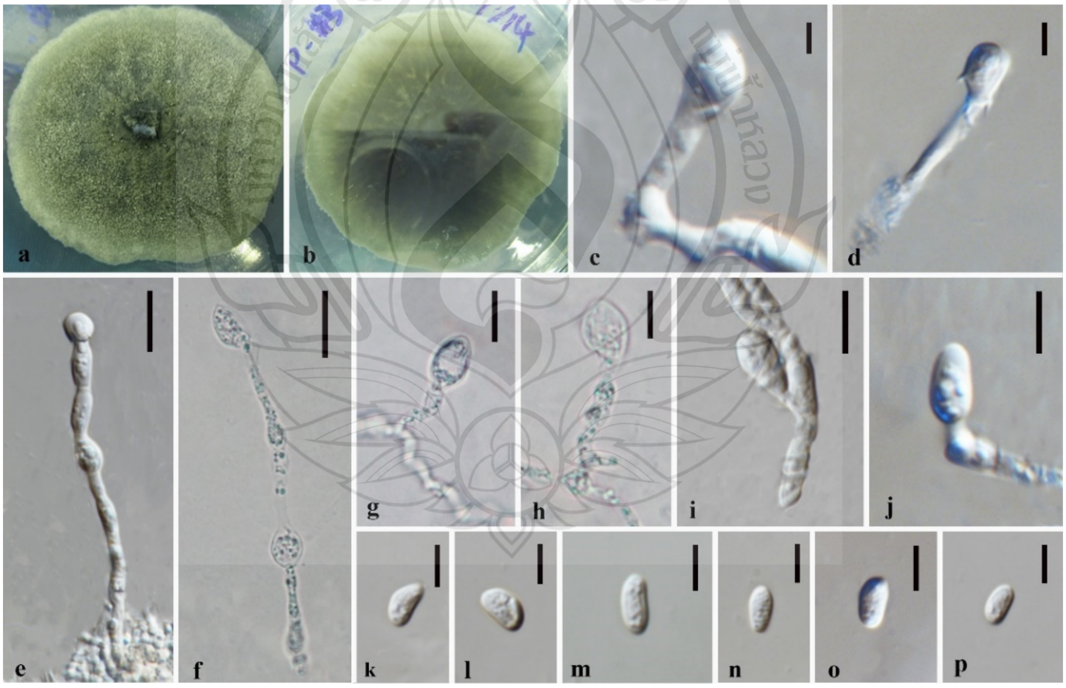
Culture characteristics – Colony on PDA surface superficial, with flat margin, rough, grained, radiated, dense, jungle green front, reverse greenish black with a growth rate of 2.5 mm/day at 28°C.

Material examined – China, Guizhou Province, Xingyi City, orchid nursery, from leaves of *Dendrobium chrysanthum* Wall. ex Lindl., 16 October 2016, Bao-Wen Chen, MFLUCC 20-0220; Thailand, Chiang Rai Province, Wat Phra That Doi Tung (Temple of Doi Tung Pagodas), from leaves of *Dendrobium chrysanthum* Wall. ex Lindl., 19 December 2013, Sureeporn Nontachaiyapoom, Natdanai Aewsakul, Xiao-Ya Ma, MFLUCC 14-0088.

GenBank submissions – ITS: MW084361 (MFLUCC 14-0088), MW084362 (MFLUCC 20-0220); ACT: MW092167 (MFLUCC 14-0088), MW092168 (MFLUCC 20-0220); TEF1-α =MW160396 (MFLUCC 14-0088), MW160397 (MFLUCC 20-0220).

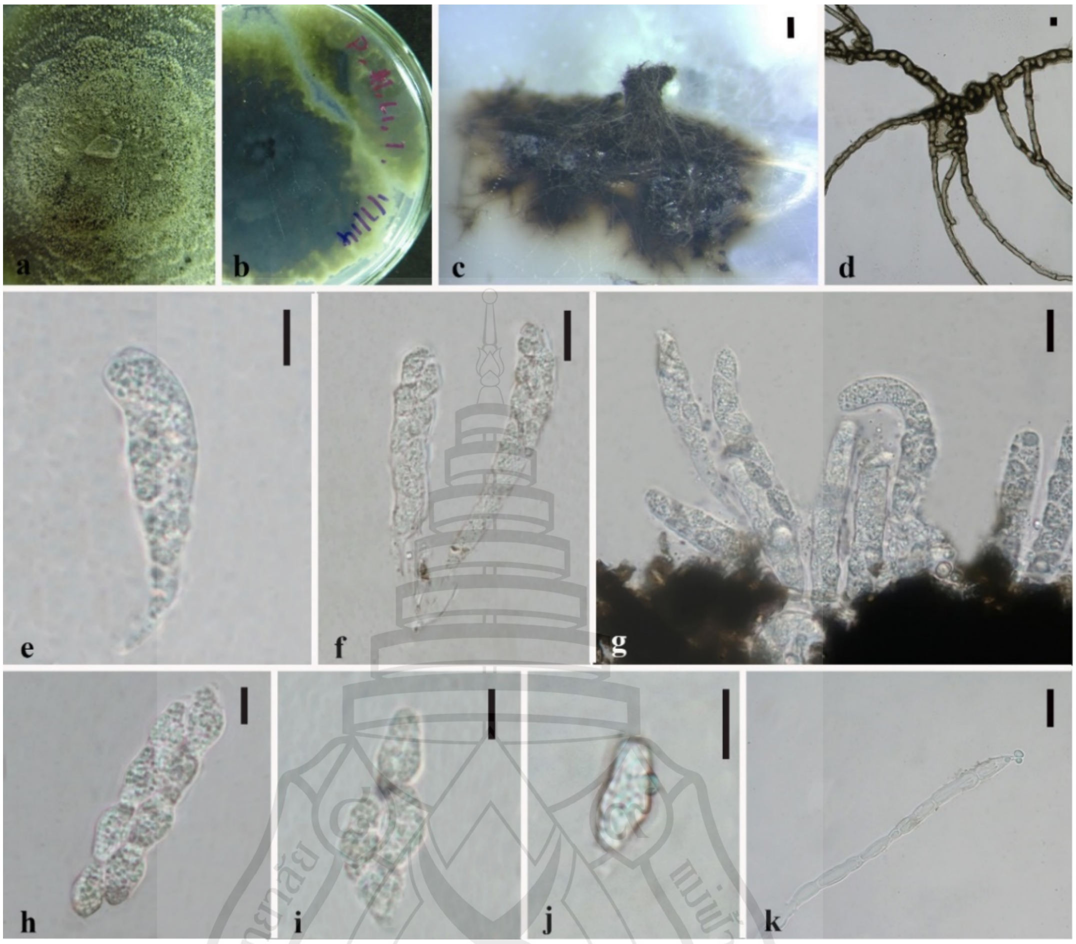
Known distribution (based on molecular data) – Worldwide (Okane et al., 2003, Glienke et al., 2011, Wikee et al., 2013a, Lin et al., 2017).

Notes – Isolates MFLUCC 14-0088 and MFLUCC 20-0220 have identical sequences, so we regard them as the same species. Both clustered with *Phyllosticta capitalensis* isolates together with its ex-type strain CBS 128856. There are only three base pair differences between MFLUCC 14-0088 and *P.* strains, and ascospores are also smaller. The conidia are shorter and wider than that of *P. mangifera-capitalensis* CBS 128856. The asci of MFLUCC 14-0088 are bitunicate and smaller than that of other *indica* (6–)9(–13) × (4–)5(–6) µm. No mucilaginous sheath was found around the conidia.



Note a–b Colony on PDA (a Surface, b Reverse). c–j Conidiophores with conidia. k–p Conidia. Scale bars: c–f = 10 µm, g–p = 5 µm.

Figure 5.6 Asexual morph of *Phyllosticta capitalensis* (MFLUCC 14-0088)



Note a–b Colony on PDA (a Surface, b Reverse). c Fruiting body on WA slide culture. d Mycelia on slide culture. e–i Asci. j Ascospore. k Mycelium with nucleates. Scale bars: c=500 μm, d–g=10 μm, h–j=5 μm, k=10 μm

Figure 5.7 Sexual morph of *Phyllosticta capitalensis* (MFLUCC 14-0088)

5.4 Discussion

This is the first report of endophytic *Neofusicoccum* species in *Dendrobium harveyanum* and *D. moschatum*, and also first report of *Phyllosticta capitalensis* from *Dendrobium chrysanthum*. Other species *Neofusicoccum umdonicola* and *Neofusicoccum* sp. have also been isolated as endophytes from the epiphytic orchids *Acampe praemorsa* and *Vanilla calyculata* (Gamboa-Gaitán & Otero-Ospina, 2016; Deepthi & Ray, 2018). Both *Neofusicoccum occulatum* and *N. parvum* are frequently found as pathogens of many plants especially *Eucalyptus* and they can be transported on *Eucalyptus* germplasm (Sakalidis et al., 2011; 2013; Ismail et al., 2013; Pavlic-Zupanc et al., 2015). *Phyllosticta capitalensis* is an ubiquitous fungal endophytes discovered from many plants (Wikee et al., 2013). In this study, there is no invasive structure was observed.

Neofusicoccum parvum has been found to occur in 90 plant hosts across six continents (Golzar & Burgess, 2011; Sakalidis et al., 2013). *Neofusicoccum occulatum* has been found in *Eucalyptus* and coniferous trees and grape vines in four countries (Sakalidis et al., 2013). *Neofusicoccum parvum* is the most studied species in virulence factors in the grapevine trunk (Massonnet et al., 2018; Masi et al., 2020). Several phytotoxic metabolites including tyrosol, (R)- (−)-mellein, (3R,4S)-(−), (3R,4R)-(−)-4- and (3R)-5-hydroxymellein were isolated from a pathogenic *Neofusicocccum parvum* strain on grapevines in Australian vineyards (Masi et al., 2020). The (3R)-5-hydroxymellein separated from an endophytic strain *Neofusicoccum parvum* isolated from Vitex rotundifolia was found to possess anti-atherosclerotic activity (Kim et al., 2020). The transition of *Neofusicoccum* life mode from endophytic to pathogenic is found to be triggered by water-stressed conditions in *Eucalyptus* globulus (Barradas et al., 2018). Despite being widely studied, the role of *Neofusicocccum* species as endophytes in orchids remains unclear.

Neofusicocccum species appear to be common and widespread on a broad range of hosts representing a potential threat to susceptible plants (Lopes et al., 2016; Dissanayake et al., 2016; Hyde et al., 2020). Co-expressed gene clusters associated with cell wall degradation have been found, which suggested that dynamic co-regulation of transcriptional networks contributed to multiple aspects of N. parvum virulence. (Massonnet et al., 2018). However, pathogenicity testing is required to confirm whether they are able to be pathogens on *Dendrobium* orchids.

CHAPTER 6

PEZICULA ENDOPHYTICA SP. NOV., ENDOPHYTIC IN *DENDROBIUM* IN THAILAND[5]

Abstract

A new species, *Pezicula endophytica,* was isolated from roots and stems of two *Dendrobium* species in northern Thailand. Evidence to support the new species is based on morphology and phylogenetic analysis of the combined ITS, LSU, and RPB2 DNA sequence dataset. *Pezicula endophytica,* which constituted a clade independent from other *Pezicula* species, has 4% distinct base pair differences in all genes. *Pezicula endophytica* has larger macroconidia and longer conidiophores compared with phylogenetically neighboring species. This is the first report of an endophytic *Pezicula* species from *Dendrobium* in Thailand.

Keywords: Ascomycetes, *Cryptosporiopsis*, *Dermateaceae*, Multi-loci, *Orchidaceae*

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[5] This paper has been published in *Mycotaxon Journal*, 2021, *136*, 563–577. https://doi.org/10.5248/136.563

6.1 Introduction

Fungal endophytes in orchids may be symbionts that help plant growth and are possible alternative sources for the bioactive compounds produced in plants (Bungtongdee et al., 2019; Ma et al., 2015; Vujanovic et al., 2000). They also play a role as mycorrhizae in orchids and transfer water and nutrition to the host (Bungtongdee et al., 2019; Ma et al., 2015; Smith & Read, 2010). *Pezicula* Tul. & C. Tul. (Dermateaceae; anamorph: *Cryptosporiopsis* Bubák & Kabát) is apothecial (Ekanayaka et al., 2017, 2019). The name *Pezicula* has been proposed as the accepted name because *Cryptosporiopsis* species are the asexual morphs of both *Pezicula* and *Neofabraea* H.S. Jacks. (Chen et al., 2016; Johnston et al., 2014; Verkley, 1999; Verkley et al., 2003; Wijayawardene et al., 2017a, b). *Pezicula* is characterized by brightly colored, short-stalked apothecia with circular discs that are initially concave and surrounded by slightly elevated margins but later become convex. They occur on the bark of woody plants and are involved in wood degradation (Kowalski, 1996). Asci are inoperculate and mostly clavate or cylindric-clavate, ascospores vary in shape from broadly ellipsoid to elongate-ellipsoid to allantoid or fusoid (Ekanayaka et al., 2016; Ooki et al., 2003; Verkley, 1999; Verkley et al., 2003). The asexual morph of *Pezicula* species produces sporodochial conidiomata, with macro- and microconidia formed on different conidiophores (Ekanayaka et al., 2016; Verkley, 1999). *Pezicula* species are recorded as endophytes, plant pathogens, and saprobes from a wide range of hosts (Chen et al., 2016; Wijayawardene et al., 2017a). They have been isolated as endophytes from *Dendrobium nobile* Lindl. and *Holcoglossum wangii* Christenson (Orchidaceae) in China (Chen et al., 2011; Tan et al., 2012).

Dendrobium Sw. is one of the largest genera in *Orchidaceae* (https://www.kew.org/), and many species are important medicinal and ornamental plants (Ma et al., 2015). However, few reports concern endophytic *Pezicula* species in *Dendrobium* (Chen et al., 2011, Tan et al., 2012).

In this study, we isolated four endophytic fungal strains from two different, undetermined *Dendrobium* species sampled in northern Thailand. The strains were identified as representing a new species, *Pezicula endophytica*, based on morphological and phylogenetic analyses. This is the first report of an endophytic *Pezicula* in *Dendrobium* in Thailand.

6.2 Materials and Methods

6.2.1 Sample Collection

Healthy roots and stems of two different but undetermined *Dendrobium* species (sp.1 and sp.2) were collected from outside the temple of Wat Phra That Doi Tung (Temple of Doi Tung Pagoda), Mae Fah Luang district, Chiang Rai Province, Thailand. Fresh material was kept fresh by placing into Zip-lock bags or tubes containing silica gel on ice.

6.2.2 Fungal Isolation and Cultivation

The material was processed within 48 hours. Surface sterilization followed Nontachaiyapoom et al. (2010) with minor modifications. Healthy roots and stems were washed in tap water and then immersed for 5 minutes in a solution containing 3% (v/v) H_2O_2 and 70% (v/v) ethanol, and then rinsed with three changes of sterile distilled water. The sterilized materials were cut into 2 mm^2 pieces and placed on potato dextrose agar (PDA, Difco, BBL/USA' #213400) containing 50 µg/ml oxytetracycline, 50 µg/ml penicillin, and 50 µg/ml streptomycin (Otero et al., 2002). The surface sterilization method was checked, as described by Petrini (1991). Petri dishes were incubated at 28 °C under natural light and observed daily; mycelia on the edge of developing fungal colonies were transferred to fresh PDA to obtain pure cultures. The pure cultures were deposited in Mae Fah Luang University Culture Collection, Chiang Rai, Thailand (MFLUCC). Dry cultures of the new species were deposited in the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU).

6.2.3 Morphological Analysis

Pure cultures were cultivated on both PDA and water agar (WA) media in a dark cabinet at room temperature (28 °C) and observed every week. Sterilized toothpicks were placed on the WA surface to encourage fungal sporulation. The growth rate was evaluated after mycelia had nearly covered the whole agar surface. Any observable structures were examined using a Zeiss SteREO Discovery.V8 stereomicroscope. The colonies were examined for morphological characteristics and structures were removed and mounted in water. The mounted slides were observed using a Nikon EOS 700D compound microscope. All observed structures were measured with Image Frame Work v.0.9.7 and the photo plates were assembled using Photoshop CS 6.0.

6.2.4 DNA Extraction and Amplification

Genomic DNA was extracted from mycelium of endophytic fungi using an EZgene Fungal gDNA Kit following the manufacturer's instructions. The selected genes for amplification were partial nuclear rDNA internal transcribed spacer (ITS), partial large subunit (LSU), and partial RNA polymerase II large subunit 2 (RPB2). Each 25 µl amplification reaction contained 12.5 µl of 2*Bench TopTM Taq Master Mix (0.05 units/µl Taq DNA polymerase, 0.4 mM dNTPs and 4 mM $MgCl_2$); 2 µl forward and reverse primers; 1 µl of DNA template and 9.5 µl of threefold-distilled water. The primers and PCR protocol followed Chen et al. (2016). PCR products were visualized on 1% agarose gel stained with Realtimes Biotech Goldview. PCR products were sent to Sangon Biotech for purification and sequencing. All DNA sequences from this study were submitted to GenBank.

6.2.5 Sequence Analysis

The sequencing results were assembled using DNASTAR SeqMan v.7.1.0. The assembled sequences were subjected to nucleotide BLAST searches in GenBank (www.ncbi.nlm.nih.gov/BLAST) for establishing highly similar strains (Altschul et al., 1997). Supplemental strains of *Pezicula* and related genera were selected from Chen et al., 2016, especially ex-type and ex-epitype sequences where available. Sequence alignment was carried out by MAFFT v.7.0 (https://mafft.cbrc.jp/alignment/server/). Misaligned sequences were manually edited using AliView v.1.26 (Larsson, 2014). The three loci were combined into a concatenated dataset with SequenceMatrixa 1.7.8 (Vaidya et al., 2011).

The best fitting nucleotide substitution models for each gene were estimated by IQ-TREE (Nguyen et al., 2014) with ultrafast bootstrap (Hoang et al., 2017). Gaps were regarded as missing data. Both maximum likelihood (ML) and Bayesian (BI) trees were constructed on The CIPRES Science Gateway V. 3.3 (http://www.phylo.org/index.php/). The ML tree was constructed using RAxML-HPC2 on XSEDE. Maximum likelihood bootstrap values (MLSB) were calculated with 1000 iterations. The generalized time-reversible (GTR) was set with gamma distribution. The BI tree was implemented by MrBayes on XSEDE (3.2.6). Two sets of four simultaneous independent chains of Markov chains Monte Carlo (MCMC) simulations were run for 5,000,000 generations; 25% of trees were discarded and the remaining trees were used to calculate the Bayesian posterior probabilities (PP). Convergence was assumed when the standard deviation of split sequences was less than 0.01. The generated trees were submitted to TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S25576). The resulting trees were viewed with Figtree v.1.4.0. The layout was made with Adobe Illustrator CS 6.

6.3 Results

6.3.1 Fungal Isolation and Sporulation

Four endophytic *Pezicula* strains were isolated from healthy roots and stems of *Dendrobium* species. Isolate MFLUCC 14-0116 sporulated on PDA after one month and on WA with toothpicks after two months. The conidia, conidiomata, conidiophores and mycelia were recorded from cultures.

6.3.2 Phylogenetic Results

Sequences of the isolated *Pezicula* and reference sequences of related taxa are listed in TABLE 6.1. Combined data for the phylogenetic tree comprised 57 sequences containing 2478 sites (including 582 ITS sites, 843 LSU sites, and 1053 RPB2 sites). The selected nucleotide substitutional model for ITS and RPB2 was a symmetrical model (SYM) with gamma distribution, and for LSU was Kimura's two-parameter substitution model with invgamma distribution.

The phylogenetic tree (FIG. 1) was rooted with two selected outgroup species *Parafabraea caliginosa* (CBS 124806) and *P. eucalypti* (CBS 124810) with robust bootstrap support (ML = 100%, PP = 1). In the phylogenetic tree (RAxML), *Pezicula endophytica* strains clustered and formed an independent clade. Strains MFLUCC 14-0140, MFLUCC 14-0144 and MFLUCC 14-0116 clustered with MFLUCC 14-0118 (ML = 100%, PP = 1). *Pezicula aurantiaca*, *P. cornina* and *P. pseudocinnamomea* formed a clade close to *P. endophytica*. The neighboring genera *Neofabraea* H.S. Jacks., *Phlyctema* Desm. and *Rhizodermea* Verkley & Zijlstra were well separated from *Pezicula* (ML = 94%, PP = 1).

Pezicula endophytica (MFLUCC 14-0116, ex-type) differs from *Pezicula diversispora* (CBS 185.50, ex-type) in 4% of base pairs [2.9% in ITS (17/582bp), 1.1% in LSU (9/843bp)] and from *Pezicula cornina* (CBS 285.39) in 8.6% of base pairs [3.3% in ITS (19/582bp); 1.5% in LSU (13/843bp); 3.8% in RPB2 (40/1053bp)].

6.3.3 Taxonomy

Pezicula endophytica X.Y. Ma, K.D. Hyde & J.C. Kang, sp. nov. FIGURE 6.2
IF558634

Table 6.1 Fungal strains and sequences of *Pezicula* and related taxa sourced from the present study, BLAST searches in GenBank, or Chen et al. (2016)

Species	Strain	GenBank accession No.		
		ITS	LSU	RPB2
Neofabraea.inaequalis	**CBS 326.75**	KR859081	KR858872	KR859321
N. krawtzewii	CBS 102867	KR859084	KR858875	KR859324
N. malicorticis	CBS 102863	KR859085	KR858876	KR859325
	CBS 122030	KR859086	KR858877	KR859326
Parafabraea caliginosa	**CBS 124806**	KR859090	KR858881	KR859330
Pa. eucalypti	**CBS 124810**	KR859091	KR858882	KR859331
Pezicula acericola	CBS 239.97	KR859093	KR858884	KF376214
	CBS 245.97	KR859098	KR858889	KF376213
P. aurantiaca	CBS 201.46	KR859102	KR858893	KF376210
P. brunnea	**CBS 120291**	KR859103	KR858894	—
P. californiae	**CBS 124805**	KR859104	KR858895	KR859332
P. carpinea	**CBS 923.96**	KR859108	KR858899	KF376158
	CBS 921.96	KR859107	KR858898	KF376159
P. chiangraiensis	**MFLUCC 15-0170**	KU310621	KU310622	KU310623

Table 6.1 (continued)

SPECIES	STRAIN	GENBANK ACCESSION NO.		
		ITS	LSU	RPB2
P. cinnamomea	CBS 626.96	KR859152	KR858944	KF376162
	CBS 239.96	KR859124	KR858915	KF376165
P. cornina	CBS 285.39	KR859163	KR858955	KR859333
P. corticola	CBS 259.31	KR859164	KR858956	—
	CBS 260.31	KR859165	KR858957	—
P. corylina	CBS 140.22	KR859166	KR858958	KR859334
	CBS 249.97	KR859168	KR858960	KF376161
P. diversispora	**CBS 185.50**	KR859170	KR858962	—
	CBS 282.47	KR859171	KR858963	—
P. endophytica	**MFLUCC 14-0116**	MN908669	MN908642	MT371880
	MFLUCC 14-0118	MN908666	MN908640	—
	MFLUCC 14-0140	MN908667	MN908641	—
	MFLUCC 14-0144	MN908668	—	—
P. ericae	**CBS 120290**	KR859173	KR858965	—
	CBS 120292	KR859174	KR858966	—
P. eucrita	CBS 259.97	KR859179	KR858971	KF376205
	CBS 656.96	KR859185	KR858977	KF376208
P. fagacearum	**CBS 112400**	KR859201	KR858993	KR859335
	CBS 112402	KR859203	KR858995	KR859336
P. frangulae subsp. *frangulae*	CBS 100244	KR859204	KR858996	KF376211
	CBS 778.96	KR859209	KR859001	KF376212
P. heterochroma	**CBS 199.46**	KR859210	KR859002	—
P. melanigena	**CBS 898.97**	KR859211	KR859003	—
P. microspora	**CBS 124641**	KR859212	KR859004	KR859337
P. neocinnamomea	**CBS 100248**	KR859213	KR859005	KF376209
P. neoheterochroma	**CBS 127388**	KR859221	KR859013	KR859338
P. neosporulosa	**CBS 101.96**	KR859223	KR859015	KF376193
	CBS 102.96	KR859224	KR859016	KF376181
P. ocellata	CBS 268.39	KR859232	KR859024	KR859339
	CBS 949.97	KR859233	KR859025	KF376215
P. pruinosa	CBS 292.39	KR859234	KR859026	—
P. pseudocinnamomea	**CBS 101000**	KR859235	KR859027	KR859340
P. querciphila	**CBS 134525**	JX144750	—	—
P. radicicola	**CBS 640.94**	KR859236	KR859028	—
	CBS 681.83	KR859237	KR859029	—
P. rubi	CBS 253.97	KR859250	KR859042	KF376204
	CBS 593.96	KR859253	KR859045	KF376203
P. sporulosa	**CBS 224.96**	KR859261	KR859053	KF376201
	CBS 225.96	KR859262	KR859054	KF376202
Phlyctema vagabunda	CBS 109875	KR859275	KR859069	KR859346
	CBS 304. 62	KR859276	KR859070	KR859347
Rhizodermea veluwensis	**CBS 110605**	KR859282	KR859076	KR859353
	CBS 110615	KR859283	KR859077	KR859354

Note *Ex-type, ex-epitype or other type strains are in bold

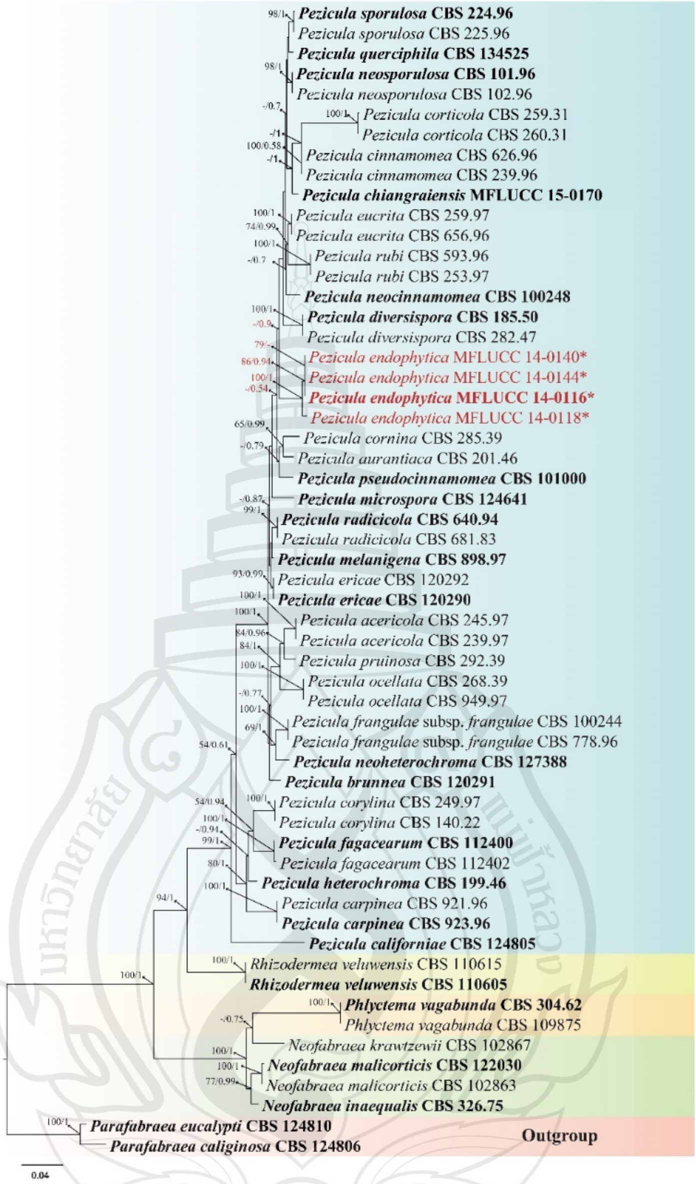


Figure 6.1 The consensus phylogram resulting from a RAxML analysis of the combined three loci (ITS-LSU-RPB2) of *Pezicula* and related taxa. *Parafabraea caliginosa* and *P. eucalypti* were selected as outgroup taxa. Isolates generated in this study are labelled with *. Holotype and ex-type isolates are in bold. Support values from maximum likelihood (MLBS) and Bayesian posterior probabilities (PP) are labelled at the end of nodes (50% majority rule). Dashes indicate values ≤50%. Scale bar represents 0.04 substitutions per site

Differs from *Pezicula diversispora* by its longer conidiophores and narrower macroconidia.

Type—Thailand, Chiang Rai Province, Mae Fah Luang district, outside Temple of Doi Tung Pagoda, endophytic in the roots of *Dendrobium* sp. 1, 19 December 2013, Nontachaiyapoom S, Aewsakul N & Ma XY (**holotype**, MFLU 17-2789; ex-type living culture, MFLUCC 14-0116; **isotype**, MFLU 20-0442; ex-type living culture, MFLUCC 14-0118.

Etymology—in reference to its endophytic habit.

Sexual morph undetermined. Asexual morph observed on both PDA and (with toothpicks) WA.

Colonies on WA superficial, white with light brownish grey mycelia, sparse, irregular edge, fluffy, reverse light brown, growth rate 0.8 mm/day. Mycelium hyaline to light brown, smooth, 2.5–4.4 µm diam. Conidiomata on toothpicks on WA aggregated or scattered, with aerial and fluffy hyphae, yellow-grey, globose.

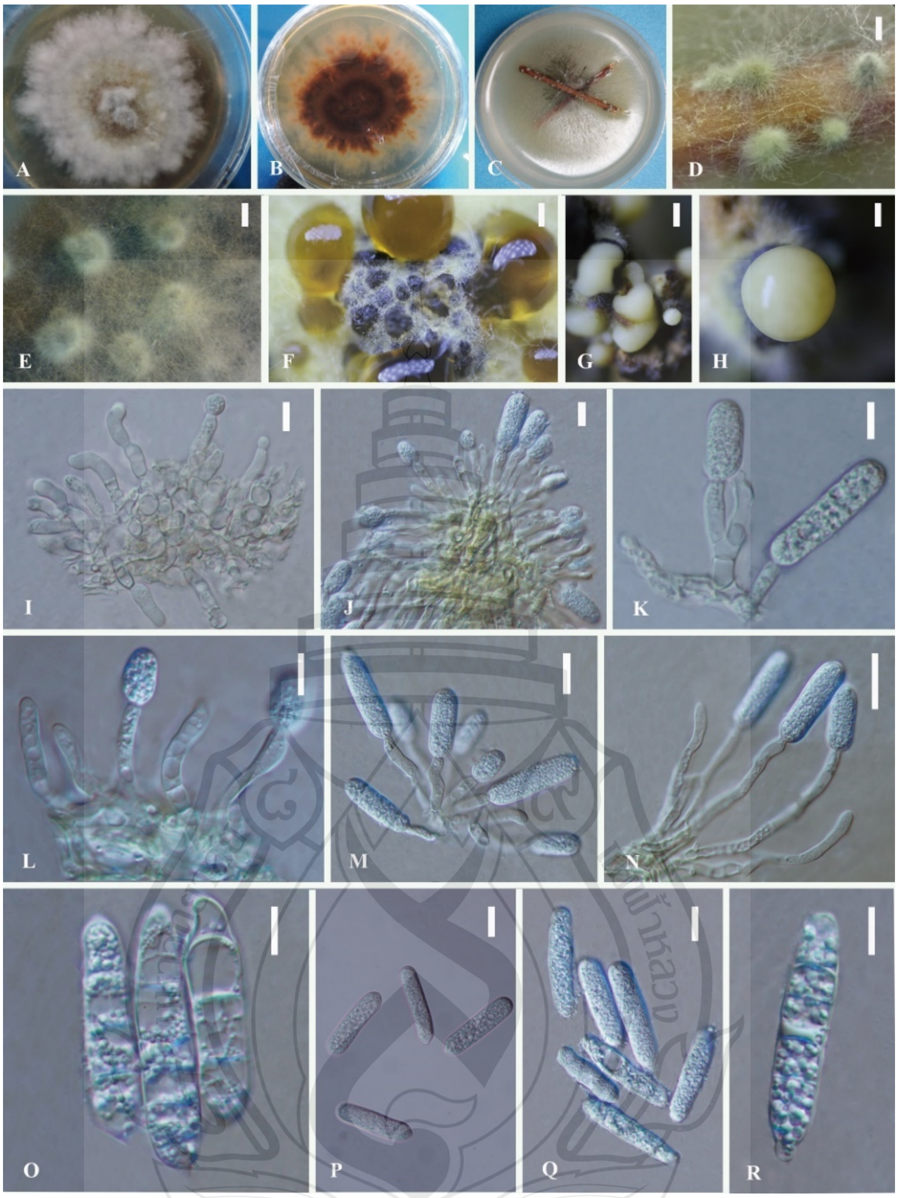
Colonies on PDA medium superficial, white and brown in the center, lobate edge, dense, fluffy, concentric ring close to the edge, reverse brown, growth rate: 1.2 mm/day. Conidiomata erumpent on surface, aggregated or scattered, with irregular sporodochia, with brown to milky white conidial masses, globose to irregular. Macroconidiophores (16–)22–30(–40) × (2–)3–4(–5.5) µm (x = 26 × 3.5 µm, n=5), arising from the inner wall layers of conidiomata, hyaline, cylindrical, straight to flexuous, septate, unbranched or branched, smooth-walled. Macroconidiogenous cells hyaline, enteroblastic, phialidic, determinate, proliferating percurrently, sometimes with a minute collarette, cylindrical, integrated, determinate, smooth-walled. Macroconidia (30–)31–43(–44) × (5–)7–9 (–10) µm (x = 37 × 8 µm, n=16), L/W=4.4, hyaline, elongated ellipsoid, rounded at the apex, narrow and slightly truncate at the base, sometimes attenuated and with a protruding scar at the base, septate when mature, straight, smooth-walled, with guttules.

Additional specimens examined—THAILAND, Chiang rai Province, Mae Fah Luang district, outside Temple of Doi Tung Pagoda, endophytic in roots and stems of *Dendrobium* sp. 2, 19 December 2013, Nontachaiyapoom S, Aewsakul N & Ma XY. (MFLUCC 14-0140, GenBank MN908667, MN908641; MFLUCC 14-0144, GenBank MN908668).

COMMENTS—*Pezicula endophytica* formed an independent clade differing from other species in the phylogenetic tree, and is adjacent to *P. aurantiaca*, *P. cornina*, *P. diversispora,* and *P. pseudocinnamomea*. *Pezicula endophytica* is distinguished from those species by its longer macroconidiophores and narrower macroconidia with septa (*P. aurantiaca* and *P. cornina* are aseptate; Chen et al., 2016, Robak, 1950, Verkley, 1999). *Pezicula endophytica* (ex-type MFLUCC 14-0116) has >4% different gene sequences than *P. diversispora* (ex-type CBS 185.50). Compared with their cultures on MEA, *Pezicula endophytica* produces no diffusing pigment on WA (Verkley, 1999). *Pezicula aurantiaca*, *P. cornina*, *P. diversispora,* and *P. pseudocinnamomea* were isolated from dead branches, bark, and twigs of dicotyledonous woody plants from European countries (Chen et al., 2016, Robak, 1950, Verkley, 1999), whereas *P. endophytica* is an endophytic fungus from monocotyledonous *Dendrobium* orchids.

6.4 Discussion

The taxonomy and identification of *Pezicula* is complicated because many species have been introduced based on single-morph taxa, some lack molecular data, and their morphology may vary depending on the growing medium (Verkley, 1999, Verkley et al., 2003). Of the 136 species listed in *Pezicula* (http://www.speciesfungorum.org), only 29 (including *P. endophytica*) have DNA sequence data available in the 2021 NCBI database (Yuan et al., 2015, Ekanayaka et al., 2016; http://www.ncbi.nlm.nih.gov/). A revision of the genus based on comprehensive morphological and phylogenetic data is necessary. The asexual morphs of most *Pezicula* species produce both macro- and microconidia on the natural substrata and in cultures (Verkley, 1999). In this study, only macroconidia were observed in cultures.



Note a, b Colony on PDA (a. top; b. reverse). c Colony on WA with toothpicks. d–e Conidiomata on toothpicks on WA. f–h. Conidiomata with conidial masses on PDA. i–n. Macroconidiophores, macroconidiogenous cells and macroconidia. o–r. Macroconidia. NOTE: i–r from PDA. Scale bars: d–h = 500 µm; i–n, p, q = 10 µm; o, r = 5 µm.

Figure 6.2 *Pezicula endophytica* (Holotype)

Macroconidia of *P. endophytica* resemble *P. diversispora,* which differs by its longer conidiophores, narrower macroconidia, and a 4% gene sequence difference. *Pezicula endophytica,* which only produced its asexual morph in cultures, differs from *P. carpinea* (the type species) by lacking conidia along the conidiophore sides and large gene sequence differences. Presumably *P. endophytica* microconidia will not be produced on WA after two sporulation months.

Chen et al. (2011) first reported an endophytic *Pezicula* sp. from *Dendrobium* orchids in China. *Pezicula endophytica* was the first *Pezicula* endophyte identified in Thailand. *Pezicula* species are frequently isolated endophytes in angiosperms, particularly in roots of *Abies*, *Calluna*, *Erica*, *Larix*, *Pleurothallis*, *Pseudorchis*, *Stelis,* and *Vaccinium* (Chen et al., 2016; Devi & Joshi, 2014; Herrera et al., 2010; Kohout et al., 2013; Noble et al., 1991; Schulz et al., 2002; Strobel et al., 1999; Talontsi et al., 2012; Verkley et al., 2003; Wang et al., 2014; Yuan & Verkley, 2015; Zilla et al., 2013). They have also been found in peridermal bark of living branches of temperate trees such as *Abies*, *Acer*, *Alnus*, *Betula*, *Carpinus, Castanea, Fagus*, *Fraxinus*, *Picea*, *Pinus*, *Quercus,* and *Sequoia* (Espinosa-Garcia & Langenheim, 1990; Kowalski & Kehr, 1992; Matsumura et al., 2013; Petrini & Müller, 1979; Schulz et al., 1995; Sieber, 1988; Sieber & Dorworth, 1994; Verkley, 1999).

Although many *Pezicula* species are reported from temperate and boreal forests of the northern hemisphere, such as Canada, Germany, and the Netherlands (Kowalski & Kehr, 1992; Chen et al., 2016; Verkley, 1999), few geographic and climatic limitations have been observed. Saprobic and pathogenic *Pezicula* species have been recorded from temperate to tropical and Mediterranean areas (Bergero et al., 2003; Cheewangkoon et al. 2010; Old et al. 2002). *Pezicula eucalyptigena* was isolated from leaves of *Eucalyptus* sp. in the mediterranean Western Cape, South Africa, which has smaller conidia ((23–)24–27(–30) × (6–)7(–8) µm; Crous et al. 2019) than those of *P. endophytica*. Ekanayaka et al. (2016) described a sexual morph from Thailand, *P. chiangraiensis*, saprobic on the bark of decaying wood. *Pezicula ericae* was found as ericoid mycorrhizal fungi in the roots of velvetleaf blueberry (*Vaccinium myrtilloides*) seedlings in Canada, which could enhance development of the host plant (Mu, 2020). For most endophytic *Pezicula* species, their roles remain unknown.

CHAPTER 7

ENDOPHYTIC XYLARIALES SPECIES IN *DENDROBIUM* AND THE CYTOTOXIC ACTIVITY OF *BISCOGNIAUXIA* SPECIES[6, 7]

Abstract

Many Xylariales species are culturable endophytes found in numerous plants including orchids. *Dendrobium* is one of the largest genera in Orchidaceae. Although xylarialean endophytes of *Dendrobium* and their associations have been investigated, their resolution remains extremely difficult due to poor sporulation on artificial media. Furthermore, there are few asexual descriptions for known species, insufficient characters to differentiate taxa and old type material without sequencing data. In this study, we investigated culturable Xylariales in *Dendrobium* species collected from southwestern China and northern Thailand. Forty-night endophytic xylarialean strains were isolated and identified as species belonging to Apiosporaceae, Graphostromataceae, Hypoxylaceae, Induratiaceae and Xylariaceae based on multigene analyses. Some identified taxa are also supported by morphology. Nine new species—*Annulohypoxylon moniliformis*, *Apiospora dendrobii*, *Biscogniauxia dendrobii*, *Hypoxylon endophyticum*, *H. hercoglossi*, *H. officinalis*, *Nemania dendrobii*, *Xylaria aphylli*, *X. chrysanthi* and *X. dendrobii* are introduced in this study. Fourteen known fungal species from *Biscogniauxia*, *Hypoxylon*, *Nemania*, *Nigrospora* and *Xylaria* were also identified. Two strains were only identified genus and family level (*Induratia* sp. and Hypoxylaceae sp.). This study recorded 13 new hosts for xylarialean endophytes. This

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[7] This paper has been partly submitted to the *Journal of Fungi*

is the first report of Xylariales species as endophytes from *Dendrobium aphyllum*, *D. aurantiacum* var. *denneanum*, *D. cariniferum*, *D. harveyanum*, *D. hercoglossum*, *D. moniliforme* and *D. moschatum*. *Dendrobium* has associated abundant xylarialean taxa, especially species of *Hypoxylon* and *Xylaria*. The ethyl acetate crude extract of the isolate *B. petrensis* showed moderate and strong cytotoxic activity against A549 and K562 using the CCK8 assay (IC_{50} of it 13.5 and 3.5 ug/ml respectively). This is the first report of endophytic *Biscogniauxia* in *Dendrobium* species and of their cytotoxicity to A549 and K562, all of which enhances the current knowledge on the genus *Biscogniauxia*. We recommend the use of oat agar with 4% concentrations to sporulation of *Xylaria* strains. The asexual morph of xylarialean species should be recorded as much as possible in future studies.

Keywords 8 New Species, Cytotoxic, Endophytes, Multi-locus Phylogeny, Orchids, Oat Media, Xylariomycetidae

7.1 Introduction

Endophytes live in plants without causing any obvious symptom and *Xylariaceae* species such as *Xylaria* and *Hypoxylon* are common endophytes have been found in many flowering plants (Davis et al., 2003; Promputtha et al., 2005; Hyde & Soytong, 2008; Chen et al., 2013; Jeewon et al., 2013; Rampadarath et al., 2018). The roles and taxonomy of these endophytes have always been a subject of debate among mycologists (Liu et al., 2012; Doilom et al., 2017; Jeewon et al., 2017; 2018). Some studies have documented their roles as saprobes (Hyde et al., 2001; Okane et al., 2008; Vinit et al., 2018) while others postulated that they are dormant or latent pathogens in plant tissues and become active saprobes when plants die (Choi et al., 2005; Sieber, 2007; Promputtha et al., 2007; Chareprasert et al., 2012). There have been several studies on endophytes from orchids as the latter are widely used ornamental plants (Yuan et al., 2009; Chen et al., 2013; Ma et al., 2018).

Dendrobium Sw. is one of the three largest genera in Orchidaceae (Moudi & Go, 2017). Many *Dendrobium* orchids have important medicinal and ornamental values (Wu et al., 2009; Kabir et al., 2013). However, majority *Dendrobium* species are endangered due to low germination rates, habitat destruction and over-exploitation as reported by IUCN (International Union for Conservation of Nature) Red List of Threatened Species. Fungal endophytes play an important role in orchid development and defense against stress (Arnold et al., 2003; Fracchia et al., 2016; Rashmi et al., 2019; Xie et al., 2019). Extracts of *Dendrobium* such as alkaloids, bibenzyls, fluorenones, phenanthrenes, phenylpropanoids polysaccharides and sesquiterpenoids possess angiogenesis inhibitory, anti-cancer, anti-inflammatory, anti-mutagenic and anti-oxidative bioactivities (Luo et al., 2010; Song et al., 2012; Lin et al., 2013; Xu et al., 2013; Chen et al., 2014; Ramesh et al., 2015; Divate & Chung, 2017; Becker et al., 2020; Lambert et al., 2021).

Xylariales was introduced by Nannfeldt in 1932, which is a large order in Sordariomycetes of perithecial Ascomycota with eight-spored unitunicate asci, usually with an apical J+ ring apparatus and ellipsoidal, dark ascospores (Smith et al., 2003; Maharachchikumbura et al., 2016). Fifteen families and 44 accepted genera have been recorded in this order (Maharachchikumbura et al., 2016; Wendt et al., 2018; Hyde et al., 2020; Wijayawardene et al., 2020). Xylariaceous taxa present polyphyletic topology in the phylogeny and many old xylarialean species lack sequence data or further verification with herbarium defined types and repeated collections (Smith et al., 2003; Jaklitsch & Voglmayr, 2012; Daranagama et al., 2018; Wendt et al., 2018; Konta et al., 2020). They are common endophytes also be found as hemibiotrophs, necrotrophs, pathogenes and saprobes that occur on monocotyledons, dicotyledons, dung and even in some arthropod animals with diverse lifestyles in both terrestrial and aquatic environment (Whalley, 1996; Fröhlich & Hyde, 2000; Osono et al., 2011; Li et al., 2015; Senanayake et al., 2015; Maharachchikumbura et al., 2016; Daranagama et al., 2018). Xylariales is the best-investigated filamentous group regarding wide distribution and the production of bioactive secondary metabolites that possess various bioactivities (Stadler & Hellwig, 2005; Xu et al., 2008; Pažoutová et al., 2013; Aly et al., 2010; Becker & Stadler, 2021). Xylariaceae is one of the largest families of Xylariales, which was introduced by Tulasne and Tulasne in 1863 (Hyde et al., 2020; Wijayawardene et al., 2020). Hyde et al. (2020) verified the Xylariaceae placement in the subclass Xylariomycetidae and estimated the subclass divergence time at 278 Mya.

Dendrobium species contain a large community of xylariaceous endophytes (Yuan et al., 2009; Chen et al., 2011, 2013). Several xylariaceous genera, *viz. Hypoxylon*, *Nemania* and *Xylaria* have been isolated from *Dendrobium* orchids in tropical and subtropical areas (Yuan et al., 2009; Chen et al., 2003; Parthibhan et al., 2017; Sarsaiya et al., 2020). However, few reports on endophytic species resolution and most was carried out by using solo gene-based phylogeny (Yuan et al., 2009; Kasmir et al., 2011; Chen et al., 2011; Ma et al., 2015). A single gene is inadequate for xylariaceous species delimitation (Doilom et al., 2017; Hyde et al., 2011, 2014, 2019; Maharachchikumbura et al., 2016; Daranagama et al., 2018). A considerable number of sterile mycelia from endophytic isolations makes the accurate identification difficult, particularly the typical genera *Xylaria*, morphology on artificial media hardly success and lack of comparative reference (Rogers & Ju, 1992; Rodrigues et al., 1993; Wang et al., 2005; Tejesvi et al., 2006; Hsieh et al., 2010; Vemireddy et al., 2020). Therefore, a multi-gene phylogenetic approach is recommended while it is encouraged to break through the endophyte sporulation for a better resolution of taxa (Doilom et al., 2017; Hyde et al., 2019).

The classification and phylogeny of *Biscogniauxia* Kuntze, Revis. have been discussed in depth (e.g. Tang et al., 2009) and currently it belongs to the family Graphostromataceae (Daranagama et al., 2018; Wendt et al., 2018; Wijayawardene et al., 2018). The species in this genus are characterised by bipartite stromata that do not release KOH extractable pigments, the asci are 8-spored, unitunicate (some species have hyaline cellular appendage), cylindrical, short-pedicellate with J+ apical ring, ascospores are overlapping uniseriate, uni-bicellular with rounded ends and nodulisporium-like asexual morphs (Daranagama et al., 2015, 2016, 2018; Wendt et al., 2018). There are 125 species recorded worldwide in Indexfungorum (2019) and more than 52 species reported exclusively as parasites of angiosperms including *Acacia*, *Acer*, *Alnus*, *Artocarpus*, *Carya*, *Celtis*, *Coprosma*, *Eucalyptus*, *Fagus*, *Fraxinus*, *Gluta*, *Lithocarpus*, *Padus*, *Phyllirea*, *Pisnia*, *Populus*, *Psidium*, *Quercus*, *Rhamnus*, *Rubus* and *Tilia* (Raimondo et al., 2016). The type species *B. nummularia* is a common fungal endophyte of Europe beech and it can be a possible bioindicator of beech health conditions (Luchi et al., 2015). Many species of *Biscogniauxia* have also been reported as pathogens cause white or soft rot and charcoal canker on woody plants such as beech and oak trees (Lee, 2000; Granata & Sidoti, 2004; Reed et al., 2017). There are over 40 secondary metabolites isolated from *Biscogniauxia* spp. and found to be possessed with anti-Alzheimer’s disease, antigerminative, antimicrobial, antimycobacterial, antioxidant, cytotoxic (towards Hela and SW480 cell lines) activities and inhibitory effects on several enzymes (Cheng et al., 2012; Amand et al., 2012; Wu et al., 2016; Zhao et al., 2016; Nguyen et al., 2018; Liu et al., 2019; Sritharan et al., 2019).

In the present study, we investigated Xylariales from 23 *Dendrobium* samples (including eight unidentified *Dendrobium* species) collected from southwestern China and northern Thailand. The concatenated sequence data of ITS-LSU-TUB2-TEF-1α and ITS-LSU-TUB2-RPB2 were used for phylogenetic species analysis in Apiosporaceae and Hypoxylaceae/Xylariaceae respectively. *Dendrobium cariniferum* Rchb. f., *D. chrysotoxum* Lindl., *D. fimbriatum* Hook., *D. harveyanum* Rchb.f., *D. hercoglossum* Rchb. f., *D. loddigesii* Rolfe, *D. moniliforme* (L.) SW, *D. moschatum* (Buch-Ham.) SW and *D. primulinum* Lindl. are native species to Southeastern Asia (http://www.orchidspecies.com/), while *Dendrobium catenatum* Lindl. (Tie Pi Shi Hu in China, also known as *D. officinale* Kimura & Migo) is endemic to China (Ding et al., 2008). In this study, we provided descriptions and illustrations of 47 endophytic xylariaceous strains belonging to Apiosporaceae, Hypoxylaceae, Induratiaceae and Xylariaceae.

We identified two endophytic *Biscogniauxia* taxa isolated from *Dendrobium aphyllum* and *D. harveyanum* collected from northern Thailand and southwestern China respectively. The cytotoxic activity of ethyl acetate crude extracts towards adenocarcinomic human alveolar basal epithelial (A549) and human immortalised myelogenous leukemia (K562) cell lines were tested via Cell Counting Kit-8 (CCK8). This is the first study to identify endophytic *Biscogniauxia* species from *Dendrobium* orchids using both morphological and phylogenetic methods and investigate its cytotoxic activities.

7.2 Materials and Methods

7.2.1 Sample Collection

Healthy leaves, roots and stems of 23 *Dendrobium* samples (containing eight unidentified species labelled from 1-8) were collected from six different collecting sites in southwestern China and northern Thailand (Table 7.1). Materials were packed in zip-lock bags or tubes containing silica gel on ice. Fungal isolation was carried out within 48 hours following collection.

Table 7.1 Information on *Dendrobium* orchids from this study

Code	Sample site	***Dendrobium* species**
1	Orchid nursery 2, Luodian, Guizhou, China	*Dendrobium aphyllum* (Roxb.) C. E.
2	Orchid nursery 2, Luodian, Guizhou, China	*Dendrobium aurantiacum* Rchb. f. var. *denneanum*
3	Wat Phra That Doi Tung (Temple of Doi Tung Pagodas), Mae Fah Luang District, Chiang Rai, Thailand	*Dendrobium cariniferum* Rchb. f.
4	Orchid nursery 1, Luodian, Guizhou, China	*Dendrobium catenatum* Lindl.
5	Orchid nursery 2, Luodian, Guizhou, China	*Dendrobium catenatum* Lindl.

Table 7.1 (continued)

Code	Sample site	*Dendrobium* species
6	Animal Husbandry and Veterinary Institute, Guiyang, Guizhou, China	*Dendrobium chrysanthum* Lindl.
7	Orchid nursery 2, Luodian, Guizhou, China	*Dendrobium chrysotoxum* Lindl.
8	Orchid nursery 2, Luodian, Guizhou, China	*Dendrobium fimbriatum* Hook.
9	Wat Phra That Doi Tung (Temple of Doi Tung Pagodas), Mae Fah Luang District, Chiang Rai, Thailand	*Dendrobium harveyanum* Rchb.f.
10	Orchid nursery 2, Luodian, Guizhou, China	Dendrobium *hercoglossum* Rchb. f.
11	Orchid nursery, Xingyi, Guizhou, China	*Dendrobium huoshanense* C. Z. Tang et S. J. Cheng
12	Orchid nursery, Xingyi, Guizhou, China	*Dendrobium loddigesii* Rolfe
13	Orchid nursery 2, Luodian, Guizhou, China	*Dendrobium moniliforme* (L.) Sw.
14	Wat Phra That Doi Tung (Temple of Doi Tung Pagodas), Mae Fah Luang District, Chiang Rai, Thailand	*Dendrobium moschatum* (Buch.Ham.) Sw
15	Orchid nersury 2, Luodian, Guizhou, China	*Dendrobium primulinum* Lindl.
16	Wat Phra That Doi Tung (Temple of Doi Tung Pagodas), Mae Fah Luang District, Chiang Rai, Thailand	*Dendrobium* sp. 1
17	Wat Phra That Doi Tung (Temple of Doi Tung Pagodas), Mae Fah Luang District, Chiang Rai, Thailand	*Dendrobium* sp. 2
18	Wat Phra That Doi Tung (Temple of Doi Tung Pagodas), Mae Fah Luang District, Chiang Rai, Thailand	*Dendrobium* sp. 3
19	Steep forest, Doitung, Mae Fah Luang District, Chiang Rai, Thailand	*Dendrobium* sp. 4
20	Orchid nursery, Xingyi, Guizhou, China	*Dendrobium* sp. 5
21	Orchid nursery, Xingyi, Guizhou, China	*Dendrobium* sp. 6
22	Orchid nursery, Xingyi, Guizhou, China	*Dendrobium* sp. 7
23	Orchid nursery, Xingyi, Guizhou, China	*Dendrobium* sp. 8

7.2.2 Fungal Isolation and Cultivation

Surface sterilization of orchid tissues and endophytic fungal isolation was carried out as described by Nontachaiyapoom et al. (2010) with necessary modifications. The tissues were first washed with running water. Leaves, roots and stems were then soaked in a solution containing 3% (v/v) H_2O_2 and 70% (v/v) ethanol for 5 minutes, and then rinsed with sterile distilled water three times. Sterilized tissues were cut into 2 mm^2 pieces and put on potato dextrose agar (PDA) containing 50 μg/mL oxytetracycline, 50 μg/ mL penicillin and 50 μg/ mL streptomycin (Otero et al., 2002). The surface sterilization was tested via the print method described in Petrini (1991) and Hyde (2008). Samples were incubated at 28 °C in the dark. Vegetative marginal mycelia were cut and transferred to fresh PDA to obtain pure cultures. The growth rates were evaluated by measuring the colony diameter and growth time. The pure cultures were deposited at China General Microbiological Culture Collection Center (CGMCC), the Culture collection of Guizhou Agricultural College, Guizhou University (GZAC) and Mae Fah Luang University Culture Collection (MFLUCC).

7.2.3 DNA Extraction and Amplification

DNA samples were extracted from fresh mycelia scraped from pure fungal cultures using EZgene^{TM} Fungal gDNA Kit (GD2416, Biomiga, USA) following the manufacturer’s protocol. The amplification reactions were performed using reagents purchased from BIOMIGA (San Diego, USA). Each 25 μL amplification reaction contained 12.5 μL of 2*Bench Top^{TM} Taq Master Mix (0.05 units/μL Taq DNA polymerase, 0.4mM dNTPs and 4mM $MgCl_2$); 2μL of forward and reverse primers; 1μL of DNA template and 9.5μL of threefold-distilled water. Five loci were selected for DNA amplification including a ca. 900 bp section of the internal transcribed spacer (ITS), a ca. 1.5k bp segment of large subunit (LSU), a ca. 1.2k bp fragment of RNA polymerase II core subunit (RPB2), a ca. 250 bp fraction of the elongation factor 1-alpha (TEF-1α), a ca. 0.5k bp fragment of β-tubulin (TUB2) (Wang et al., 2017; Daranagama et al., 2018). The primers used in this study and PCR thermal cycling conditions are listed in Table 7.2. The PCR products were viewed on 1% agarose gel electrophoresis stained by 4S green nucleic acid (Sangon Biotech (Shanghai) Co., Ltd. China) for checking quality and then sent to Sangon Biotech Co. Ltd. (Shanghai, China) for purification and sequencing.

7.2.4 Phylogenetic Analysis

The original DNA sequence data were manually trimmed and assembled into contigs using ContigExpress (Vector NTI suite 6.0, Informax). The consensus sequences were subjected to BLASTn in the NCBI GenBank database for the initial screening of the most similar sequences, particularly for those of ex-type/ex-epitype strains (www.ncbi.nlm.nih.gov/BLAST). All the newly sequences in this study were deposited in GenBank (Supplementary table 7.9 and table 7.9).

The selected sequences were aligned using MAFFT version 6 (Katoh & Toh, 2008, mafft. cbrc. jp/ alignment/server/). Aligned datasets were visually inspected and misaligned regions were trimmed by TrimAL v. 1.2 in PhyloSuite v. 1.2.2 (Capella-Gutierrez et al., 2009; Zhang et al., 2020) and AliView (Larsson, 2014). All base pairs differences excluding gaps were calculated by MEGA 7.0 (Kumar et al., 2016). Gaps were treated as missing data in maximum likelihood and Bayesian inference trees. For the Apiosporaceae tree, the general time-reversible (GTR) model with the gamma distribution option was implemented for TEF-1α and TUB2 datasets. The GTR model with invgamma distribution was selected for the ITS and LSU datasets. For another tree, the GTR model with the invgamma distribution option of nucleotide substitution was selected for ITS, LSU and RPB2 dataset. The Hasegawa, Kishino & Yano (HKY) model with invgamma distribution was used for the TUB2 datasets. The ML tree was constructed using RAxML-HPC2 on XSEDE in CIPRES Science Gateway V. 3.3 (http://www.phylo.org/index.php/). The BI tree was achieved by MrBayes version 3.1.2 via the public resource platform CIPRES Science Gateway V3.3 (Ronquist & Huelsenbeck, 2003, https://www.phylo.org/). The concatenated dataset was partitioned and the ultrafast bootstrap (Minh et al., 2013) implemented in the IQ-TREE software (Nguyen et al., 2014) was used to estimate the best fitting models according to the Bayesian information criterion (BIC).

Two sets of six simultaneous independent chains of Markov Chains Monte Carlo (MCMC) simulations were run for 5,000,000 (For Apiosporaceae phylogenetic tree) and 400,000,000 (For another phylogenetic tree) generations, 25% of trees were discarded and the remaining trees were used to calculate the posterior probabilities. Convergence was assumed when the standard deviation of split sequences was less than 0.01. The best scoring RAxML trees with bootstrap values of maximum likelihood

(ML) (Equal to or above 70%) and Bayesian posterior possibilities (BPP) from MCMC analysis trees (equal to or above 90%) are shown in phylogenetic trees. The two phylogenetic trees were submitted to TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S28326; http://purl.org/phylo/treebase/phylows/study/TB2:S28327). All trees were viewed in FigTree v.1.4.3 and edited in Adobe Illustrator v. 25.2.

7.2.5 Morphological Study

Pure strains were cultivated on 2% malt extract agar (MEA). Fresh mycelia were transferred to potato dextrose agar (PDA), 4% oat agar (OA) and water agar (WA) using sterilized toothpicks and slide culture for sporulation (Rodrigues et al., 1993). Cultures were kept in a dark cabinet at room temperature (28 °C) and observed every seven days or less. The growth rate was evaluated when fungal mycelia nearly covered the whole plate. Fungal structures were observed and captured with a stereomicroscope (SteREO Discovery. V8, Carl Zeiss Microscopy GmBH, Germany). Cross-sections of conidiomata or ascomata-like structures were mounted in 10% hydrogen peroxide and observed using a compound microscope (EOS Y-TV55, Nikon, Japan). The size of morphological characteristics was measured with Image Frame Work (IFW) v.0.9.7 and the photo plates were made by Adobe Photoshop CS 22.2.0.

Table 7.2 PCR thermal cycling protocols

Region/gene	**Primers**	**Cycle number**	**Denaturation**	**Annealing**	**Elongation**	**Reference**
ITS	ITS1/ITS4	30	95°C 1 min	53°C 1 min	72°C 1 min	White et al. (1990)
LSU	LR0R/LR5	35	94°C 1 min	53°C 50 s	72°C 1 min 30s	Vilgalys and Hester (1990)
RPB2	Frpb-5f/frpb-7cr	35	95°C 45 s	57°C 50 s	72°C 1 min 30s	Liu et al. (1999)
TEF-1α	728F/986R	36	95°C 45 s	56˚C 1 min	72°C 1 min	Carbone and Kohn (1999)
TUB2	Bt2A/Bt2B	35	94°C 1 min	55°C 55 s	72°C 1 min	Glass and Donaldson (1995)

7.2.6 Crude Extraction and Cytotoxic Assay

The pure mycelia were cut and put in 5 × 1000 ml Erlenmeyer flasks each containing 200 ml of potato dextrose broth under static innoculation with 28 ℃ with daylight for 5 days. And then the vigorous mycelia with broth were transferred to 12 × 1000 ml glass bottle each containing 300g of sterile rice (The final pH is 7) and cultivated under 28 ℃ and daylight for 40 days. The fermented rice substrate was extracted with four kinds of reagents — petroleum ether (PE), dichloromethane (DM), ethyl acetate (EA) and methanol (MeOH) (3 × 1000 ml respectively). The organic solvents were evaporated to dryness under vacuum to afford 15.4g, 4.5g, 2.1g and 143.3g of respective crude extracts. And all of them were stored at 4℃.

The cytotoxic assay was carried out using CCK8 assay, which was purchased from Dojindo, Kumamoto, Japan. The two cancer cell lines A549 and K562 were bought from Kunming Institute of Zoology, Chinese Academy of Sciences (CAS, cell code of A549, KCB200434YJ) and China Center for Type Culture Collection (CCTCC, cell code of K562, GDC037). The cells were cultivated with RPMI-1640 (Gibco Laboratoties, Grand Island, NY, America) supplemented with 10 % heat-inactivated fetal bovine serum (AusGeneX, Pty Ltd, Brisbane, Australia) under a humidified atmosphere with 5 % of CO_2 at 37 °C. The cells passed on 2—4 generations for stable proliferation. Then, the number of 1×10^5 cells were seeded into a 96-well microplate with equal volumes. After 24 hours, different concentrations of crude extracts and the positive control drug Doxorubicin were added to the well and the plates were cultivated for 24 hours. The total volume for each well was 100 ul. Finally, CCK8 were added 10 ul to each well and the plates were incubated for 2 hours under the same condition. The living cells were measured at 450 nm with Thermo Scientifc™ Multiskan™ FC Filterbased Microplate Photometer (Termo Fisher Scientifc). The blank control were set as the well without cell and containing only medium. The negative control were designed as the well with cells but no tested substances. The experiments were perfomed in triplicate and three independent experiments were carried out. The cell proliferation inhibitory rate was calculated as: $I\% = [(A_{negative} – A_{blank} – A_{sample})/(A_{negative} – A_{blank})] \times 100\%$. The half maximal inhibitory concentration (IC_{50}) of all tested components and related statistical analysis were evaluated using Microsoft Excel 2010.

7.3 Results

7.3.1 Fungal Isolation and Identification

Among the 49 isolates from 23 *Dendrobium* orchid samples (13 are from leaves, 23 are from roots, 13 are from stems), a total of 25 species (including 2 undetermined species– *Induratia* sp. and Xylariaceae sp.) were identified based on phylogenetic analysis and morphology (Table 7.3). *Xylaria* species account for 51% of isolates. Nine new species from *Annulohypoxylon*, *Hypoxylon*, *Nemania* and *Xylaria* were intoduced. The newly introduced species evidence is listed in Table 7.4.

7.3.2 Phylogenetic Analysis

The concatenated dataset of Apiosporaceae generated from four loci comprised 129 taxa with 2044 characters including gaps (666 bp of ITS, 796 bp of LSU, 366 bp of TEF-1ɑ, 216 bp of TUB2). The RAxML analysis for Apiosporaceae resulted in a final ML optimization likelihood value of - 18368.610931. The Bayesian tree converged at 1,045,000th generations with an average standard deviation of split frequencies 0.009928. Another consensus phylogenetic tree generated from four loci concatenated datasets of other xylarialean strains with 404 taxa and 4107 characters including gaps (847 bp of ITS, 1432 bp of LSU, 1298 bp of RPB2, 530 bp of TUB2). The RAxML analysis resulted in a final ML optimization likelihood value of - 188105.524536. The Bayesian tree converged at 287,695,000th generations with the average standard deviation of split frequencies 0.015.

The phylogram of Apiosporaceae presented three major monophyletic clades – *Apiospora*, *Arthrinium* and *Nigrospora* (Figure 1). *Pseudomassaria* species were used as an outgroup with 94% ML/1.0BPP support. In *Apiospora*, *Apiospora dendrobii* (MFLUCC 14-0152) formed a sister clade to *Apiospora xenocordella* (ex-type CBS 478.86 and CBS 595.66) with 85% ML/1.0BPP support. In *Nigrospora*, *Nigrospora chinensis* comprising four strains—CGMCC 3.18127 (ex-type), LC4593, MFLUCC 14-0109 and MFLUCC 18-1215 closed to *Nigrospora camelliae-sinensis* LC4460 with 100% ML/1.0BPP support. *Nigrospora sphaerica* represented by three strains LC13523 (ex-type), LC7259 and GZAC O37S13 formed an independent clade.

Five genera including *Kretzschmaria*, *Lunatiannulus*, *Podosordaria*, *Poronia* and *Xylaria* of the Xylariaceae were included in the phylogenetic analyses. The selected species used for phylogenetic tree are listed in Table 7.10. The symmetrical (SYM) model with gamma distribution was selected for ITS sequence data, the Hasegawa, Kishino & Yano (HKY) model with gamma distribution was selected for both RPB2 and TUB2 sequence data, the symmetrical (SYM) model with equal distribution was used for LSU sequence data in Bayesian tree construction.

The topology of Bayesian and ML trees were identical. The tree was rooted with *Annulohypoxylon cohaerens* (YMJ310) and *Jackrogersella multiformis* (CBS 119016). The isolate MFLUCC 17-2607 formed an independent linage close to *B.atropunctata*. Another isolate MFLUCC 14-0151 clustered with *B. petrensis* with strong support values 100MLB/1.0PP (Fig. 1).

Table 7.3 Endophytic Xylariaceous isolates from *Dendrobium* orchids in this study

Xylariales species	**Strain Code**	**Host *Dendrobium* orchids**	**Tissues**
Annulohypoxylon moniliformis sp. nov.	**MFLUCC 18-1214**^{HT}	*Dendrobium moniliforme*	Leaf
Apiospora dendrobii sp. nov.	**MFLUCC 14-0152**^{HT}	*Dendrobium harveyanum*	Root
Biscogniauxia dendrobii sp. nov.	**MFLUCC 17-2607**^{HT}	*Dendrobium aphyllum*	Stem
Biscogniauxia petrensis	**MFLUCC 14-0151**	*Dendrobium* sp. 5	Root
Hypoxylon endophyticum sp. nov.	**MFLUCC 18-1206**^{HT}	*Dendrobium aphyllum*	Root
	MFLUCC 18-1209	*Dendrobium huoshanense*	Stem
	MFLUCC 18-1211	*Dendrobium chrysotoxum*	Leaf
	MFLUCC 18-1208	*Dendrobium* sp. 5	Root
	MFLUCC 18-1210	*Dendrobium loddigesii*	Root
	MFLUCC 18-1207	*Dendrobium hercoglossum*	Leaf
Hypoxylon officinalis sp. nov.	**MFLUCC 14-0075**^{HT}	*Dendrobium* sp. 1	Root
	MFLUCC 14-0078	*Dendrobium* sp. 1	Root
	MFLUCC 21-0060	*Dendrobium catenatum*	Root
Hypoxylon investiens	MFLUCC 15-1155	*Dendrobium moschatum**	Stem
Hypoxylon pulicicidum	GZAC O37S13	*Dendrobium hercoglossum**	Root
Hypoxylaceae sp.	MFLUCC 14-0141	*Dendrobium* sp. 3	Root
Induratia sp.	MFLUC C 15-1218	*Dendrobium* sp. 4	Stem
Nemania dendrobii sp. nov.	**MFLUCC 18-1213**^{HT}	*Dendrobium* sp. 7	Stem
	MFLUCC 18-1212	*Dendrobium* sp. 6	Root
Nemania diffusa	MFLUCC 14-0139	*Dendrobium* sp. 2	Root

Table 7.3 (continued)

Xylariales species	Strain Code	Host *Dendrobium* orchids	Tissues
Nemania bipapillata	MFLUCC 14-0138	*Dendrobium* sp. 3	Root
	MFLUC C 14-0105	*Dendrobium cariniferum**	Stem
Nigrospora chinensis	MFLUCC 14-0109	*Dendrobium cariniferum**	Stem
	MFLUCC 18-1215	*Dendrobium catenatum*	Root
Nigrospora sphaerica	GZAC O37S13	*Dendrobium hercoglossum**	Leaf
Xylaria aphylli sp. nov.	**MFLUCC 21-0059**$^{\mathbf{HT}}$	*Dendrobium aphyllum*	Root
Xylaria chrysanthi sp. nov.	**MFLUCC 21-0014**$^{\mathbf{HT}}$	*Dendrobium chrysanthum*	Stem
Xylaria dendrobii sp. nov.	**MFLUCC 14-0137**$^{\mathbf{HT}}$	*Dendrobium* sp. 3	Root
	MFLUCC 14-0110	*Dendrobium cariniferum*	Leaf
Xylaria berteri	MFLUCC 14-0095	*Dendrobium cariniferum**	Root
Xylaria berteri	MFLUCC 14-0102	*Dendrobium cariniferum*	Stem
	MFLUCC 14-0143	*Dendrobium* sp.	Stem
	MFLUCC 14-0117	*Dendrobium* sp. 2	Root
	MFLUCC 14-0126	*Dendrobium* sp. 2	Leaf
	MFLU CC 14-0143	*Dendrobium* sp.3	Stem
	MFLUCC 14-0150	*Dendrobium harveyanum**	Root
	MFLUCC 14-0158	*Dendrobium harveyanum*	Leaf
	MFLUCC 21-0061	*Dendrobium* sp. 2	Leaf
Xylaria curta	GZAC O36L23	*Dendrobium catenatum*	Leaf
Xylaria feejeensis	GZAC O30S21	*Dendrobium aphyllum**	Stem
Xylaria grammica	MFLUCC 14-0093	*Dendrobium* sp. 1	Leaf
	MFLUCC 14-0146	*Dendrobium* sp. 3	Leaf
Xylaria laevis	GZAC O33L12	*Dendrobium aurantiacum* var. *denneanum**	Leaf
	GZAC O6LA2	*Dendrobium catenatum*	Leaf
Xylaria papulis	GZAC O32S24	*Dendrobium chrysotoxum*	Stem
Xylaria venosula	MFLUCC 14-0114	*Dendrobium* sp. *2*	Root
	MFLUCC 21-0013	*Dendrobium fimbriatum**	Root
	MFLUCC 21-0015	*Dendrobium aurantiacum* var. *denneanum**	Root
	MFLUCC 21-0016	*Dendrobium primulinum**	Stem
	MFLUCC 21-0017	*Dendrobium* sp. 8	Stem

Note Ex-type strains are in **bold**. HT are abbreviations of holotype. The label ‘HT’ means that their dry cultures are designed as holotype herbarium. * is labeled at the right upper conner of host species indicates new host record.

Another phylogenetic tree was mainly composed of Induratiaceae, Hypoxylaceae and Xylariaceae species (Figure 1). A total of 43 strains were identified to 20 species distributed in five genera—*Annulohypoxylon* (1 species), *Hypoxylon* (4 species), *Induratia* (1 unresolved species), *Nemania* (3 species), *Xylaria* (10 species) and one unresolved species in genus *incertae sedis* of Xylariaceae.

Induratiaceae was a monophyletic group close to the genera *Anthostomella*, *Anthostomelloides* and *Clypeosphaeria*. *Induratia* sp. (MFLUCC 15-1218) formed a single clade with low support. The Hypoxylaceae lineage presented a monophyletic clade comprising ten genera. *Annulohypoxylon*, *Daldinia* and *Hypoxylon* were polyphyletic groups incorporating with thirteen studied strains. *Annulohypoxylon moniliformis* (ex-type: MFLUCC 18-1214) and *An. annulatum* (ex-type: CBS 140775) clustered together with 99% ML/1.0BPP support. In the *Hypoxylon* I, *H. endophyticum* (ex-type MFLUCC 18-1206) encompassing six strains developed an independent clade adjacent to *H. investiens* with 100% ML/1.0BPP support. *H. officinalis* (ex-type MFLUCC 14-0075) represented by three isolates separated from other species with 100% ML/1.0BPP support. *H. investiens* containing four strains with low support among each other. *H. pulicicidum* (ex-type MUCL 49879) consisting of four isolates formed a sister clade to *H. hinnuleum* (CBS 286.62). Hypoxylaceae sp. (MFLUCC 14-0141) was basal to a mixed genera including *Anthocanalis*, *Daldinia* II and III, *Rhopalostroma* and *Thamnomyces* with 74% ML/0.97BPP support.

In the Xylariaceae phylogram, according to Hsieh et al. (2010), four separated lineages representing *Xylaria* 'PO' clade, *Nemania* + *Rosellinia* 'NR' clade, *Xylaria* 'HY' clade and the genus *Pseudoxylaria* 'TE' clade naturally developed. *Xylaria hypoxylon* is the core species in 'HY' clade. 'NR' clade consists mainly of *Nemania* and *Rosellinia* species. 'PO' clade formed by *X. polymorpha* A total of 29 isolates distributed in *Nemania* and *Xylaria*. In the *Nemania* + *Rosellinia* 'NR' clade, *Nemania bipapillata* encompassing three isolates (90080610 HAST, MFLUCC 14-0105 and MFLUCC 14-0138) and *Nemania dendrobii* (ex-type MFLUCC 18-1213) clustered together with 100% ML/1.0BPP support, which formed a sister clade to *Nemania diffusa.* In the *X. cubensis* aggregate I of *Xylaria* 'PO' clade, *Xylaria berteri* including 11 strains separated from *Xylaria crozonensis* (398HAST) sustained by 99% ML/1.0BPP. *Xylaria dendrobii* (ex-type MFLUCC 14-0137) and *X. cubensis* (GENT

159, 477 HAST and JDR 860) grouped and developed an independent clade with low support. *Xylaria laevis* containing four strains was at the root of this aggregate. *Xylaria chrysanthi* (ex-type MFLUCC 21-0014) and *X. phyllocharis* clustered with 75% ML/0.97 BPP support. In the *X. corniformis* aggregate II, *Xylaria feejeensis* (GZAC O30S21) and *X. frustulosa* (92092010 HAST) gathered sustained by 77% ML/0.99 BPP. In the *X. corniformis* aggregate III, *X. curta* containing three isolates (GAZC O36L23, 494HAST and 92092022 HAST) well separated from *X. karyophthora* (DRH059) with 100% ML/1.0BPP. In the *Xylaria* 'HY' clade, *X. arbuscula*, *X. apiculata* and *X. venosula* formed a monophyletic clade close to *X. arbuscula* var. *plenofissura* (93082814 HAST) with 100% ML/1.0BPP support in the *X. arbuscula* aggregate. *Xylaria aphylli* (ex-type MFLUCC 21-0059) was a single branch sister to *X. bambusicola* sustained by 93% ML/0.97 BPP. Six strains pertaining *Xylaria grammica* constituted an independent clade adjacent to *X. liquidambaris* (93090701 HAST) with low support. *Xylaria hongkongensis* (ex-type GDGM 40058) and *Xylaria papulis* 5118 adjoined to *X. mali* (CBS 385.35) with 100% ML/1.0BPP support.

Table 7.4 Evidence for novel species introduction

New species names and ex-type strains	**Compared type species name and strains**	**Morphological differences**	**Phylogenetic differences (Exclude gaps)**	**References**
Annulohypoxylon moniliformis MFLUCC 18-1214	*Annulohypoxylon* annulatum CBS 140775	No difference observed	Close to *Annulohypoxylon annulatum* CBS 140775 (98% ML/1.0BPP). Differ by 1.53% (13/847bp) of ITS and 7.92% (42/530bp) of TUB2	Ju and Rogers (1996), Sir et al. (2016)
Apiospora dendrobii MFLUCC 14-0152	*Apiospora xenocordella* CBS 478.86	Conidiogenous cell: straight vs. verruculose, globose to clavate to doliiform.	Close to *Apiospora xenocordella* (100% ML/1.0BPP). Differ by 1.95% (13/666bp) of ITS, 3.24% (7/216bp) of TUB2	Crous and Groenewald (2013)

Table 7.4 (continued)

New species names and ex-type strains	**Compared type species name and strains**	**Morphological differences**	**Phylogenetic differences (Exclude gaps)**	**References**
Hypoxylon endophyticum MFLUCC 18-1206	No ex-type available (Compared with *Hypoxylon investiens* YMJ 89062905)	No difference observed	Close to *Hypoxylon investiens* (100%ML/1.0BPP). Differ by 3.54% (30/847bp) of ITS and 1.89% (15/530bp) of TUB2	Ju and Rogers (1996), Platas et al. (2009)
	Hypoxylon investiens CBS 118185	No difference observed	Different lineages. Differ by 2.13% (18/847bp) of ITS, 13.8% (198/1432bp) of LSU, 4.93% (64/1298BP) of RPB2 and 2.83% (15/530bp) of TUB2	Ju and Rogers (1996), Platas et al. (2009)
Hypoxylon officinalis MFLUCC 14-0075	*Hypoxylon lateripigmentum* MUCL 53304	No difference observed	Close to *H. lateripigmentum* (100%ML/1BPP). Differ by 5.08% (43/847bp) of ITS, 2.03% (29/1432bp) of LSU, 8.3% (44/530bp) of TUB2	Bills et al. (2012)
Nemania dendrobii MFLUCC 18-1213	No ex-type available (Compared with *Nemania bipapillata* 90080610 (HAST))	No difference observed	Close to *N. bipapillata* 90080610 (100% ML/1.0BPP). Differ by 2.48% (21/847bp) of ITS; 4.15% (22/530bp) of TUB2, 4.93% (64/1298bp) of RPB2	Ju and Rogers (1999), Hsieh et al. (2010), Database for Rice Seed-borne Fungi-Nemania sp. 15008
Xylaria aphylli MFLUCC 21-0059	*Xylaria bambusicola* WSP 205	Morphological characters are not sufficient for characterisation	Close to *X. bambusicola* (100%ML/1BPP). Differ by 5.31% (45/847bp) of ITS, 6.04% (32/530bp) of TUB2 and 4.93% (64/1298bp) of RPB2	Rodrigues et al. (1993), Hsieh et al. (2010), Dai et al. (2016)

Table 7.4 (continued)

New species names and ex-type strains	Compared type species name and strains	Morphological differences	Phylogenetic differences (Exclude gaps)	References
Xylaria chrysanthi MFLUCC 21-0014	No ex-type available (Compared with *Xylaria phyllocharis* 528 (HAST))	Morphological characters are not sufficient for characterisation	Close to *X. phyllocharis* (75% ML/0.97BPP). Differ by 6.85% (58/847bp) of ITS; 16.6% (215/1298bp) of RPB2; 7.17% (38/530bp) of TUB2	Rodrigues et al. (1993), Hsieh et al. (2010)
Xylaria dendrobii MFLUCC 14-0137	No ex-type available (Compared with *Xylaria cubensis* JDR 860)	In *X. cubensis*, Conidiogenous cell has round to denticulate secession scars; Conidia have flat basal abscission scar	Close to *X. cubensis.* Differ by 4.37% (37/847bp) of ITS and 5.28% (28/530bp) of TUB2	Rogers 1984, Rodrigues et al. (1993), Hsieh et al. (2010)
	No ex-type available (Compared with *Xylaria cubensis* GENT 159	*X. cubensis* has smaller conidia size: (3.6-)5-6.3 x 1.8-3.6 um vs. 4.5–9 × 2.5–4.5 µm	Close to *X. cubensis* GENT 159 (86% ML/0.99BPP). Differ by 1.13% (6/530bp) of TUB2	Rogers 1984, Rodrigues et al. (1993), Hsieh et al. (2010)

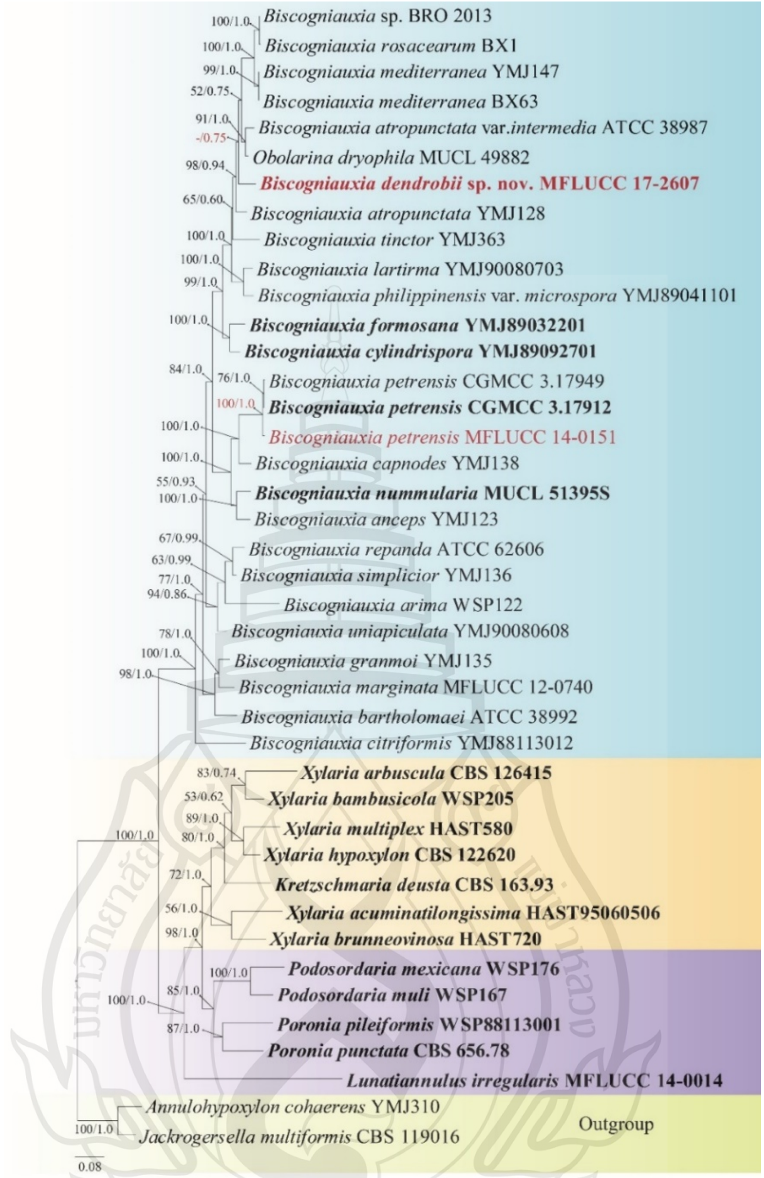


Figure 7.1 Consensus phylogram of 1,000 trees resulting from a RAxML analysis of the (ITS+TUB2+RPB2+LSU) alignment of the analysed *Biscogniauxia* related genera aequences. The isolates in this study are in red. The holotype and ex-type isolates are in **bold**. Maximum likelihood (MLB) and Bayesian posterior probabilites (PP) are labelled at the end of nodes (50% majority rule). Dashes are indicated values lower than 50%. Scale bar corresponds to 0.08 substitutions per site

Figure 7.2 A multi-locus phylogenetic tree based on the combined ITS-LSU-TUB2-TEF-1α sequences of Apiosporaceae species resulting from a maximum likelihood analysis. The isolates from this study are in red. The ex-type isolates are in **bold**. Equal or greater than 70 maximum likelihood values (ML) and/or 0.9 Bayesian posterior probabilities (BPP) are labelled at the end of nodes. Dashes are indicated values lower than 70% ML and/or 0.9BPP. Scale bar corresponds to 0.09 substitutions per site

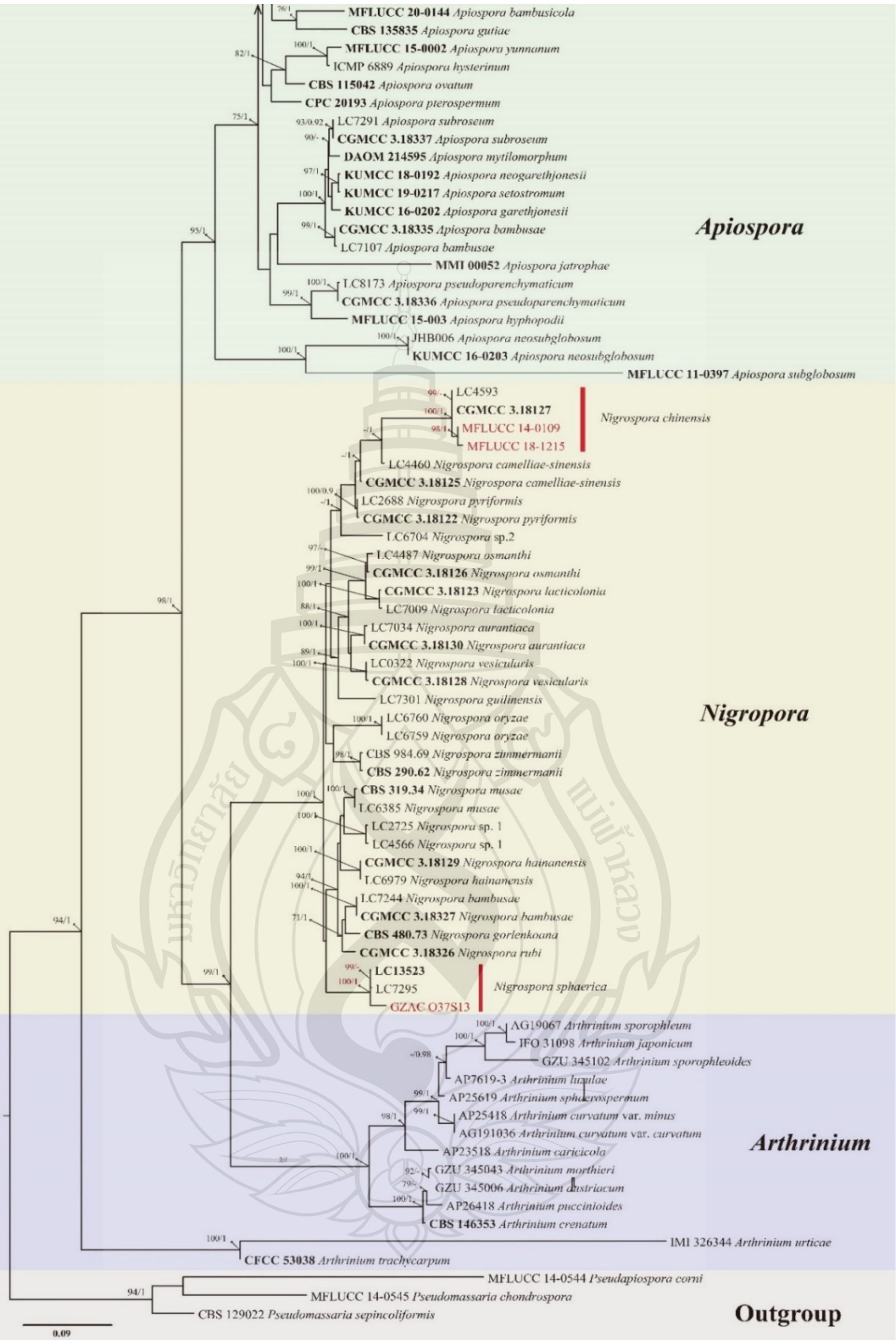


Figure 7.2 (continued)

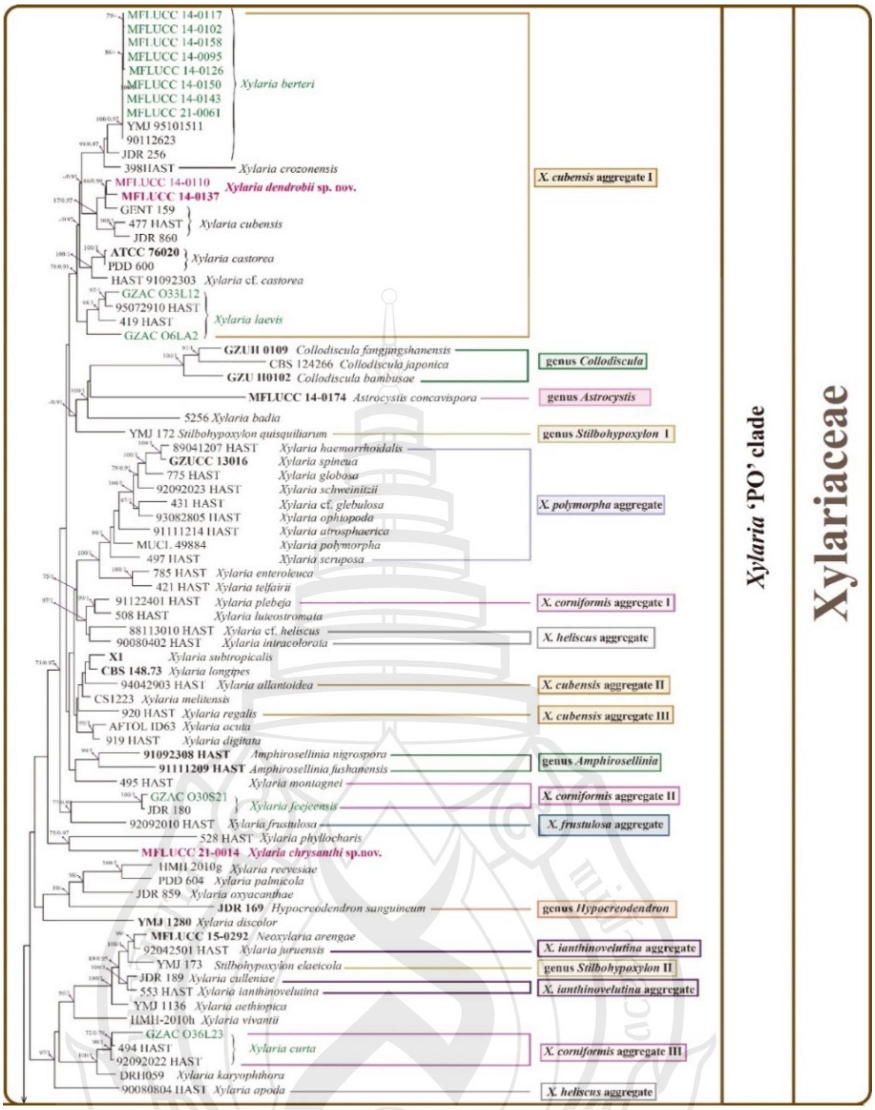


Figure 7.3 A multi-locus phylogenetic tree based on the combined ITS-LSU-TUB2-RPB2 sequences of related Xylariales species resulting from a maximum likelihood phylogenetic analysis. The isolates from this study are in red (new species) and green (known species). Strians need revising are in purple. Different aggregate partition is based on their various stromata types

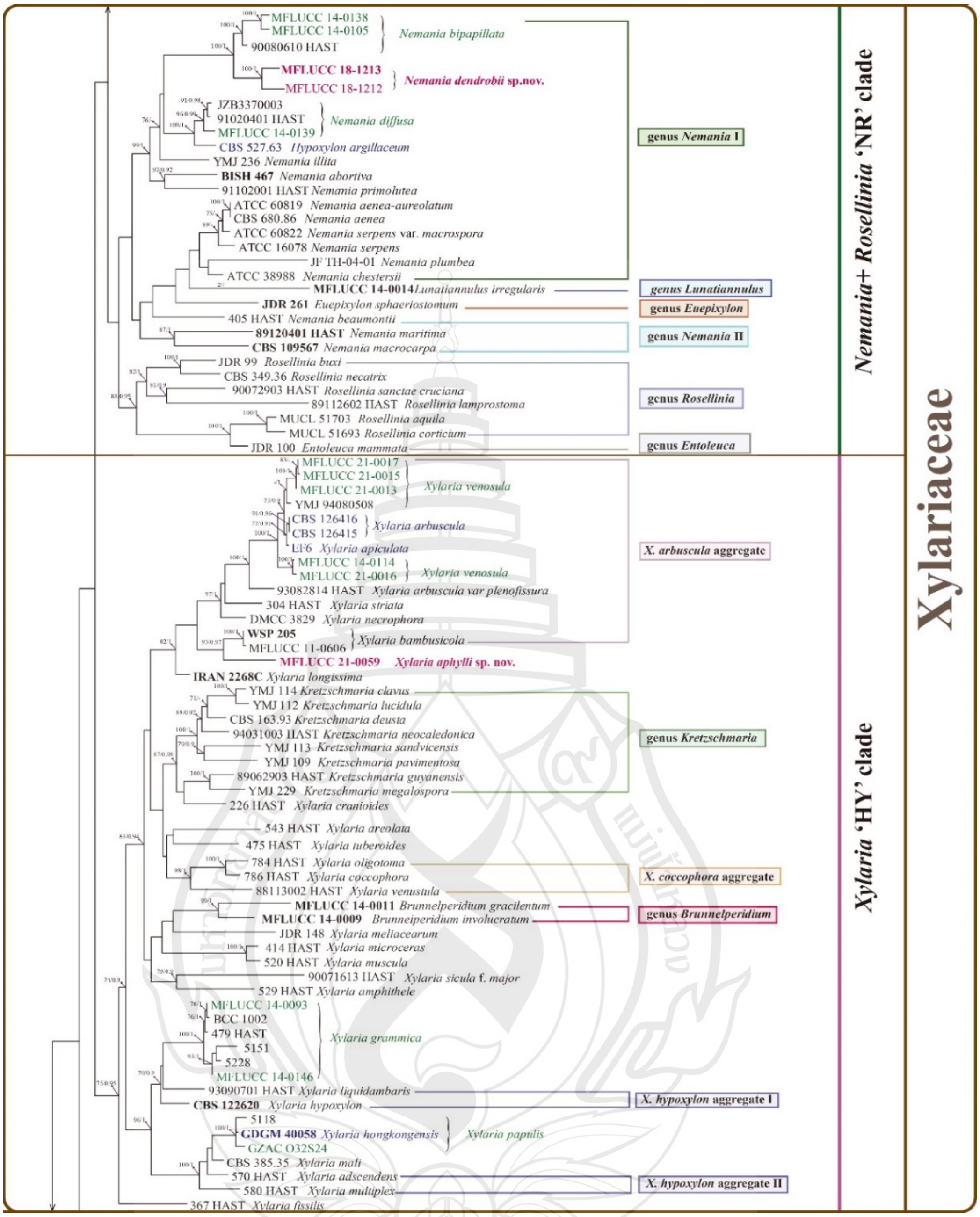


Figure 7.3 (continued)

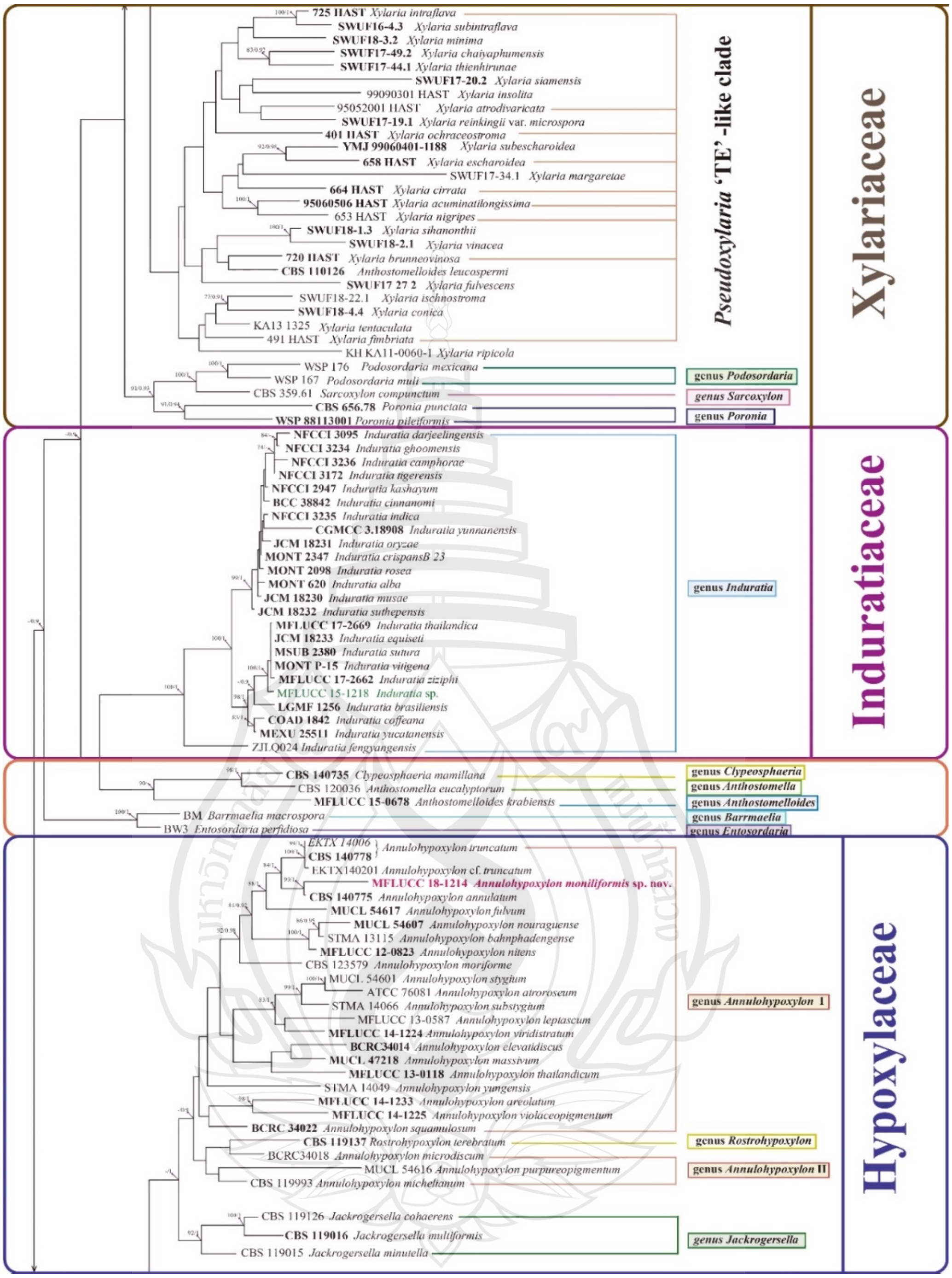


Figure 7.3 (continued)

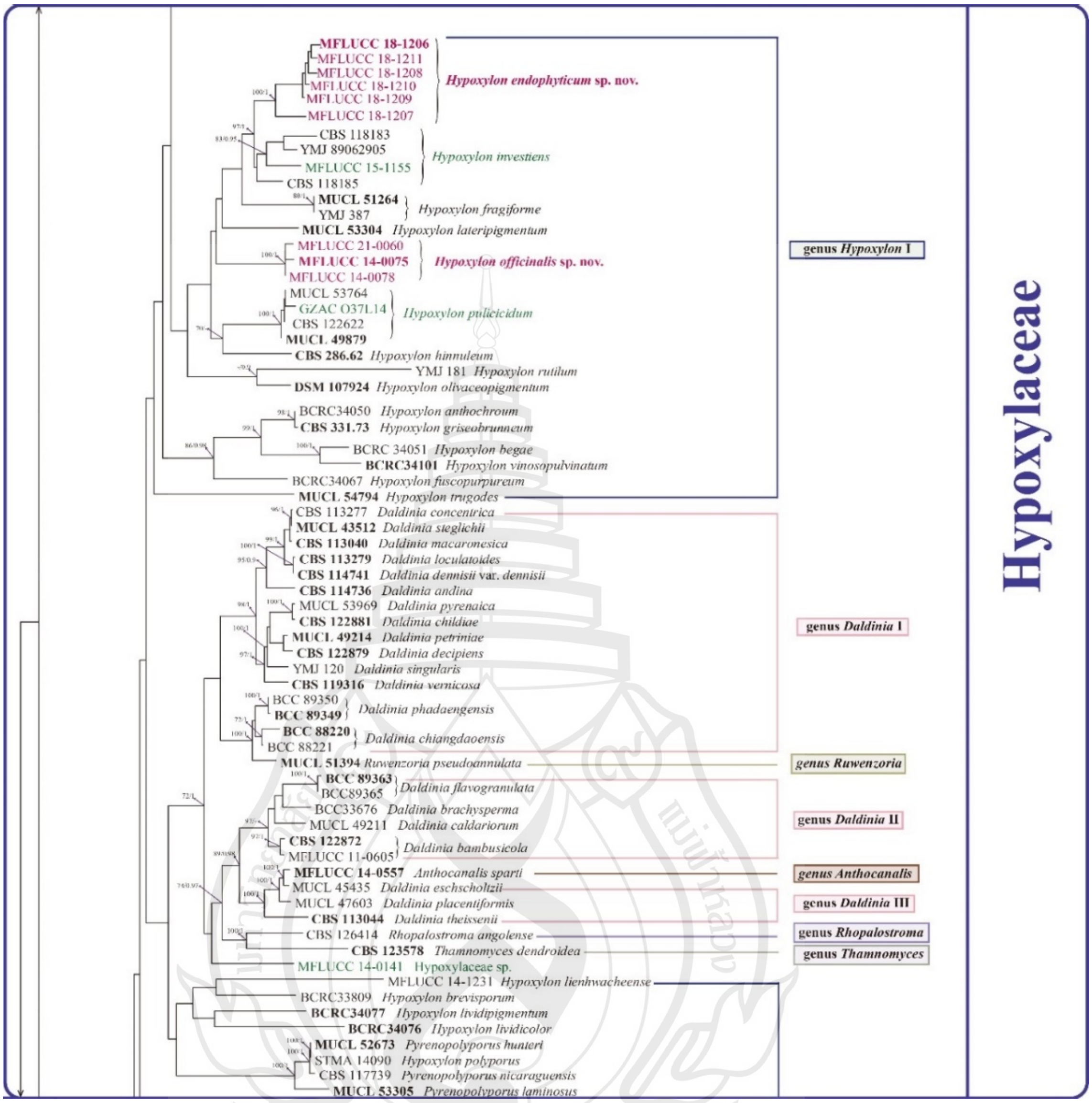


Figure 7.3 (continued)

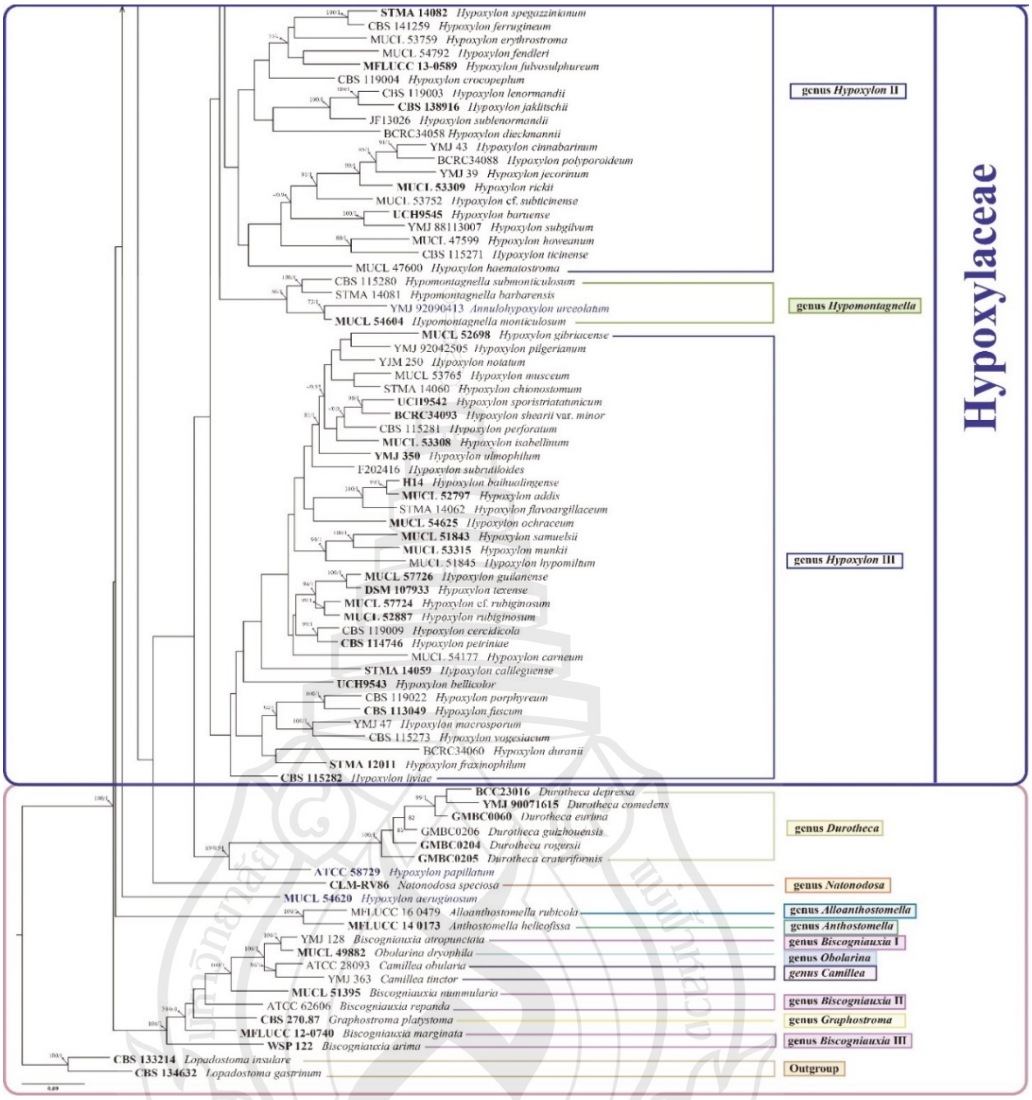


Figure 7.3 (continued)

7.3.3 Taxonomy

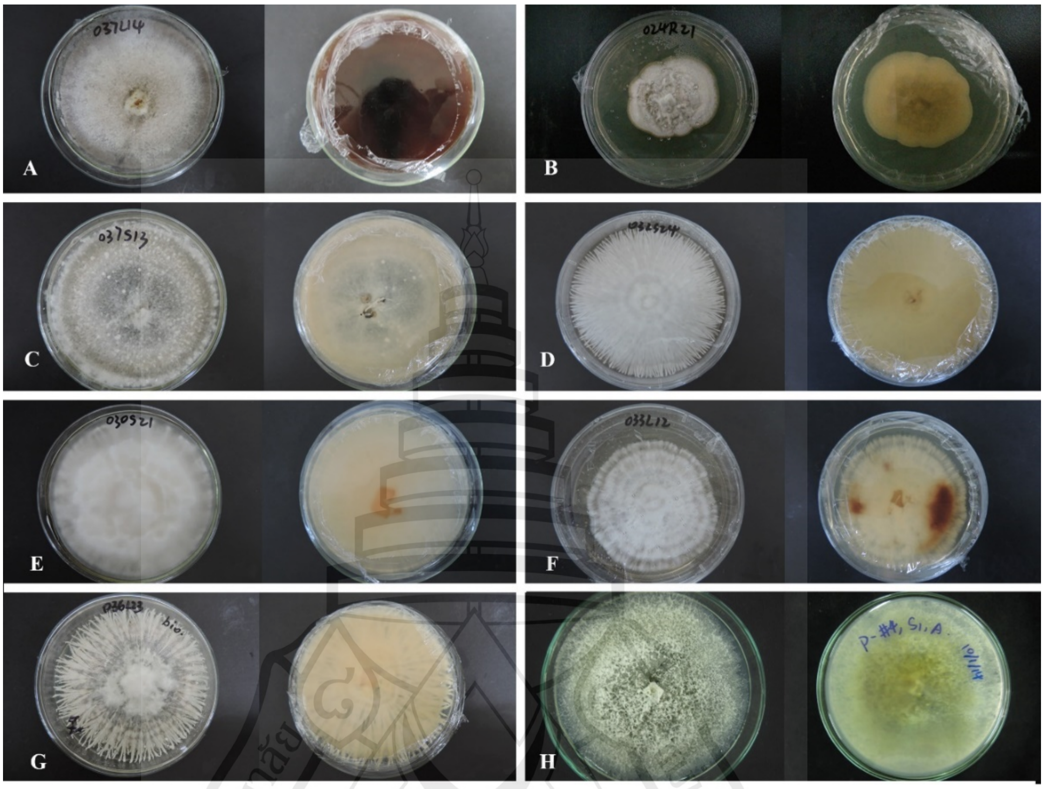


Figure 7.4 Front and reverse view of the isolates on PDA, isolated *Dendrobium* species; A. *Hypoxylon pulicicidum* (GZAC O37L14). B. *Induratia* sp. (MFLUCC 15-1218). C. *Nigrospora sphaerica* (GZAC O37S13). D. *Xylaria papulis* (GZAC O32S24). E. *Xylaria feejeensis* (GZACA O30S21). F. *Xylaria laevis* (GZACA O33L12). G. *Xylaria curta* (GZAC O36L23). H. *Xylaria berteri* (MFLUCC 14-0142)

Xylariales Nannf. Nova Acta R. Soc. Scient. upsal., Ser. 4 8(no. 2): 66 (1932)

Xylariales are commonly found as fungal endophytes in herbaceous plants, saprobes or pathogens on rotting wood with a preference for humid, shady habitats and have a worldwide distribution (Daranagama et al., 2018; Zhou et al., 2019). Hyde et al. (2020) accepted 15 families in this order. Stroma is an important characteristic for distinguishing most of the genera in Xylariales, however, some genera are astromatic and there are asexual Xylariales (Wendt et al., 2018). Many novel structures of interesting secondary metabolites have been discovered in species of this order, which indicates a great potential for investigating bioactive compounds (Becker & Stadler, 2021).

Apiosporaceae K.D. Hyde, J. Fröhl., Joanne E. Taylor & M.E. Barr, Sydowia 50(1): 23 (1998)

Apiosporaceae was introduced by Hyde et al. (1998) to accommodate *Apiospora* and *Appendicospora* according to the exclusive sexual morphology and special asexual morphs, such as basauxic conidiophores with terminal and intercalary polyblastic conidiogenous cells, and unicellular conidia with germ slits. Crous & Groenewald (2013) analyzed nrLSU DNA and confirmed Apiosporaceae taxonomic placement in Xylariales (Maharachchikumbura et al., 2015; Hyde et al., 2020). They are common fungal endophytes of various plants, and they can be saprobic and pathogenic for mostly monocotyledons and grasses (Hyde et al., 2020). Presently, six genera viz. *Appendicospora*, *Arthrinium*, *Dictyoarthrinium*, *Endocalyx*, *Scyphospora* and *Spegazzinia* are accepted (Wijayawardene et al., 2017; Hyde et al., 2020).

Apiospora Sacc., Atti della Società Veneto-Trentino di Scienze Naturali Residente in Padova 4: 85 (1875) Fig.4

Apiospora Sacc. was proposed in 1875 and synonymized with *Arthrium* Kunze based on their basauxic conidiogenesis, large upper cell and small basal cell (sexual morph), LSU based (mainly) phylogenetic analysis following the one fungus-one name policy (Hughes, 1953; Hawksworth et al., 2011; Crous & Groenewald, 2013; Dai et al., 2016; Pintos et al., 2019). However, *Nigrospora* phylogenetically splits the original *Arthrinium* species into two parts, which resulted in forming three monophyletic groups mainly due to their different sequences of ITS, LSU, TEF-1α and TUB2 exons (Pintos & Alvarado, 2021). *Apiospora* was proposed to accommodate the species containing conidia that generally more or less rounded in face view and lenticular in side view (Pintos & Alvarado, 2021). *Apiospora montagnei* (= *Sphaeria apiospora*) was the type species, however, the ex-type strain and its sequences is absent (Pintos et al., 2019; Pintos & Alvarado, 2021). This group of fungi are endophytes, pathogens and saprobes associated with multiple plant host families worldwide (Maharachchikumbura et al., 2015, Dai et al., 2016; Pintos & Alvarado, 2021). *Apiospora phaeospermum* (published as *Arthrinium phaeospermum*) was reported as a human pathogen (Li et al., 2020).

Apiospora dendrobii XY Ma & JC Kang Figure 7.5

Index Fungorum number: IF551811; Facesoffungi number: FoF 10263

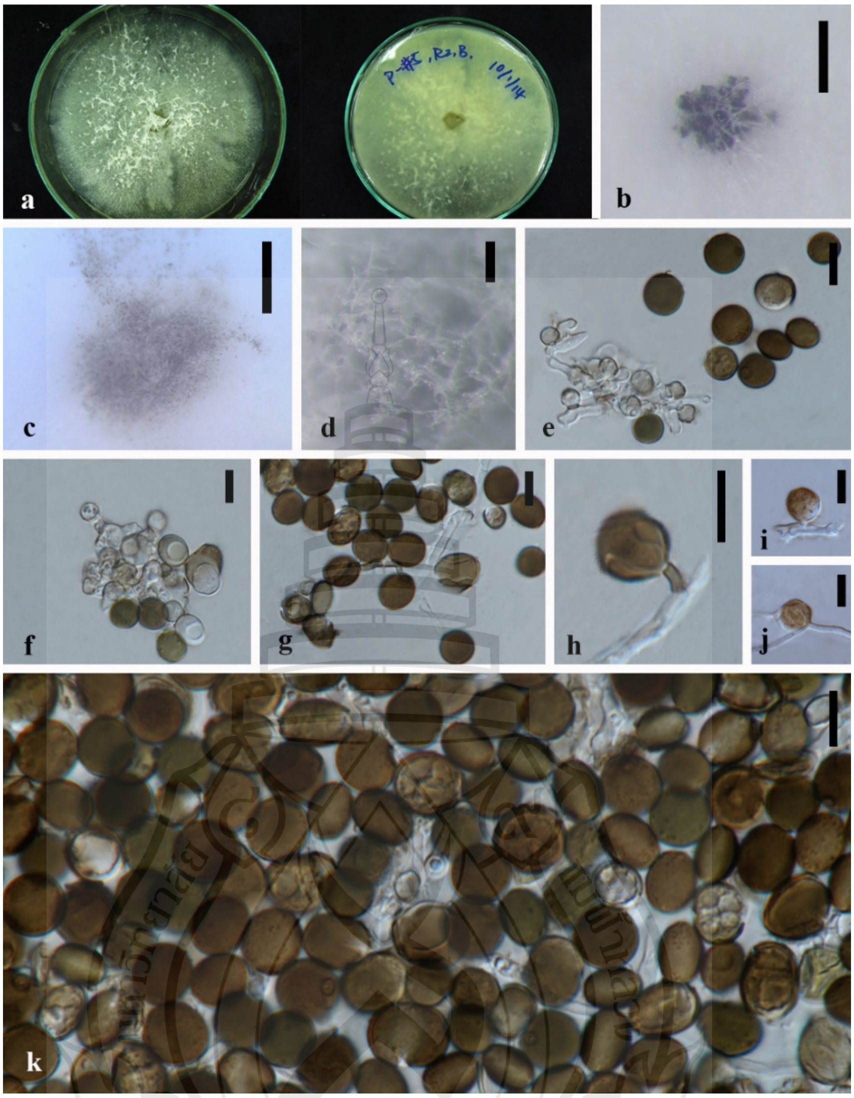
Etymology – Name after its host species-genus name.

Culture characteristics – Colonies on PDA superficial, white to light yellow hypha, circular, lightly undulate, radiant from the middle; reverse yellow. Vegetative hyphae septate, branched, hyaline, thick-walled. Growth rate: 5mm/day

On 4% OA, vegetative hyphae hyaline, 1–4 µm diam., septate, smooth, branched, thick-walled. Conidiophores reduced to conidiogenous cells, hyaline to brown, thick-walled. Conidiogenous cells erect from mature mycelia, aggregated, hyaline to brown, straight to curved. Conidiomata 20 µm diam., superficial or immersed in aerial mycelia, globose to irregular, dark brown to black, aggregated. Conidia 7–11.5 × 9–12.5 µm ($\bar{x}$ = 9.5 × 10.3 µm, n=50), hyaline to light brown, solitary, in surface view, ellipsoid to globose or sub-globose, lenticular in side view.

Material examined – THAILAND, Chiang Rai Province, Mae Fah Luang District, outside Temple of Doi Tung Pagoda, in the roots of *Dendrobium harveyanum*, 19 December 2013, S. Nontachaiyapoom, N. Aewsakul and X.Y. Ma. MFLU 21-0153 (Holotype); ex-holotype, MFLUCC 14-0152.

Notes – The conidiogenous cell of *Apiospora dendrobii* is straight, which differs from that of *A. xenocordella* (verruculose, globose to clavate to doliiform). *Apiospora dendrobii* forms an individual branch clustered with *A. xenocordella* sustained by100% ML/1.0BPP. The ex-type MFLUCC 14-0152 differs the ex-type CBS 478.86 of *Arthrinium xenocordella* by 1.95% (13/666bp) of ITS and 3.24% (7/216bp) of TUB2 sequences. The ITS blast search indicates that the most similar species is Sordariomycetes sp. (Sequence similarity 99%). Both LSU and TUB2 blast searches indicated that MFLUCC 14-0152 was the most similar to *A. xenocordella* with sequence similarity 99% and 94% respectively).



Note a Colony on PDA (left-front view, right-reverse view). b–d Conidiomata mass. e–g Conidiophores with conidia. h–j Chlamydospores. k Conidia. Notes: b–k on 4% OA. Scale bars: b–D=100 µm, e–k=10 µm.

Figure 7.5 *Apiospora dendrobii* (Holotype)

Biscogniauxia dendrobii X.Y. Ma, K.D. Hyde & J.C. Kang, sp. nov. Figure 7.6

Facesoffungi: FOF 06258

MycoBank. MB 831129

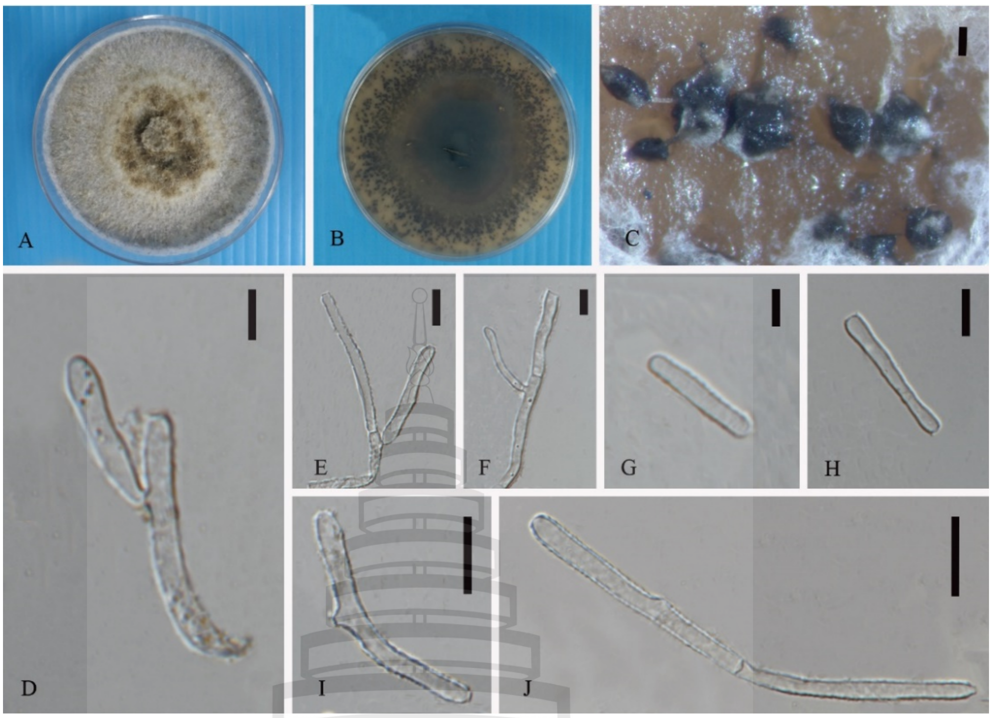
Etymology: In reference to the host genus *Dendrobium*.

Description on PDA and WA: Vegetative *hyphae* 2–4 µm diam, hyaline to brown, septate, rough–walled. *Conidiomata* 300 × 250 µm, black, globose to subglobose, solitary or aggregate. *Conidiophores* 2–4 µm diam, hyaline to brown, septate, cylindrical, thick-walled, composed of one main axis, one or more major branches, with conidiogenous cell arising terminally or laterally. *Conidiogenous cell* holoblastic, cylindrical with guttules, integrated, smooth, thick-walled, bearing poroid conidial secession scars. *Conidia* 11–45 × 2–4.5 µm, hyaline, cylindrical to clavate, with round or truncate apex, thick-walled, aseptate.

Culture characteristics: Colonies on PDA white to brown, darker brown from the center to the concentric ring, with felty aerial mycelia, reverse dark brown, darker in the center, dense conidiomata, growth rate: 4.0 mm/day under 28°C. Colonies on WA white, dense fluffy mycelia, reverse white, conidia scaterred under mycelia, growth rate: 3.4 mm/day under 28°C.

Material examined: China, Guizhou Province, Xingyi (Orchid nursery), isolated as a fungal endophyte on *Dendrobium aphyllum* on 27 Novermber 2015, Collector: Natdanai Aewsakul, Sureeporn Nontachaiyapoom, Xiao-Ya Ma (Holotype: MFLU 17-2791, Ex-type living culture: MFLUCC 17-2607).

Diagnose: The strain MFLUCC 17-2607 morphologically differs from *B. atropunctata* by the shape and size of conidia (globose to subglobose vs. cylindrical; 4–5.5 × 3–4.5 µm vs. 11–45 × 2–4.5 µm (Vasilyeva et al., 2007). The *Biscogniauxia* species morphology were recorded in table 7.7. In phylogenetic analysis, there is no RPB2 sequence data of *B. dendrobii*, LSU sequences of *B. atropunctata* (YMJ128), LSU and RPB2 of *B. atropunctata* var.*intermedia* (ATCC 38987). The ITS, LSU and TUB2 BLAST results indicate that MFLUCC 17-2607 is most similar with several different *Biscogniauxia* isolates (Identity: 94%-99%). In the phylogenetic tree, it formed a single clade that adjoining to *B. atropunctata* YMJ128 and *Obolarina dryophila* (MUCL 49882) (Only RPB2 sequence is available) with week support values. In the available gene sequences, the *B. dendrobii* have more than 4% different base pairs (ITS and TUB2) compared with that of *B. atropunctata* (YMJ128) and 7% distinguished base pairs (ITS) compared with that of *B. atropunctata* var.*intermedia* (ATCC 38987). Therefore, we assigned the isolate MFLUCC 17-2607 as a new species.



Note A–B. Colony on PDA (A from above view, B from below view). C. Conidiomata. Conidiophores. D–F. Conidiophores with conidia. G–J. Conidia. Scale bars: C=200 µm, D–J=10 µm.

Figure 7.6 *Biscogniauxia dendrobii* (Holotype)

***Biscogniauxia petrensis* Z.F. Zhang, F. Liu & L. Cai Figure 7.7**

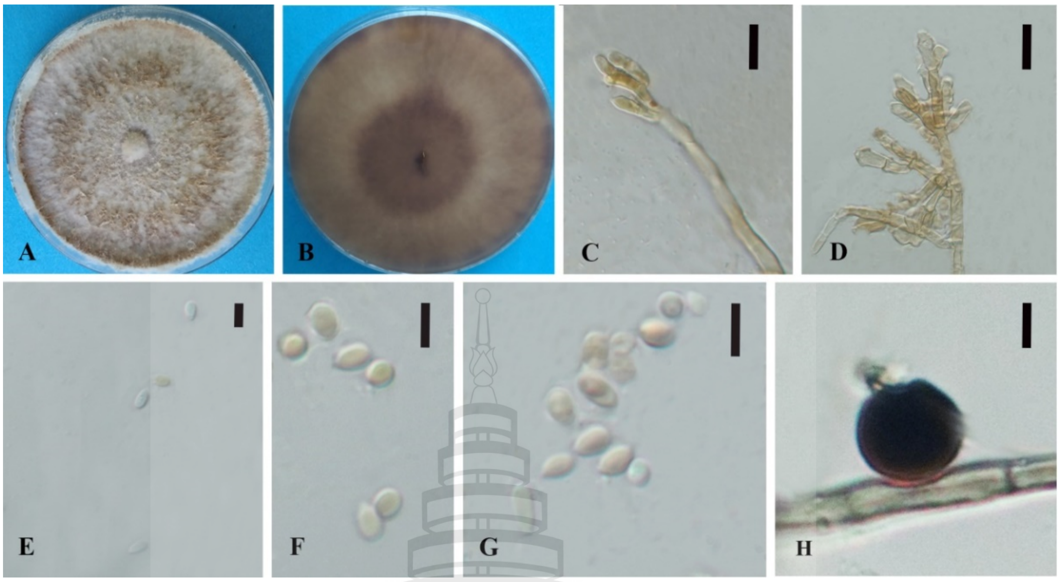
Facesoffungi: FOF 06259

Description on PDA: Vegetative *Mycelia* 3.5–5 µm diam, hyaline to light brown, septate, dense. *Conidiophore* 2.3–4.5 µm diam, nodulisporium-like, hyaline to slightly brownish, finely roughed, composed of one main axis, one or more major branches, with conidiogenous cell arising terminally or laterally. *Conidiogenous cells* (8–) 9–12.5 (–13.5) × (2.5–) 3 – 4 (–5) µm, mean ± SD= 11 ± 1.5 × 3.5 ± 0.8 µm, hyaline to light brownish, holoblastic, determinate, integrated, finely roughed, cylindrical to oblong, swollen at the apex with conidia secession scars. *Conidia* (3–) 3.5 – 5 (–5.5) × (2.0–) 2.5 – 3.0 (–3.5) µm, mean ±SD=4 ± 0.8 × 2.5 ± 0.4 µm, obovoid to clavate with obtuse or acute truncated apex, hyaline to light brown, smooth-walled.

Culture characteristics: Colonies on PDA white with dark orange dense aerial mycelia masses around the center, felty, azonate, diffuse margins, reverse light brown to dark brown, growth rate: 7.0 mm/day under 28℃.

Sample examined: Thailand, Chiang Rai, Wat Phra That Doi Tung (Temple of Doi Tung Pagodas), isolated as fungal endophyte on *Dendrobium harveyanum*, 19 December 2013. Collector: Natdanai Aewsakul, Sureeporn Nontachaiyapoom, Xiao-Ya Ma (Living culture: MFLUCC 14-0151).

Diagonosis: The strain MFLUCC 14-0151 was isolated from the root of *Dendrobium harveyanum* sampled in northern Thailand. The conidia size of MFLUCC 14-0151 is smaller than that of *B. petrensis* (3–5.5 × 2– 3 µm vs. 4.5–7.5 × 2.5–4.5 µm), MFLUCC 14-0151 has hyaline to light brown conidia but hyaline in *B. petrensis*. MFLUCC 14-0151 has hyaline to light brown conidia. However, the conidia of *B. petrensis* were hyaline in publications (Ju, 1998). Other morphological characteristics of MFLUCC 14-0151 match the description for *Biscogniauxia petrensis* (CGMCC 3.17912, type) (Zhang et al., 2017). It is phylogenetically closed to two *Biscogniauxia petrensis* strains (CGMCC 3.17912 and CGMCC 3.17949) isolated from Karst cave in southwestern China with strong support values (100MLB/1.0PP). The strain MFLUCC 14-0151 has totally 17 (5 in ITS, 8 in LSU, 3 in RPB2 and 1 in TUB2) and 18 (6 in ITS, 9 in RPB2 and 3 in TUB2) different base pairs with CGMCC 3.17912 and CGMCC 3.17949 respectively (<1%). The four-gene BLAST results show that MFLUCC 14-0151 is most similar with *B. petrensis* isolates (Identity: 99%–100%). Therefore we identified it as *B. petrensis*.



Note A–B. Colony on PDA (A from above view, B from below view). C–D. Conidiophores. E–G. Conidia. H. Chlamydospore. Scale bars: C–H=5µm.

Figure 7.7 *Biscogniauxia petrensis* (MFLUCC 14-0151)

Nigrospora Zimm., Centbl. Bakt. ParasitKde, Abt. I 8: 220 (1902)

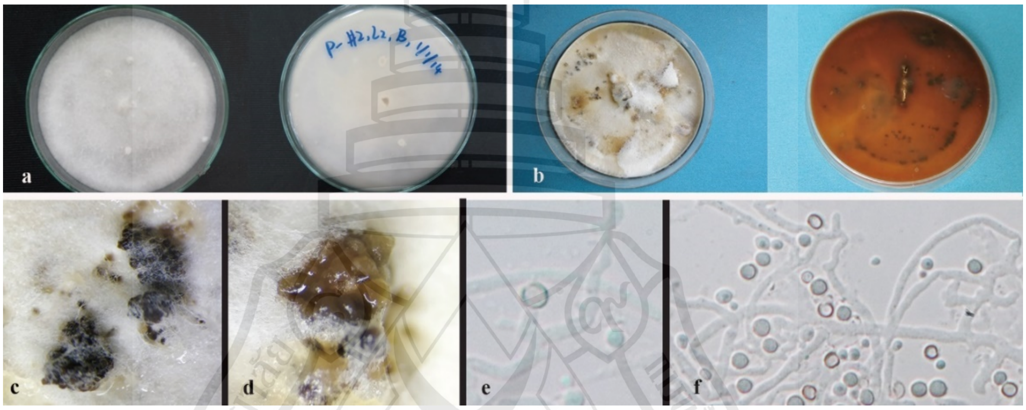
Nigrospora as ubiquitous endophytes and saprobes, and some species are human and plant pathogens, with exhibiting a global distribution (Wang et al., 2017). *Nigrospora* species are characterized by spherical to subspherical conidiogenous cells, black and globose to subglobose conidia (Seifert et al., 2011; Wang et al., 2017). Numerous secondary metabolites isolated from this genus have promising bioactive potentials, such as plant growth-inhibiting nigrosporolide and anti-mosquitoes phomalactone (Meepagala et al., 2015; Huang et al., 2016).

Nigrospora chinensis Mei Wang & L. Cai, Persoonia 39, 2017: 118–142, Figure 7.8

Culture characteristics – colonies on PDA superficial, white, cottony, entire edge. Reverse white. Growth rate: 5 mm/day. Colonies on MEA white to brown, leathery, brown to black conidiomata masses scattered; reverse deep brown. Growth rate: 6 mm/day.

On 2% MEA, vegetative hyphae hyaline, 1.2–2.4 µm diam., septate, smooth, branched, thick-walled. Conidiomata jelly-like, brown to black, aggregated, irregular. Conidia solitary, globose to subglobose, hyaline to light brown.

Notes – *Nigrospora chinensis* (MFLUCC 14-0109 and MFLUCC 18-1215) was isolated from leaves of *Dendrobium cariniferum* collected from northern Thailand and southwestern China. The two strains clustered with *Nigrospora chinensis* (CGMCC 3.18127 and LC4593) from *Camellia sinensis* in Guang Dong, China with 100% ML/1.0BPP support. The blast search also indicates that their most similar species is *Nigrospora chinensis* (Sequence similarity 99%).



Note a Colony on PDA (left-front view, right-reverse view). b Colony on MEA. c–d Conidiomata masses. e Mycelia. f Conidia. Notes: c–f on 2% MEA.

Figure 7.8 *Nigrospora chinensis* (MFLUCC 14–0109)

Nigrospora sphaerica (Sacc.) E.W. Mason, Trans. Brit. Mycol. Soc. 12: 158. 1927 Fig. 3, C

Culture characteristics – colonies on PDA superficial, white to light brown, velvety, zonate with one concentric circle, entire edge; reverse light brown. Vegetative hyphae septate, branched, hyaline, thick-walled. Growth rate: 6.4mm/day

Notes – *Nigrospora sphaerica* (GZAC O37S13) was isolated from *Dendrobium hercoglossum* from Xingyi, Guizhou in southwestern China. It is failed to sporulate on PDA after one month. It exhibited a strong phylogenetic relationship with two *Nigrospora sphaerica* strains isolated from *Musa paradisiaca* in Hai Nan, China.

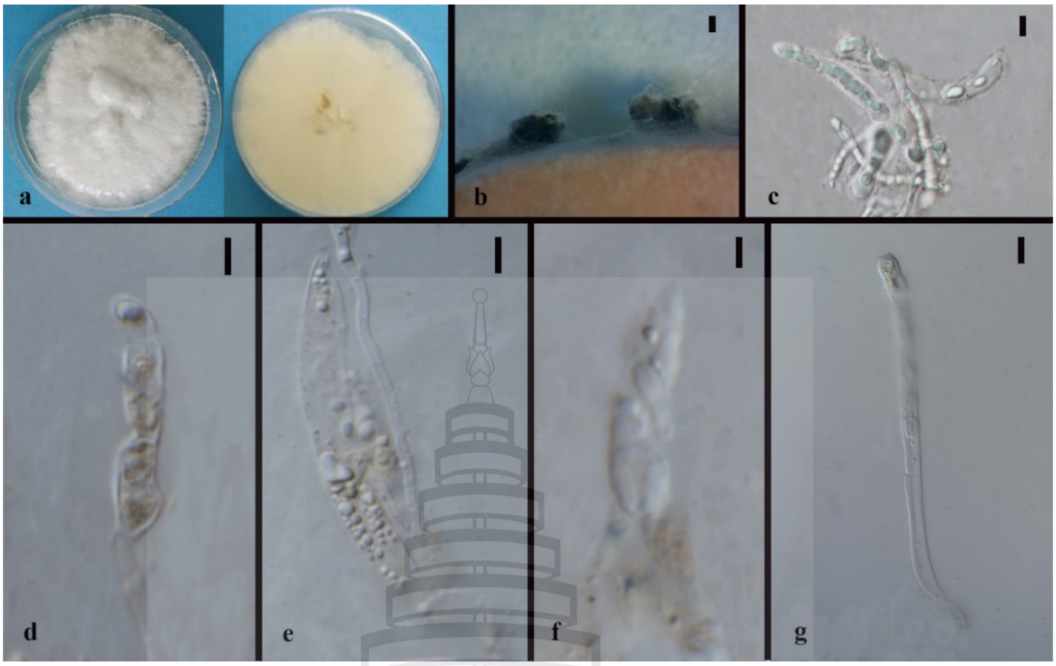
Hypoxylaceae DC. Fl. Franc., Edn 3 (Paris) 2: 280 (1805), emend. M. Stadler & L. Wendt. 2018

Hypoxylaceae was resurrected by Wendt et al. (2018) following a multi-locus phylogeny to accommodate nodulisporium-like asexual genera, which was accepted by Daranagama et al. (2018) and Hyde et al. (2020). Most Hypoxylaceous species are endophytes and saprobes on herbaceous and woody plants, while some species are linked with insect vectors (Pažoutová et al., 2010, 2013; Chen et al., 2013; Wendt et al., 2018).

Annulohypoxylon Y.M. Ju, J.D. Rogers & H.M. Hsieh, Mycologia 97(4): 855 (2005), emend L. Wendt, Sir, Kuhnert & M. Stadler. 2018

Annulohypoxylon was introduced by Ju et al. (2005) to accommodate the section *Annulata* of *Hypoxylon*. Most *Annulohypoxylon* species are saprobic on decorticated and corticated wood, and some species are endophytes of herbaceous plants (Chen et al., 2013; Daranagama et al., 2018). Wendt et al. (2018) amended the morphological characteristics of *Annulohypoxylon* and established the genus *Jackrogersella* as a separate group from *Annulohypoxylon* based on chemotaxonomy, morphology and multigene genealogy. The *Annulohypoxylon* becomes polyphyletic since the *Rostrohypoxylon* was introduced by Fournier et al. (2010). *Annulohypoxylon sensu stricto* is mostly characterized by ostioles encircled by an annulated disc, binaphthalenes as major stromal metabolites and lack of azaphilones (Wendt et al., 2018; Hyde et al., 2020). As endophyte, *Annulohypoxylon* spp. have been isolated from *Dendrobium chrysotoxum*, *D. crystallinum*, *D. falconer* and *Stanhopea trigrina* (Chen et al., 2013)

Annulohypoxylon moniliformis XY Ma & JC Kang, sp.nov. Figure 7.9



Note a Colony on PDA (left-front view, right-reverse view). b Ascomata on WA (slide cultre). c–g Asci with ascospores on the robber band (for supporting slide) in slide culture. Scale bars: b=200 µm, c–g=10 µm.

Figure 7.9 *Annulohypoxylon moniliformis* (Holotype)

Index Fungorum number: IF551812; Facesoffungi number: FoF 10264

Etymology – Named after its host species.

Culture characteristics – colony on PDA superficial, white, cottony, undulate edge; reverse white to light brown. Growth rate: 5.7 mm/day.

On WA vegetative hyphae 1.7–3.5 µm diam., hyaline to brown, septate, branched, smooth to rough from young to old, thick-walled. Ascomata 200 × 600 µm wide, black, on rubber band of slide culture, superficial, cylindrical to irregular. Ostioles 69–142 × 6.3–8.4 µm, hyaline to light brown, lower than the stromal surface. Asci cylindrical, thick-walled. Ascospores 7–8.5 × 4–6 µm ($\bar{x}$ = 7.5 × 5 µm, n=5), hyaline, ellipsoidal to fusiform with narrowly rounded ends, thick-walled.

Material examined – CHINA, Guizhou Province, Gui Yang City, Luodian County, in the roots of *Dendrobium aphyllum*, 27 November 2015, J.C. Kang. MFLU GZAC O35L22 (Holotype); ex-holotype strain: MFLUCC 18-1214.

Notes – *Annulohypoxylon moniliformis* and *An. annulatum* cluster with 98% ML/1.0BPP support. It is difficult to judge their morphology because our characteristics are insufficient and unclear, although they differ by color and size of ascospores (hyaline 7–8.5 × 4–6 µm in 1 month vs. brown to dark brown, unicellular, 7.5–11 × 3.6–6 µm in 2–3 weeks) on different media (WA of slide culture vs. OA). The blast search of all sequences indicated that *An. annulatum* was the most similar species to *An. moniliformis* (Sequence similarity 99%). However, a pairwise nucleotide comparison between the MFLUCC 18-1214 and *An. annulatum* (ex-type CBS 140775) revealed 1.53% (13/847bp) of ITS and 4.51% (42/932bp) of TUB2 sequence differences. Therefore, here we introduce MFLUCC 18-1214 is a new species.

Hypoxylon Bull., Histoire des champignons de la France. I: 168 (1791)

Hypoxylon is the type genus of Hypoxylaceae, which mainly inhabit dead wood as saprobes and some occur as endophytes of a wide range of hosts or facultative parasites on diseased hosts, and they have *Nodulisporium*-like asexual morphs (Ju & Rogers, 1996; Hyde et al., 2020). Stomatal (including pigment) and ascospore morphology were often used for morphological delimitation of *Hypoxylon* species (Ju & Rogers, 1996). Multigene genealogy revealed that they are a polyphyletic group (Maharachchikumbura et al., 2015; Wendt et al., 2018). Many bioactive secondary metabolites have been identified from *Hypoxylon* species, especially from endophytic strains (Tomsheck et al., 2010; Bills et al., 2012; Becker & Stadler, 2021).

Hypoxylon endophyticum XY Ma & JC Kang, sp. nov. Fig. 7.10

Index Fungorum number: IF551815; Facesoffungi number: FoF 10265

Etymology – Name after its habitat as a fungal endophyte.

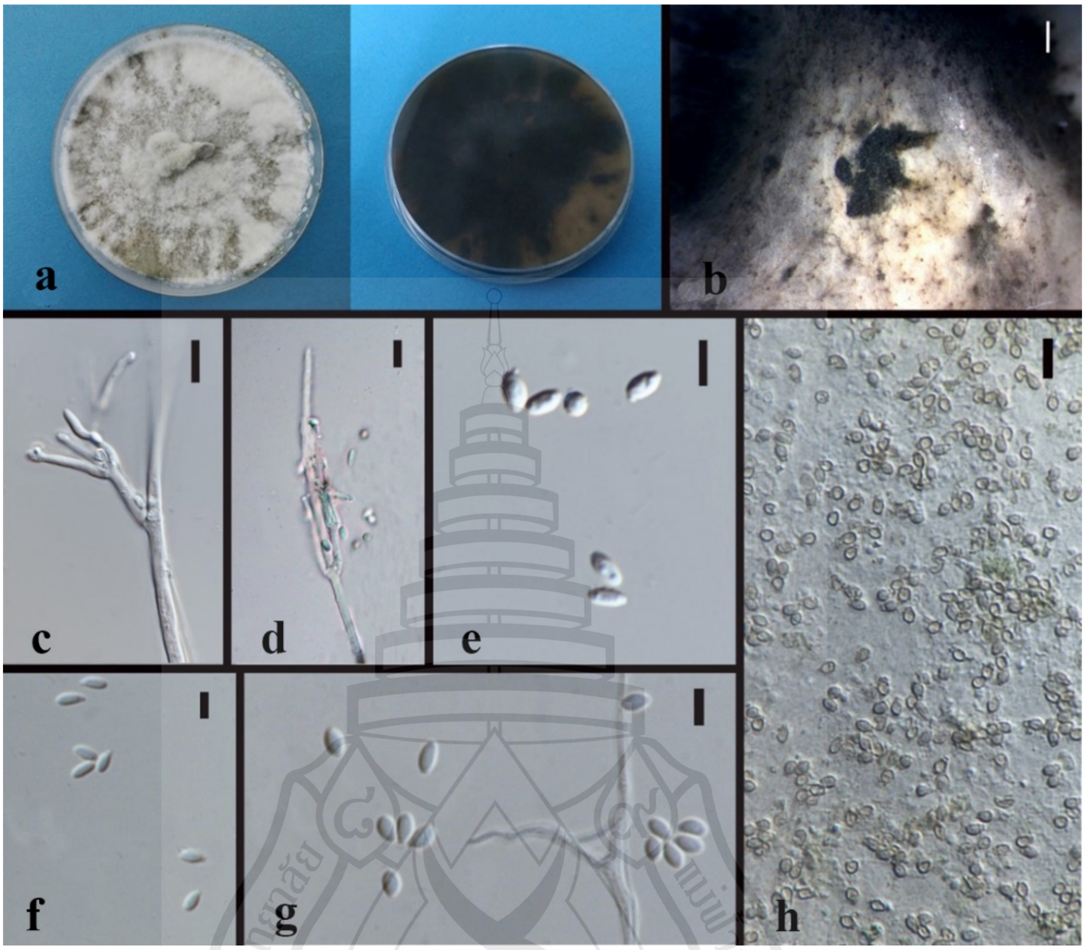
Culture characteristics – Colony on PDA superficial, greyish to white, velvety, loose and entire edge; reverse brown to black. Growth rate: 5.6 mm/day.

On PDA vegetative hyphae 1.6–4.5 µm, hyaline to brown, smooth to rough, branched. On WA (slide culture), conidiomata superficial, black, irregular. Conidiophores macronematous, cylindrical, hyaline to brown, septate, branched, finely roughed. Conidia 3–5 × 1.5–3.5 µm ($\bar{x}$ = 4 × 2.5 µm, n=30), globose to subglobose, hyaline to brown.

On WA (slide culture), vegetative hyphae 2–5 µm, hyaline to brown, smooth to rough, branched. Conidiomata 200–500 µm wide, light brown, irregular. Conidiophores mononematous or macronematous, *Periconiella*-like, cylindrical, hyaline to brown, integrated, septate, branched, finely rough. Conidia 3.5–6.5 × 2–5.5 µm ($\bar{x}$ = 5.5 × 3 µm, n=20), hyaline, globose to ellipsoid, sympodially, smooth.

Material examined – CHINA, Guizhou Province, Luodian County, Orchid nursery, in the roots of *Dendrobium loddigesii* and stems of *D. huoshanense*, 4 April 2016, B.W. Chen, living cultures, MFLUCC 18-1209, MFLUCC 18-1210, MFLUCC 18-1206; China, Guizhou Province, Xingyi City, Orchid nursery, in the roots of *Dendrobium aphyllum* and *Dendrobium* sp., leave of *D. hercoglossi* and *D. chrysotoxum*, 27 November 2015, J.C. Kang, MFLUCC 18-1207, MFLUCC 18-1208, MFLUCC 18-1211. MFLU 21-0154 (Holotype); ex -holotype strain: MFLUCC 18-1206.

Notes – *Hypoxylon endophyticum* formed an independent clade close to *H. investiens*. The sequence variation among the six strains is less than 1% except for the MFLUCC 18-1207 that may be caused by the crossing contamination. The differences between *Hypoxylon endophyticum* and *H. investiens* have been listed in Table 7.4. The big diference exists in the sequeces of LSU and RPB2. Though *Hypoxylon endophyticum* and *H. investiens* have very similar morphological characteristics, maybe caused by the different media, the size of conidia in this study are larger a bit than that of *Hypoxylon investiens* in Ju & Rogers (1996) (3.5–6.5 × 2–5.5 µm on WA in 1 month vs. 2.5–3.5 × 2.2–3.5 µm on OA in 2 weeks). Blast searches for the six isolates showed that *Hypoxylon investiens* is the most similar species with sequence similarity from 96–97%.



Note a Colony on PDA (left-front view, right-reverse view). b Conidiomata. c–d Conidiophores with conidia. e–h Conidia. Notes: b–g on WA, slide culture, h on PDA. Scale bars: b=200 μm, c–d=10 μm, e–g=5 μm, h=10 μm.

7.10 *Hypoxylon endophyticum* (Holotype)

Hypoxylon officinalis XY Ma & JC Kang, sp. nov. Figure 7.11

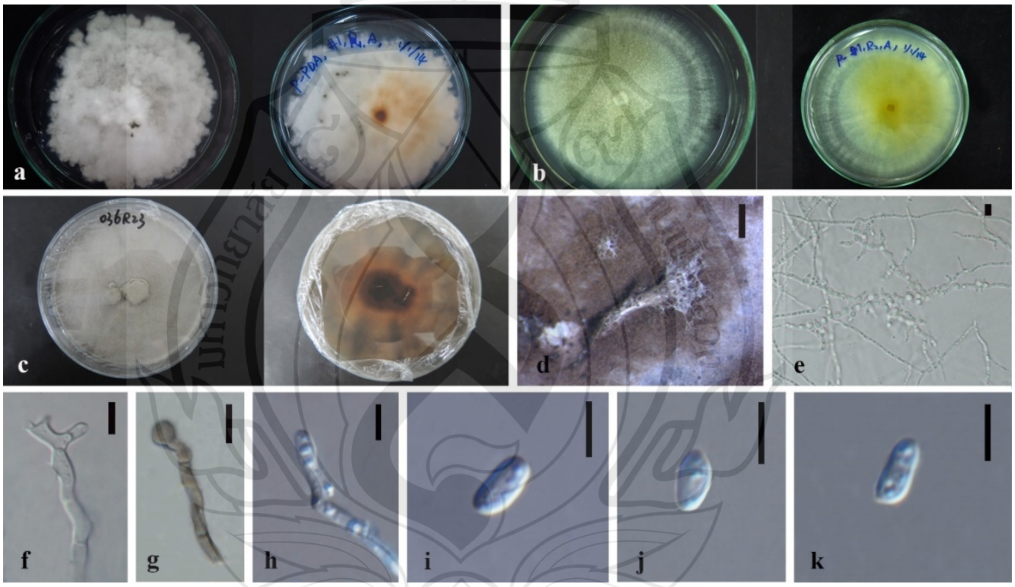
Index Fungorum number: IF551818; Facesoffungi number: FoF 10268

Etymology – Named after it host epithet (*Dendrobium*).

Culture characteristics – Colony on PDA superficial, white or buff or amber, velvety or cottony, sometimes radial, with or without a concentric ring, denticulate with an entire or loose edge; reverse unevenly white to brown. Growth rate: 6.7 mm/day.

On PDA, vegetative hyphae 1.7–3.5 μm diam., hyaline to brown, smooth to finely roughed, branched, septate, swollen at mycelia nodes. On MEA, conidiomata 1 cm high., aggregates white to brown, cylindrical. Conidiophores mononematous or macronematous, cylindrical, hyaline to brown, integrated, septate, finely roughed. Conidia 5.5–7 × 2.5–3.5 µm ($\bar{x}$ = 6.1 × 3 µm, n=3), hyaline to brown, ellipsoidal to reniform, smooth-walled.

Material examined–THAILAND, Chiang Rai Province, Mae Fah Luang District, outside Temple of Doi Tung Pagoda, in the roots of unidentified *Dendrobium* sp., 19 December 2013, S. Nontachaiyapoom, N. Aewsakul and X.Y. Ma, MFLUCC 14-0075, MFLUCC 14-0078; China, Guizhou Province, Gui Yang City, Luodian County, in the roots of *Dendrobium aphyllum*, 27 November 2015, J.C. Kang, MFLUCC 21-0060. MFLU 21-0152 (Holotype); ex-holotype strain: MFLUCC 14-0075.



Note a Colony on PDA (MFLUCC 14-0075). b Colony on PDA (MFLUCC 14-0078). c Colony on PDA (MFLUCC 21-0060]). d Conidiomata. e Mycelia. f–h Conidiophores with conidia. i–k Conidia. Notes: d, f–g on MEA, e on WA, h–k on 4% OA. Scale bars: d=500 µm, e–k=5 µm.

Figure 7.11 *Hypoxylon officinalis* (Holotype)

Notes – *Hypoxylon officinalis* represented by MFLUCC 14-0075, MFLUCC 14-0078 and MFLUCC 21-0060 have an independent clade adjacent to *Hypoxylon lateripigmentum* (MUCL 53304). Their discrepancy has been listed in table 7.4. Interestingly, the three strains are all from *Dendrobium* roots.

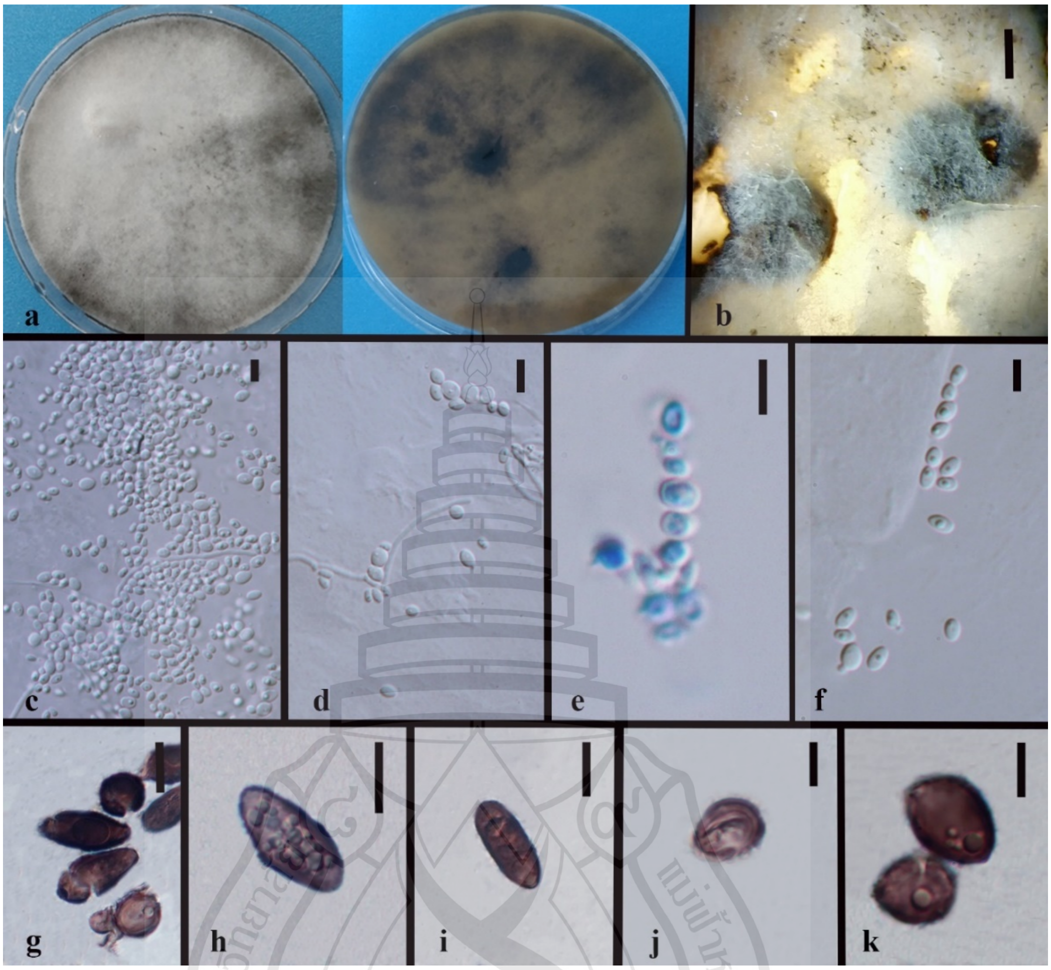
Hypoxylon investiens (Schwein.) M.A. Curtis, North Carolina Geological and Natural History Survey Papers 3: 140 (1867) Figure 7.12

Culture characteristics – Colony on PDA superficial, white to brown, velvety, mycelia gather unevenly, with an entire edge; reverse brown to black, radial. Growth rate: 7 mm/day.

On MEA and WA (slide culture), vegetative hyphae 1.2–3.2 µm diam., hyaline to brown, smooth to rough, branched. Conidiomata superficial or immersed, scattered, black, globose to irregular. Conidia on WA 2–4.5 × 1.5–3 µm ($\bar{x}$ = 3.5 × 2.2 µm, n=20), hyaline, globose to ellipsoidal, smooth-walled, thick-walled, with or without guttules, Ascospores-like structures on MEA, light brown to brown, sub-globose or ellipsoidal to fusiform, some with germ slits in the center, with guttules.

Material examined – THAILAND, Chiang Rai Province, Mae Fah Luang District, outside Temple of Doi Tung Pagoda, in the stem of *Dendrobium moschatum*, 11th May 2015, S. Nontachaiyapoom, B. Mala and X.Y. Ma

Notes – The isolate MFLUCC 15-1155 was isolated from the stems of *Dendrobium moschatum* collected in northern Thailand. It tangled with three *Hypoxylon investiens* strains sustained by low values. However, the ITS sequence discrepancy among the four *Hypoxylon investiens* strains—YMJ 89062905, CBS 118185, CBS 118183 and MFLUCC 15-1155 can be up to 3.5%. We doubt that it is likely to be a complex which needs further verification with more type species and molecular data. The blast search shows that *Hypoxylon investiens* has the highest 96% sequence similarity with strain MFLUCC 15-1155. Although they have a few comparable conidial morphology and variant sequences, based on the limited information, we identify this strain as *Hypoxylon investiens.*



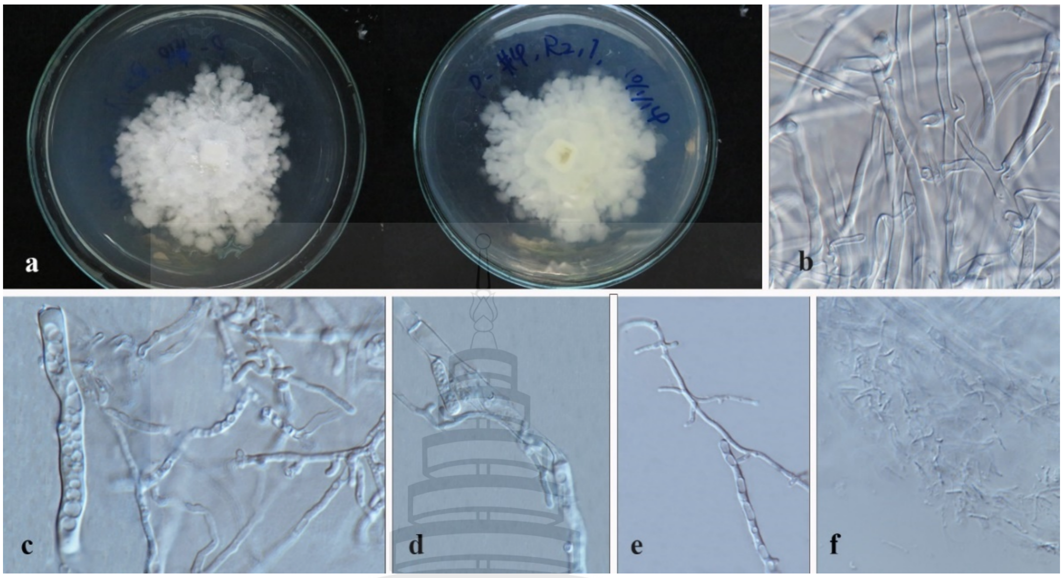
Note a Colony on PDA (left-front view, right-reverse view). b Aggregated conidiomata. c–f Conidia. g–k Ascospores. Notes: b on PDA, c–f on WA (slide culture), g–k on MEA. Scale bars: b=1cm, c–f=5 µm, g–k=10 µm.

Figure 7.12 *Hypoxylon investiens* (MFLUCC 15-1155)

Hypoxylon pulicicidum J. Fournier, Polishook & Bills, PloS ONE 7 (10): 10 (2012) Figure 7.4, C

Culture characteristics – Colony on PDA superficial, white to amber, velvety, entire edge; reverse brown to black. Growth rate: 6 mm/day

Hypoxylaceae sp. Figure 7.13



Note a Colony on PDA (MFLUCC 14-0141) (left-front view, right-reverse view). b–c Mycelia. d–e Conidiophore-like structures. f β conidia-like structures Notes: b–f on 4% OA.

Figure 7.13 Hypoxylaceae sp. (MFLUCC 14-0141)

Notes–The strain GZAC O37L14 was isolated from leaves of *Dendrobium hercoglossum* collected from southern China. Although there are no morphological characteristics from this strain, there are little sequence differences between GZAC O37L14 and *Hypoxylon pulicicidum* (ex-type MUCL 49879) (1.3% (8/589bp) of ITS and 0.38% (1/265bp) of TUB2).

Culture characteristics – Colonies on PDA superficial white, irregular, with lobbed concentric ring and undulate margin, flossy, velvety; reverse white, with the ununiform concentric ring. Growth rate: 5mm/day.

On 4% OA, Vegetative hyphae, 2.14–3.49 μm diam., hyaline, smooth, septate, branched, thick-walled. Conidiophore-like structures, branched, interminate, crooked. β conidia-like structures straight or curved.

Material examined: Thailand, Chiang Rai Province, Mae Fah Luang District, outside Temple of Doi Tung Pagoda, in roots and stems of *Dendrobium* spp., 19 December 2013, S. Nontachaiyapoom, N. Aewsakul and X.Y. Ma, MFLUCC 14-0141.

Notes: Hypoxylaceae sp. (MFLUCC 14-0141) is a solo branch taxon basal to *Daldinia* II& III and another three genera with 74% ML/0.97BPP support in Hypoxylaceae. Sporulation failed with CMA, PDA, MEA, OA, SNA and WA. Compared with *Thamnomyces dendroidea* (CBS 123578), It has 8.03 % (68/847bp) of ITS, 4.47% (64/1432 bp) of LSU and 19.4% (103/530 bp) of TUB2 different sequences. The blast search for three gene sequences showed different results— *Xylaria enteroleuca* (CBS 128357) with 100% ITS sequence similarity, *Rhopalostroma indicum* (CBS 113035) with 99.6% LSU sequence similarity) and *Daldinia brachysperma* (BCC 33676) with 90.4% TUB2 sequence similarity. We speculate that the isolate MFLUCC 14-0141 is contaminated during DNA extraction or amplification, or it is a cryptic taxon needing further research. Therefore, we failed to resolve its generic taxonomic placement

Induratiaceae Samarak., Thongbai, K.D. Hyde & M. Stadler, Fungal Diversity 2020

Induratiaceae was introduced by Smarakoon et al. (2020) to accommodate *Emarcea* and *Induratia* (= Muscodor) with apiospores and independent phylogenetic clade trees. This group is usually saprobic on dead wood, leaves, and endophytic on leaves, stems and bark (Smarakoon et al., 2020). The hyphae of Induratiaceae are rope-like with cauliflower-like hyphal bodies (Smarakoon et al., 2020).

Induratia Samuels, E. Müll. & Petrini, Mycotaxon 28 (2): 482 (1987) emend. S Samarak., Thongbai, K.D. Hyde & M. Stadler, Fungal Diversity 2020

= ***Muscodor*** Worapong, Strobel & W.M. Hess, Mycotaxon 79: 71 (2001)

Moscodor is an endophyte genus without morphological record and established only based on molecular data, which can produce volatile antibiotics and distinguished from each other by their chemical profiles (Daranagama et al., 2018). However, the genus was not accepted by Stadler et al. (2013) and Wendt et al. (2018). It was listed as Xylariales genera *incertae sedis* by Maharachchikumbura et al. (2016) and Daranagama et al. 2018. *Induratia* and *Muscodor* were thought to be the same genus corresponding to the sexual and asexual states in phylogenetic studies (Samarakoon et al., 2020). Induratia was adopted as the genus name according to the One-Fungus-One-Name proposal (Samarakoon et al., 2020). This group is saprobic on dead wood, endophytic on bark, leaves, roots and stems (Samarakoon et al., 2020). The asexual morph is characterized by terminal conidiogenous cells bearing inconspicuous denticles, conidia narrowly ellipsoidal to subglobose, hyaline, smooth-walled, with a flat, wide, basal scar (Samarakoon et al., 2020).

***Induratia* sp.** Figure 7.4, G

Culture characteristics – Colony on PDA white, cottony, undulate edge; reverse light brown. Growth rate: 3.6 mm/day

Notes – *Induratia* rarely produces morphological structures on artificial media. *Induratia* sp. was isolated as a fungal endophyte from root of *Dendrobium nobile* (Chen et al., 2012). The endophytic strain MFLUCC 15-1218 was isolated from roots of *Dendrobium* sp. collected from a steep forest in northern Thailand. It clustered with *Induratia brasiliensis* and *I. ziziphi*. However, it is hard to discern its taxonomic placement based on the ITS sequences with less than 1% base pairs difference. Due to inadequate evidence, we reckon the isolate MFLUCC 15-1218 as *Induratia* sp.

Xylariaceae Tul. & C. Tul., Selecta Fungorum Carpologia, Tomus Secundus. Xylariei - Valsei - Sphaeriei 2: 3, 1863

Xylariaceae is one of the largest families of Xylariales and can be saprobe, pathogen, or endophyte on a wide range of hosts, substrates or associated with insect vectors (Daranagama et al., 2018; Hyde et al., 2020). Many xylariaceous species have been founded as endophytes and involved in the study for natural products (Chen et al., 2013; Maharachchikumbura et al., 2016; Daranagama et al., 2018; Becker & Stadler, 2021). The stromata and ascomata of Xylariaceae are variable in size (Wendt et al., 2018; Hyde et al., 2020). Their sexual morph is hyphomycetous, which are mostly geniculosporium-like (Daranagama et al., 2018; Hyde et al., 2020). Hyde et al. (2020) accepted 32 genera in Xylariaceae.

Nemania Gray, Nat. Arr. Brit. Pl. (London) 1: 516, 1821

Nemania is a large genus of Xylariaceae and characterized by stromata without bark rupturing appearance, and lack KOH-extractable pigments and finely papillate ostioles (Hyde et al., 2020). Maharachchikumbura et al. (2016) and Réblová et al. (2016) proposed the use of *Nemania* over *Geniculosporium* following Stadler et al.

(2013). They are mostly found as saprobe and endophytic on woody and herbaceous plants (Chen et al., 2013; Daranagama et al., 2018; this study).

Nemania dendrobii XY Ma & JC Kang, sp.nov. Figure 7.14

Index Fungorum number: IF551819; Facesoffungi number: FoF 10269

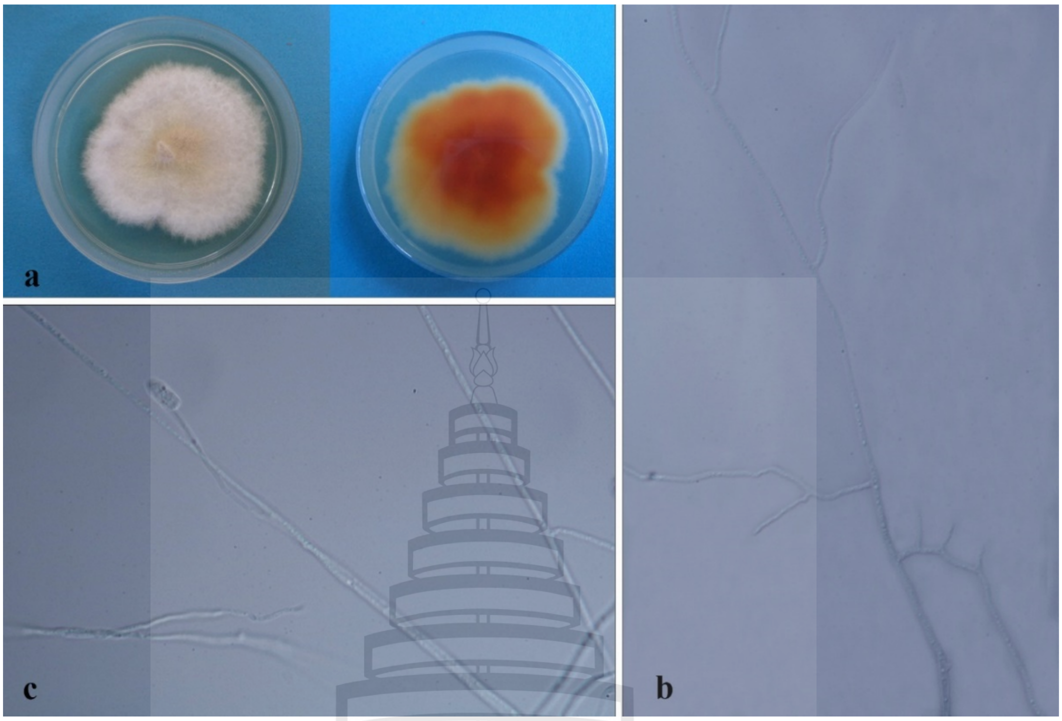
Etymology – Name after its host genus.

Culture characteristics – colonies on PDA superficial, white, felt, undulate edge, with yellow centre; reverse brown. Growth rate: 6 mm/day. Colonies on WA velvety, with concentric grey rings, a little brown spot scattered, undulate edge; reverse brown to white from the inner part to edge. Growth rate: 4.5 mm/day.

On 4% OA, vegetative hyphae, 0.7–1.2 µm diam., hyaline, straight, branched, aseptate, finely-roughed, with rich lipid droplets. Conidiophores rising from mycelia, mononematous, crooked, finely roughed. Conidia ellipsoid, hyaline, guttules.

Material examined – CHINA, Guizhou Province, Xingyi City, Orchid nursery, in the roots and stems of *Dendrobium* spp., 4 October 2016, B.W. Chen, MFLUCC 18-1212, MFLUCC 18-1213. Ex-holotype strain: MFLUCC 18-1212; Holotype: GZAC O49S1A (Holotype); ex-isotype strain: MFLUCC 18-1213.

Notes – The two isolates MFLUCC 18-1212 and MFLUCC 18-1213 have identical sequences and form a distinct clade close to *Nemania bipapillata* 90080610 (HAST) with 100% ML/1.0BPP support. The morphological characteristic is scanty for identification and comparison. The big difference can only be observed in sequence difference (Table 7.4). The blast search for each gene of MFLUCC 18-1212 and MFLUCC 18-1213 revealed *Nemania bipapillata* is the most corresponding species with similarities of 93–95%.

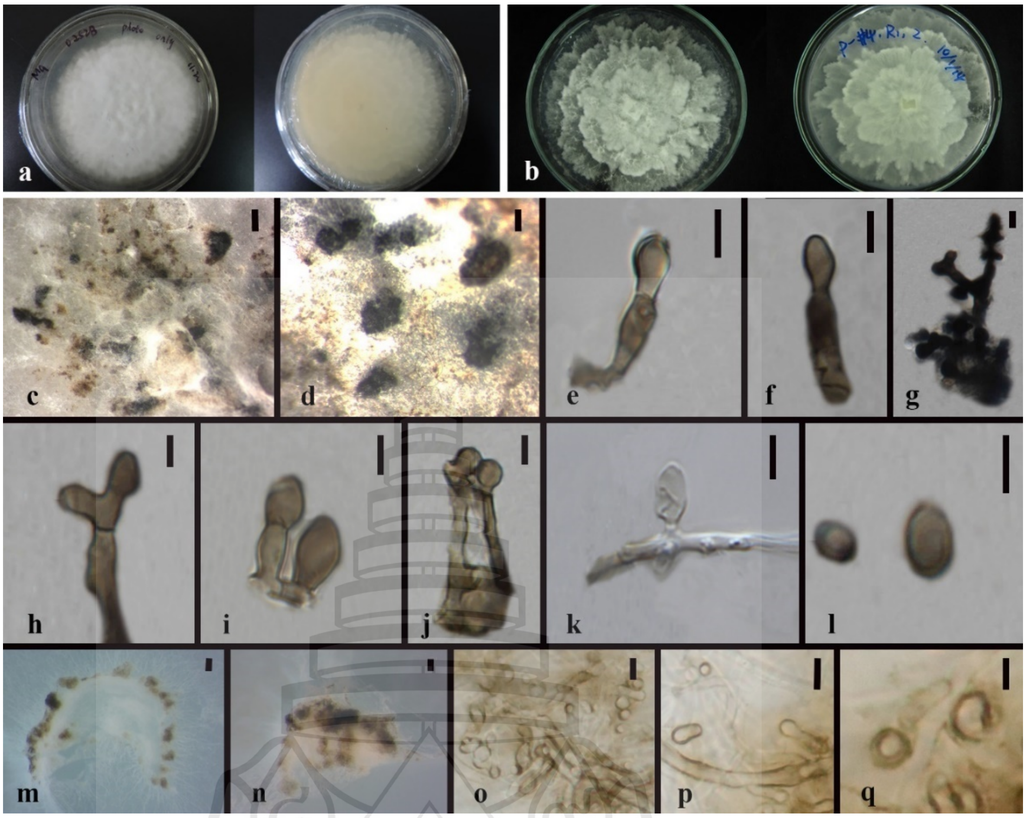


Note a Colony on PDA (MFLUCC 18-1213) (left-front view, right-reverse view). b Mycelia. c Conidiophore with conidia. Notes: b–c on WA.

Figure 7.14 *Nemania dendrobii* (Holotype)

Nemania bipapillata (Berk. & M.A. Curtis) Pouzar, Ceská Mykologie 39 (1): 24 (1985) Fig. 7.15

Culture characteristics: colonies on PDA superficial, white, leathery, lobbed and stacked, undulate edge; reverse white. Growth rate: 4.5 mm/day.



Note a Colony on PDA (MFLUCC 14-0105) (left-front view, right-reverse view). b Colony on PDA (MFLUCC 14-0138) (left-front view, right-reverse view). c–d Conidiomata. e–f, h–j Conidiophores with conidia. g Stromatic hypha with protuberances. k Chlamydospora. l Conidia. m–n Conidiomata masses. o–q Conidia. Notes: c–l on PDA, m–q on WA. Scale bars: c–d=200 µm, e–l=5 µm, m–n=500 µm, o=10 µm, p–q=5 µm.

Figure 7.15 *Nemania bipapillata* (MFLUCC 14-0105)

On PDA, vegetative hyphae 1.7–3.3 µm diam., hyaline to brown, smooth, branched, thick-walled. Conidiomata 500 × 1000 µm scattered, brown to black, clavate to irregular. Conidiophores cylindrical sometimes with swollen in the upper part, integrate, interminate, macronematous erect, septate, branched or unbranched, smooth, thick-walled. Conidiogenous cell holoblastic, brown. Chlamydospores ellipsoidal, hyaline, thick-walled. Conidia 5.5–9 × 3.5–6 µm ($\bar{x}$ = 6.7 × 4.6 µm, n=8), brown, globose to ellipsoidal, truncate at the end, thick-walled.

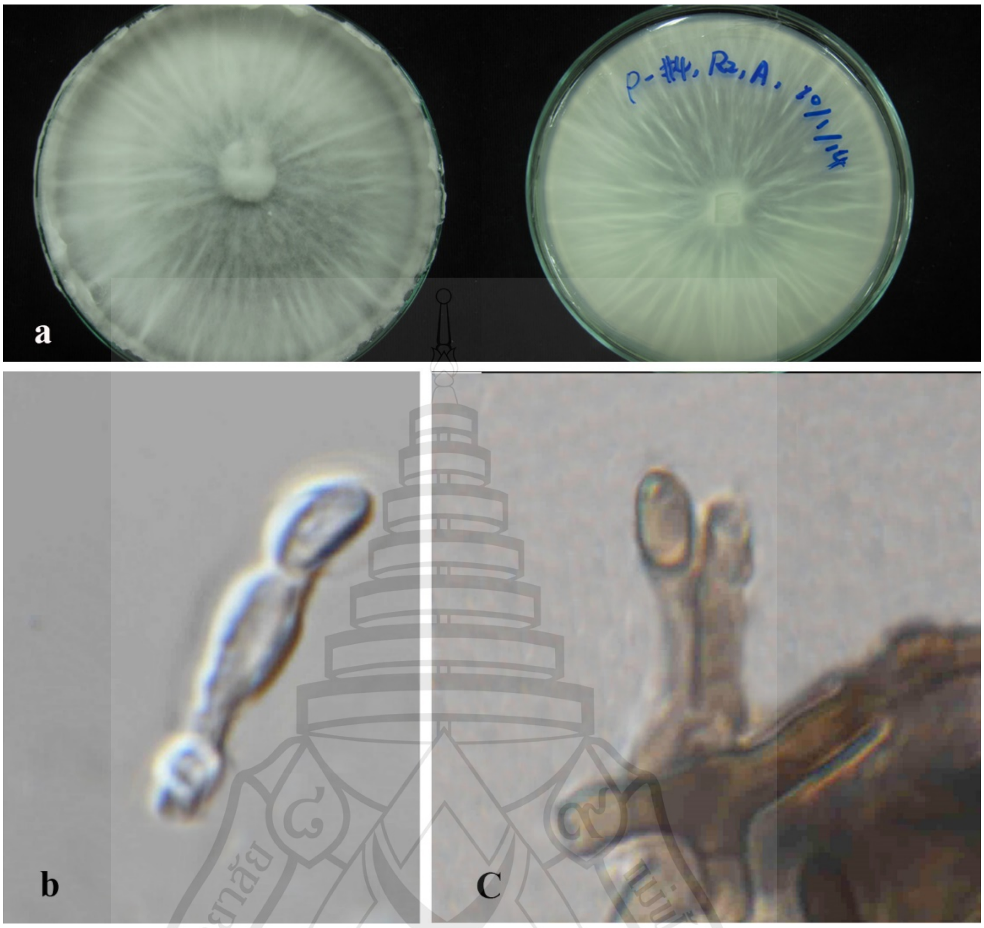
Notes – *Nemania bipapillata* comprising three isolates MFLUCC 14-0105, MFLUCC 14-0138, 90080610 (HAST) well separated with *N. dendrobii*. MFLUCC 14-0105 and MFLUCC 14-0138 were isolated from stems of *Dendrobium cariniferum* and roots of *Dendrobium* sp. collected in northern Thailand. The morphological information is scanty. The blast search showed that *Nemania bipapillata* matched their each gene with a high sequence similarity from 97% to 100%.

Nemania diffusa (Sowerby) Gray, A natural arrangement of British plants 1: 517 (1821) Figure 7.16

Culture characteristics – colonies on PDA superficial, white, cottony, radial, entire edge, exudate colorless; reverse white. Growth rate: 7 mm/day.

Description – Vegetative hyphae 1–3 µm on 2% MEA, septate, hyaline to brown, smooth, branched, thick-walled. Conidiophore-like structures cylindrical, hyaline to brown, smooth, thick-walled. Conidia-like structures ellipsoidal, hyaline to brown, thick-walled.

Note – The strain MFLUCC 14-0139 was isolated from the root of *Dendrobium* sp. collected in Thailand. *Nemania diffusa* represented by three strains 91020401 (HAST), JZB3370003 and MFLUCC 14-0139 formed a single clade close to *Hypoxylon argillaceum* (CBS 527.63) with robust support (100% ML/1.0BPP). *Hypoxylon argillaceum* (CBS 527.63) is the only hypoxylaceous species in the '*Nemania*+*Rosellinia*' clade. The same situation also occurred in U'ren et al. 2016 and re-evaluation was recommended. We speculate that CBS 527.63 should be '*Nemania argillaceum*'. Although MFLUCC 14-0139 holds 3.8% of different TUB2 sequences compared with 91020401 (HAST), considering their identical ITS sequences and scanty morphological information, incorporating with the blast search for each gene, we identified it as *Nemania diffusa*.



Note a Colony on PDA (left-front view, right-reverse view). b–c Conidiophores with conidia-like structures.

Figure 7.16 *Nemania diffusa* (MFLUCC 14-0139)

Xylaria Hill ex Schrank, Baierische Flora 1: 200, 1789

Xylaria is a type genus of Xylariaceae and is characterized by large stromata, long asci with stipes, dark ascospores and geniculosporium-like asexual morph (Ju & Rogers, 1996; Stadler et al., 2013; Hyde et al., 2020). The highly diversified nature in the genus may be the result of highly convergent evolution within the genus (Lee et al., 2000; Hyde et al. 2020). Most *Xylaria* are saprobic on deciduous dead wood and endophytes of numerous plants especially pantropical areas, some associated with termites (Daranagama et al., 2018).

Xylaria aphylli XY Ma & JC Kang, sp.nov. Fig.7.17

Index Fungorum number: IF551821; Facesoffungi number: FoF 10271

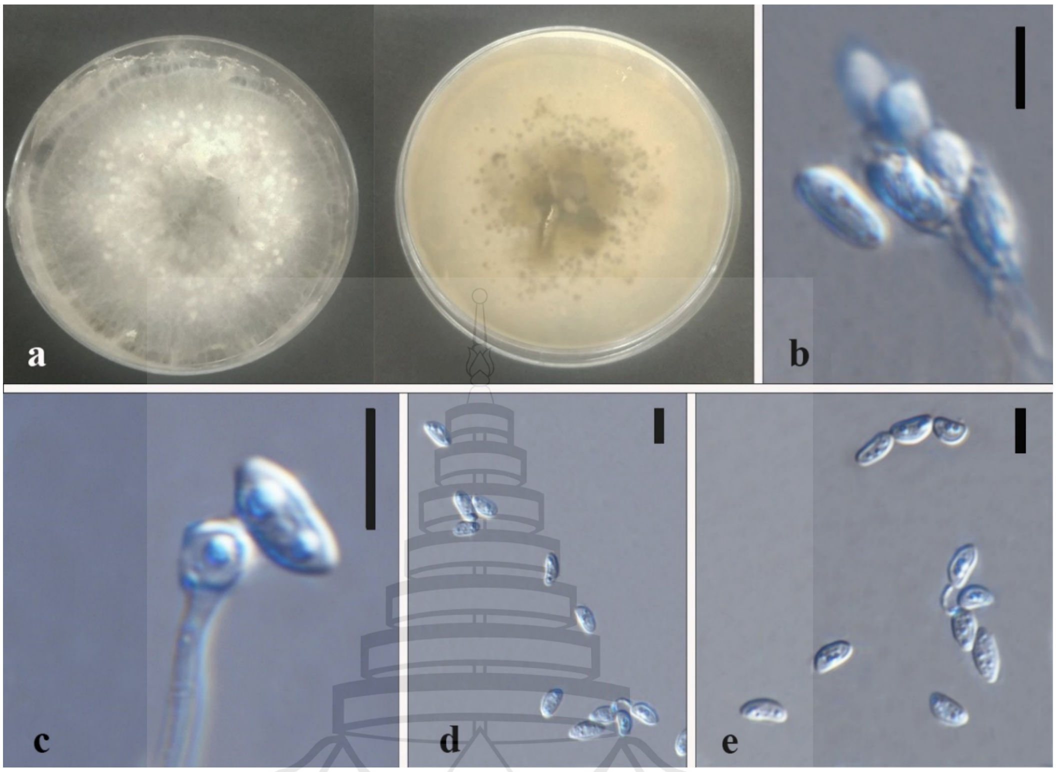
Etymology – Name after its host genus name.

Culture characteristics – colonies on OA superficial, white, velvety, mycelia aerial and radiate, forming condense white spots, entire edge; reverse light brown, brown-black masses and spots. Growth rate: 3.5 mm/day.

On PDA and 4% OA, vegetative hyphae 1–2.2 μm diam., hyaline to brown, smooth to finely roughed, branched, septate, thick-walled. On OA, vegetative hyphae 0.9–2.5 μm diam., hyaline, smooth, branched, septate, thick-walled. Conidiomata superficial, globose to irregular, aggregates, white to brown. Conidiophores mononematous or macronematous, cylindrical, hyaline to brown, integrate, smooth to finely roughed. Conidia 4.5–8 × 2–4 μm ($\bar{x}$ = 5.7 × 2.8 μm, n=25), hyaline, ellipsoidal to reniform, smooth-walled, thick-walled.

Material examined – China, Guizhou Province, Luodian County, isolated from the roots of *Dendrobium aphyllum*, 27 November 2015, J.C. Kang. GZAC O30R21 (Holotype); ex-holotype strain: MFLUCC 21-0059.

Notes – The blast search for each gene of the strain MFLUCC 21-0059 indicate that *Xylaria bambusicola* is the best match with sequence similarities from 89%–98%. Despite few available asexual morph records about *X. bambusicola*, the observed conidial characteristics resemble the asexual description for endophytic *Xylaria* (Rodrigues et al., 1993). To exclude erroneous or low-quality sequencing by checking sequence chromatogram and consensus blast results for each gene. We confirmed that MFLUCC 21-0059 represents a new species *Xylaria aphylli.*



Note a Colony on 4% OA (MFLUCC 21-0059) (left-front view, right-reverse view). b–c Conidia with conidiophores. d–e Conidia. Notes: b–e on 4% OA. Scale bars: b–e=5 μm.

Figure 7.17 *Xylaria aphylli* (Holotype)

Xylaria chrysanthi XY Ma & JC Kang, sp. nov. Figure 7.18

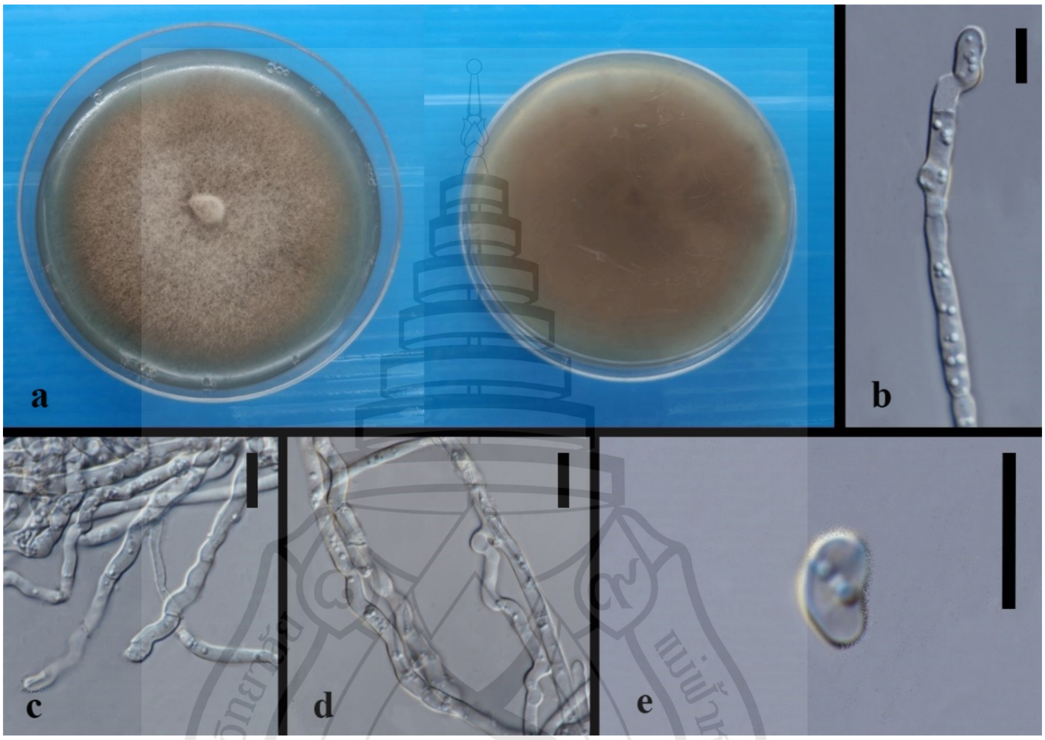
Index Fungorum number: IF551822; Facesoffungi number: FoF 10272

Etymology – Name after its host species name.

Culture characteristics – colonies on PDA superficial, white, velvety, aerial mycelia, entire edge; reverse white. Vegetative hyphae septate, branched, hyaline, thick-walled. Growth rate: 6.5 mm/day.

On 4% OA, vegetative hyphae 1–3.5 μm diam., hyaline, smooth, branched, septate, with swollen nodes and abundant lipid droplets, thick-walled. Conidiophores hyaline, mononematous, crooked, integrate, septate, smooth, thick-walled. Conidia hyaline, ellipsoidal to reniform, smooth-walled, thick-walled.

Material examined – CHINA, Guizhou Province, Gui Yang City, Animal husbandry and veterinary institute, in the stems of *Dendrobium chrysanthum*, 11 April 2016, S.X. Zhou and X.Y. Ma. MFLU 21-0150 (Holotype); ex-holotype strain: MFLUCC 21-0014.



Note. a Colony on 2% MEA (MFLUCC 21-0014) (left-front view, right-reverse view). b Conidiophore with conidium. c–d Mycelia. E. Conidia. Scale bars: b–e=5 µm. Notes: b–e on 4% OA

Figure 7.18 *Xylaria chrysanthi* (Holotype)

Notes – The conidia and conidiophores of *Xylaria chrysanthi* sporulated on 4% OA are similar to the asexual morph of *Xylaria* (Rodrigues et al., 1993). In view that only two conidia were observed in this culture, the conidia size was not measured because the sample is too small to be representative. The strong evidence supported its unique position is from the phylogenetic analysis (Table 7.4). *Xylaria cubensis*, *X. digitata* and *X. enteroleuca* are the best match in blast search for MFLUCC 21-0014 with sequence similarities from 85.5%–98% (ITS could not match a known species).

Xylaria dendrobii XY Ma & JC Kang, sp. nov. Figure 7.19

Index Fungorum number: IF 551823; Facesoffungi number: FoF 10273

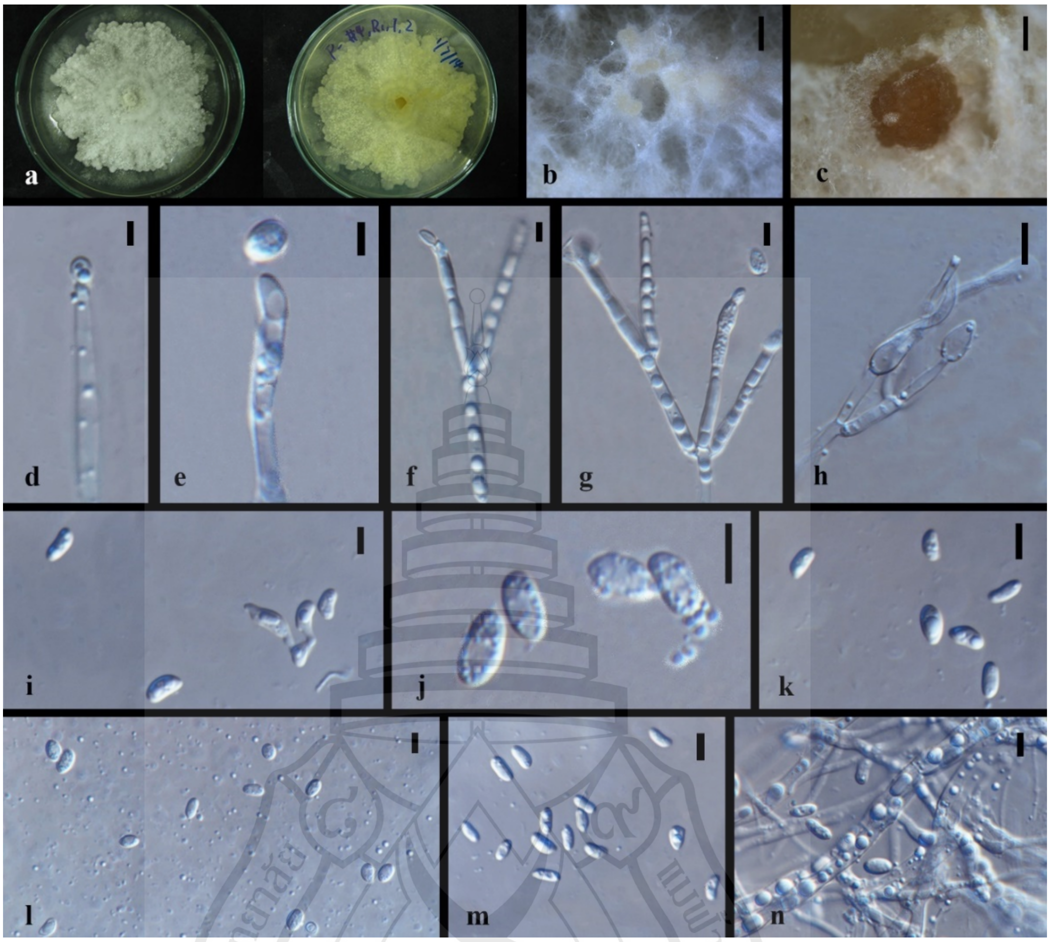
Etymology – Name after its host genus.

Culture characteristics – colonies on PDA superficial, white, cottony, undulate edge, zonate with one concentric ring, aggregated white spots scattered; reverse white to buff in the concentric ring. Growth rate: 4.7 mm/day.

On 4% OA, vegetative hyphae 1.5–3.3 µm, hyaline, smooth, septate, branched, thick-walled. Conidiomata 750 µm wide, solitary or aggregate, white orange to orange, irregular. Conidiophore hyaline, palisades, mononematous or macronematous, with abundant lipid droplets, branched near base, smooth, thick-walled. Conidiogenus cell cylindrical, terminate, Conidia 4.5–9 × 2.5–4.5 µm ($\bar{x}$ = 6.2 × 3.1 µm, n=24), hyaline, globose to ellipsoid or reniform or cone-like, guttulates, smooth.

Material examined – THAILAND, Chiang Rai Province, Mae Fah Luang District, outside Temple of Doi Tung Pagoda, in the roots of *Dendrobium* sp., 19 December 2013, S. Nontachaiyapoom, N. Aewsakul and X.Y. Ma, MFLUCC 14-0137. China, Guizhou Province, Luodian County, orchid nursery, in the leaves of *Dendrobium catenatum*, 1 December 2014, J.C. Kang, GZAC O6LA2. MFLUCC 14-0137. MFLU 21-0149 (Holotype); ex-holotype strain: MFLUCC 14-0137.

Notes – *Xylaria dendrobii* (MFLUCC 14–0137 and GZAC O6LA2) is adjacent to *X. cubensis* isolate GENT 159*,* which forms a sister group to another two *X. cubensis* isolates. Compare with asexual morph of *Xylaria cubensis* recorded by Rodrigues et al. (1993), *X. dendrobii* has much bigger conidia with no obvious denticulate secession scars on conidiogenous cell and flat basal abscission scar on conidia. In gene sequence comparison, as listed in Table 7.4, *Xylaria dendrobii* and *X. cubensis* mainly differed by the ITS and TUB2 gene sequences. Here we introduce both MFLUCC 14–0137 and GZAC O6LA2 as a new species separated with *Xylaria cubensis*. *X. cubensis* isolate GENT 159 and *Xylaria dendrobii* sex-type MFLUCC 14-0137 has only 6 distinguished base pairs in TUB2 sequence. However, GENT 159 is much more different from another two *X. cubensis* isolates. The taxonomic placement of GENT 159 perhaps needs re- evaluating with other gene sequence data.



Note a Colony on PDA (MFLUCC 14–0137) (left-front view, right-reverse view). b–c Conidiomata. d–h Conidiophores with conidia. i–m Conidia. n Mature mycelia. *b–n on 4% OA. Scale bars: b–c=500 μm, d–n=5 μm.

Figure 7.19 *Xylaria dendrobii* (Holotype)

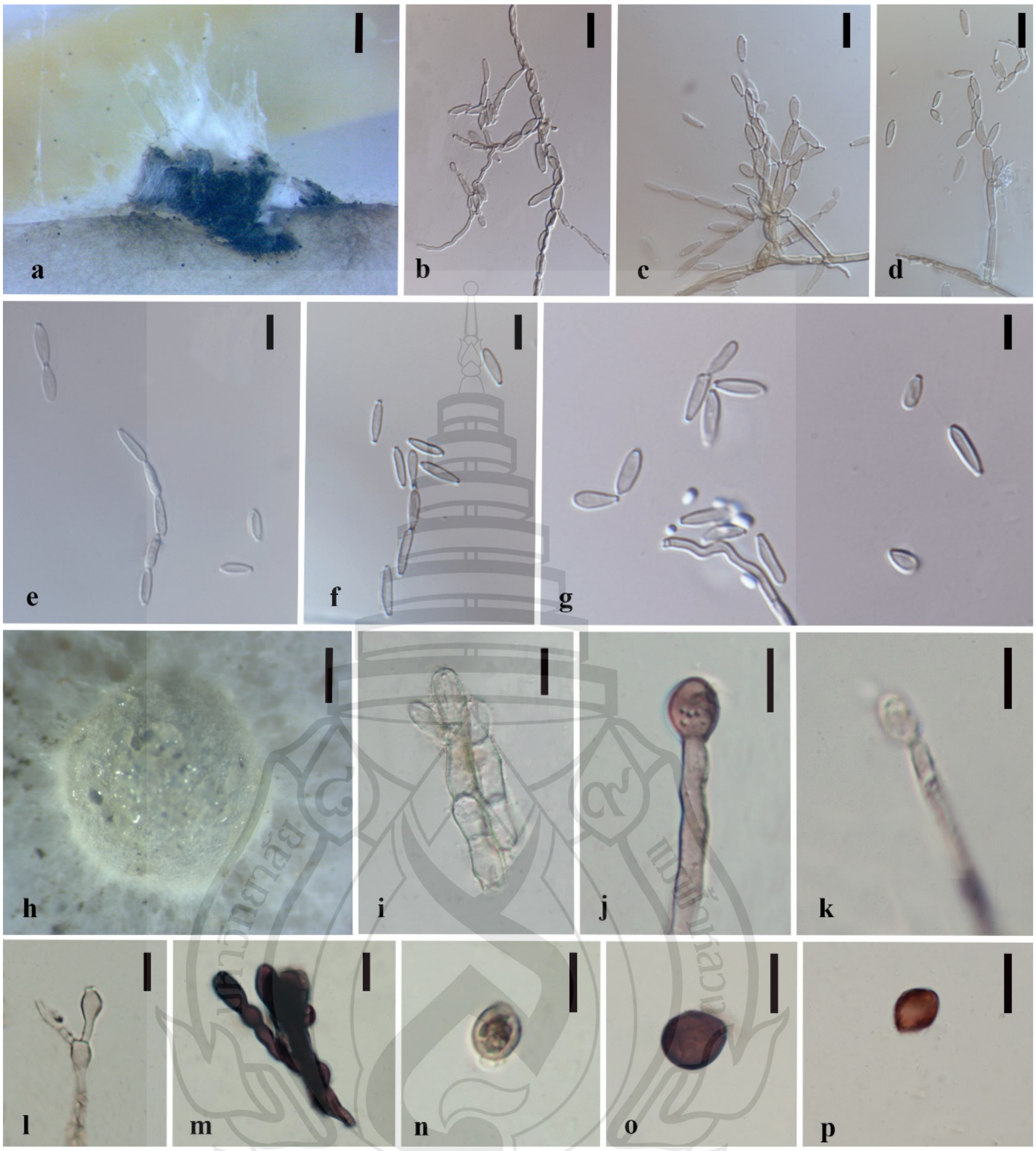
Xylaria berteri (Mont.) Cooke ex J.D. Rogers & Y.M. Ju, N. Amer. Fung., 2012, 7(9): 18. Figure 7.20 and Fig. 7.4, H

Culture characteristics – colonies on PDA superficial, white, velvety or cottony, stacked, lobbed or entire or undulate edge; reverse white to brown. Growth rate: 4 mm/day.

On PDA, vegetative hyphae 2.7–4.8 μm, hyaline to brown, septate, smooth, branched, thick-walled. Conidiomata superficial, erect through the media, globose, with flattened apex, white to light brown. Conidiophores 4.4 μm diam., cylindrical to clavate, hyaline to brown, branched or unbranched, smooth, sometimes laterally compressed into a tight layer. Conidia hyaline to brown, ovoid to ellipsoid, with granular contents, smooth-walled, thick-walled, with a flattened basal scar.

On WA ($1m^2$ WA cube on slide culture), vegetative hyphae 0.8–2.5 µm, hyaline to light brown, septate, smooth. Conidiomata semi-immersed, erect from media, irregular, surrounded by white mycelia, with concave ostioles. Conidiophores string-like and made from a bunch of consecutive fusiform cells, hyaline to light brown, branched, smooth. Conidia 8–12 × 2–4 µm ($\bar{x} = 10 \times 3$ µm, n=20), hyaline, ellipsoidal to fusiform, produced at the tip of conidiophore or laterally, single or percurrent, with one round end and blunt base, smooth-walled, another end truncated with flat basal abscission scar, thick-walled.

Notes – *Xylaria berteri* (strains MFLUCC 21-0061, MFLUCC 14-0095, MFLUCC 14-0102, MFLUCC 14-0110, MFLUCC 14-0117, MFLUCC 14-0126, MFLUCC 14-0143, MFLUCC 14-0150, MFLUCC 14-0158) were isolated from roots, stems and leaves of *Dendrobium cariniferum*, *D. harveyanum* and *Dendrobium* spp. collected from northern Thailand. All strains have identical sequences. The blast results of each region indicate that *X. berteri* is the best match species (Sequence similarity 99–100%). Based on these, we identified these strains as the species *X. berteri.*



Note a Conidiomata. b–d Conidiophores with conidia. e–g Conidia. h Conidiomata. i–m Conidiophore-like structures. n–p Conidia-like structures. Notes: a–g on WA, slide culture, h–p on PDA. Scale bars: a=200 µm, b–g=10 µm, h=500 µm, i–p=10 µm.

Figure 7.20 *Xylaria berteri* (MFLUCC 14-0095)

Xylaria curta Fr., Nova Acta Regiae Societatis Scientiarum Upsaliensis Ser. 3, 1: 126 (1851). Fig. 7.4, G

Culture characteristics – colonies on PDA superficial, white, cottony aerial hyphae and brown concentric rings in the centre, leathery outside the centre, radial with bunched mycelia, denticulate edge; reverse light brown. Growth rate: 5 mm/day

Notes – As a fungal endophyte, *Xylaria curta* was isolated from the roots of *Dendrobium aphyllum* and *D. chrysanthum* (Chen et al., 2013). Although GZAC O36L23 and 92092022 (HAST) have 7.1% of distinct ITS base pairs with 1.3% and 1.1% of sequence differences in TUB2 and RPB2 respectively, we tend to identify GZAC O36L23 as *Xylaria curta* due to the phylogenetic placement and scanty morphology. The ITS blast results showed *Xylaria* cf. *curta* (K.-L. Chen L148) was the best match species with 94.2% sequence similarity. *Xylaria* cf. *curta* (K.-L. Chen, L148) represents an endophyte isolated from leaves of lotus (Chen & Kirschner, 2018). However, the classification of *Xylaria* cf. *curta* is not clear. TUB2 and RPB2 gene sequence blast search indicate that *X. curta* is the most similar species to GZAC O36L23 (Sequence similarity 93%–99%). We speculate that GZAC O36L23 might be an intraspecific variant of *Xylaria curta*.

Xylaria feejeensis (Berk.) Fr., Nova Acta Regiae Societatis Scientiarum Upsaliensis Ser. 3, 1: 128 (1851). Fig. 7.4, E

Culture characteristics – colonies on PDA superficial, white, velvety, radial, zonate with several concentric circles, entire edge; reverse unevenly white to light brown. Vegetative hyphae septate, branched, hyaline, thick-walled. Growth rate (on PDA): 6mm/day.

Notes – *Xylaria feejeensis* is an endophyte isolated from roots of *Dendrobium fimbriatum* and *D. crystallinum* (Chen et al. 2013). In this study, *Xylaria feejeensis* (GZAC O30S21) was isolated from stems of *Dendrobium aurantiacum* Rchb. f. var. *denneanum* sampled in Xingyi City, Guizhou Province in southwestern China. It has an identical ITS sequence as JDR 180. The blast results show that the most similar species is *X. feejeensis* (Sequence similarity 97–99%).

Xylaria laevis Lloyd, Xylaria notes (7): 8 (1918) Fig. 7.4, F

Culture characteristic – colonies on PDA superficial, white, velvety, radial, zonate with several concentric circles, entire edge; reverse white, brown around some marginal part. Vegetative hyphae septate, branched, hyaline, thick-walled. Growth rate: 4.5mm/day.

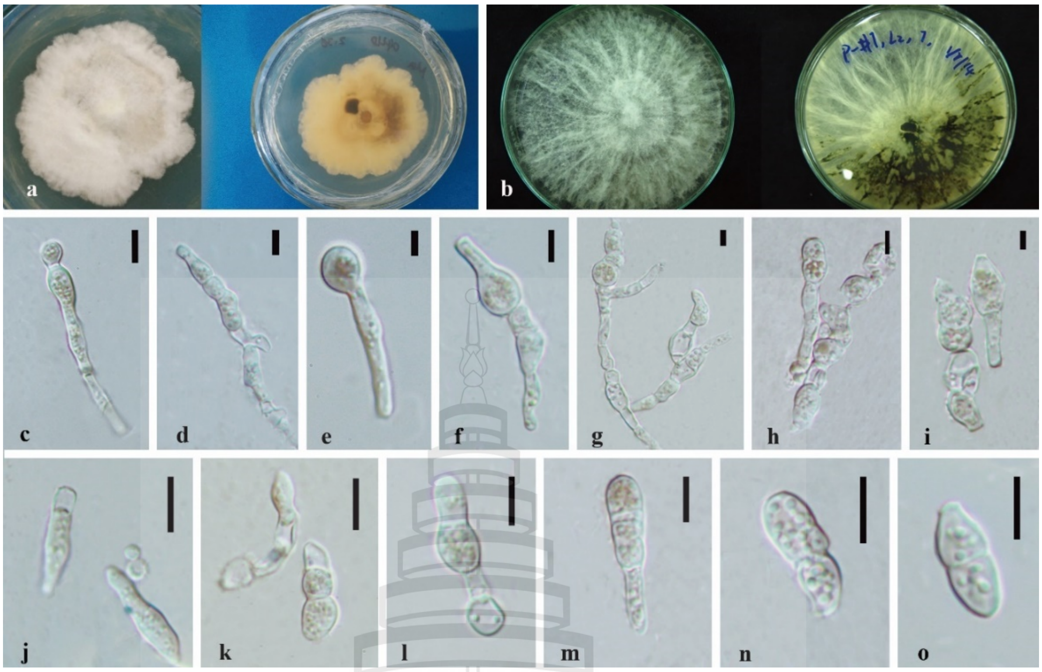
Notes – Two target strains GZAC O33L12 and GZAC O6LA2 were isolated from leaves of *Dendrobium aurantiacum* and *D. catenatum* sampled in Xingyi City, Guizhou Province in southwestern China. The gene blast search showed that *Xylaria laevis* was their best match species. (Sequence similarity 95–98%). Despite their gene sequences disparity between the two isolates, due to lacking further evidence, both of them are identified as *Xylaria laevis*.

Xylaria grammica (Mont.) Mont., Annales des Sciences Naturelles Botanique sér. 4, 3: 108 (1855). Figure 7.21

Culture characteristics – colonies on PDA superficial, white to light brown, zonate with concentric circle, with velvety to cottony, radial, entire or slightly undulate edge; reverse white to dark brown. Growth rate: 6 mm/day.

Description – Vegetative hyphae 2–3.6 µm, hyaline to brown, septate, smooth, branched, thick-walled. Conidiophores-like structures 3.2–5.1 µm wide, cylindrical, hyaline, branched, septate. Conidiogenous cell cylindrical, holoblastic. Conidia-like structures 13.5–32.5 × 5–8 µm ($\bar{x}$ = 19.5 × 6.7 µm, n=10), hyaline to brown, ellipsoid or pyriform to the gourd, septate, one end rounded and the other truncate.

Notes – *Xylaria grammica* has been isolated as endophyte from the root of *Dendrobium aphyllum*, *D. nobile*, *D. chrysanthum*, *D. chrysotoxum*, *D. crystallinum* and *D. fimbriatum* (Chen et al. 2012, 2013). In this study, *Xylaria grammica* (MFLUCC 14-0093 and MFLUCC 14-0146) were isolated from leaves of *Dendrobium* sp. collected from northern Thailand. The mononematous conidiophores and conidia with abscission scar resemble the asexual morph of *Xylaria* but conidia are much larger with more variant shapes (Rodrigues et al., 1993). They have identical sequences as *Xylaria grammica* (Strains BCC 1002 and 5228) and all cluster with well-supported values (100% ML/1.0BPP). The blast results for the two strains showed that *Xylaria grammica* has the highest sequence similarity 99–100%.



Note a MFLUCC 14-0093 colony on PDA (left-front view, right-reverse view). b MFLUCC 14-0146 colony on PDA (left-front view, right-reverse view). c–i Conidiophores with conidia-like structures. j–o Conidia-like structures. Scale bars: c–i=5 µm, j–o=10 µm. Note: c–o on WA of slide culture.

Figure 7.21 *Xylaria grammica* (MFLUCC 14-0093 and MFLUCC 14-0146)

Xylaria papulis Lloyd, Mycological Writings 6 (65): 1055 (1920) Fig. 7.4, F

Culture characteristics – colonies on PDA superficial, white, velvety in the centre, leathery outside the centre, radial with bunched mycelia, one concentric circle, denticulate edge; reverse light brown. Vegetative hyphae septate, branched, hyaline, thick-walled. Growth rate: 4.2 mm/day.

Notes – *Xylaria populis* GZAC O32S24 was isolated from the stems of *Dendrobium chrysotoxum* collected from Luodian County, Guizhou Province, southwestern China. *Xylaria papulis* 5118 as an endophyte was isolated from the roots of *Dendrobium aphyllum*, *D. chrysotoxum* and *D. fimbriatum* (Chen et al., 2013). In view that the isolate GZAC O32S24 is identified as *Xylaria papulis* with only ITS sequence in the polyphasic analysis, it may not be a stable result. Ex-type of *Xylaria* *hongkongensis* GDGM 40058 has less than 0.5% ITS sequence discrepancy with *Xylaria papulis* 5118. Therefore, *Xylaria hongkongensis* GDGM 40058 perhaps need to be re-evaluated with further gene sequences.

Xylaria venosula Speg., Boletín de la Academia Nacional de Ciencias en Córdoba 11 (4): 511 (1889). Figure 7.22

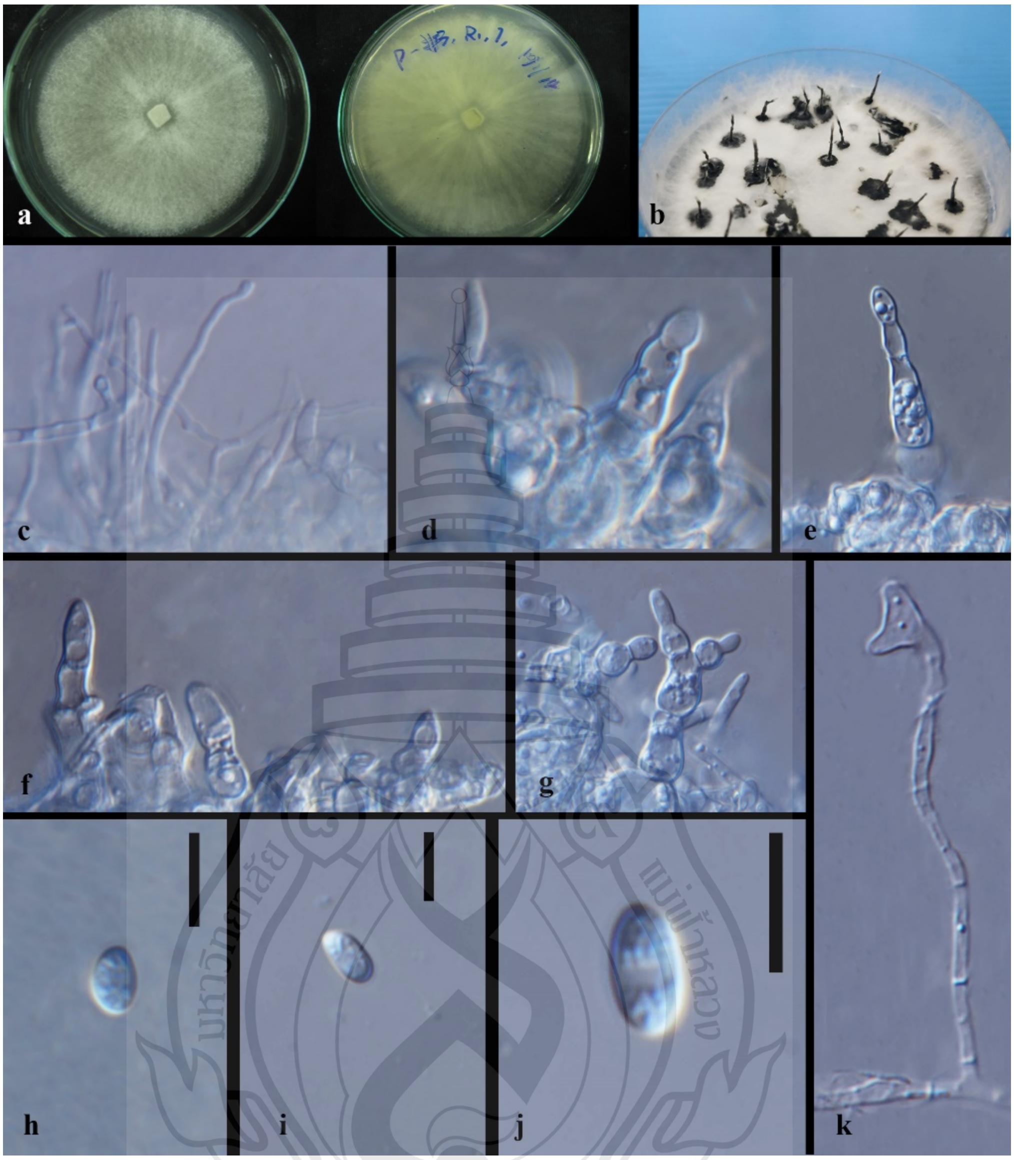
= *Xylaria arbuscula* CBS 126415, CBS 126416

= *Xylaria apiculata* EF6

Culture characteristics – colonies on PDA superficial, white to light yellow, velvety, entire or slightly undulate edge; reverse light brown to dark brown. Vegetative hyphae septate, branched, hyaline, thick-walled. Growth rate: 4.3 mm/day. Colonies on MEA white, velvety, entire edge, with 1–2 cm high charcoal stroma, stroma cylindrical with a white top; reverse white with black spots. Growth rate: 4.5 mm/day. Stroma formed after 2 months.

On 4% OA, Vegetative hyphae septate, branched, hyaline, thick-walled. Conidiophore hyaline, palisades, mononematous, smooth, thick-walled. Conidia 3–5 × 2–4 µm ($\bar{x}$ = 5.8 × 3.3 µm, n=10), hyaline, globose to ellipsoid, guttules, smooth-walled, thick-walled. Appressoria-like structure rising from mycelia, crooked, septate, with crescent head.

Notes – *Xylaria venosula* were isolated as endophytes from the root of *Dendrobium nobile* (Chen et al., 2012, 2013). In the present study, it was isolated from roots and stems of *Dendrobium fimbriatum, D. primulinum* and *Dendrobium* sp. *Xylaria venosula, X. arbuscula* and *X. apiculate* formed a monophyletic group with robust support (100% ML/1.0BPP). *Xylaria arbuscula* (CBS 126415 and CBS 126416) were isolated from the branch of *Quercus* and *Robinia* in fern greenhouse (Brandenburg, Germany) respectively (Fournier et al., 2011). *Xylaria apiculata* EF6 as an endolichenic strain was isolated and identified with ITS sequence from the lichen *Pyxine petricola* in India (Poornima et al. 2017). All nine strains in this group have very similar sequences of each gene with only 0.35%–2.5% base pairs differences. Few morphological information is available for comparison. Therefore, we regard all of them as *Xylaria venosula*.



Note a Coloy on PDA (MFLUCC 14-0114) (left-front view, right-reverse view). b Stroma on 2% MAE. c–g Conidiophores with conidia. h–j Conidia. k Appressoria-like structure. Notes: c–k on 4% OA. Scale bars: h–j=5 µm.

Figure 7.22 *Xylaria venosula* (MFLUCC 14-0114)

7.3.4 Cytotoxic Activity of *Biscogniauxia* Isolates

The ethyl acetate crude extracts of *Biscogniauxia petrensis* (MFLUCC 14-0151) was found to hold a dose-dependent inhibitory activity towards both A549 and K562 cell lines *in vitro* (As showed in Tables 4 and 5). The IC_{50} of EA crude extract from this strain are 13.5 µg/ml and 4 µg/ml in A549 and K562 cytotoxic assay respectively while that of the positive drug is 2.38 µg/ml and 5.1 µg/ml. The MeOH crude extracts of MFLUCC 14-0151 display inhibitory activity to K562 only when the concentration was up to 100 µg/ml. Other solvent crude extracts in this study showed no effect on the proliferation of the two cell lines.

Table 7.5 A549 cytotoxic assay of ethyl acetate and methanol extracts from *Biscogniauxia petrensis* MFLUCC 14-0151

A549 inhibitory rate %	**Concentration (µg/ml)**						**IC50 (µg/ml)**
	100	**58**	**5.8**	**2.9**	**1.45**	**0.725**	
EA of MFLUCC 14-0151	54.9±8%	58.8±5.8%	49.5±4.7%	49±5.9%	25.5±6.7%	25.1±6.2%	13.5
MeOH of MFLUCC 14-0151	45.50%	–	–	–	–	–	–
Doxorubicin	–	87.1±1.7%	75.9±4.1%	57.3±7.6%	41.0±8.7%	23.3±17.9%	2.38

Table 7.6 K562 cytotoxic assay of ethyl acetate and methanol extracts from *Biscogniauxia petrensis* MFLUC 14-0151

K562 inhibitory rate %	**Concentration (µg/ml)**						**IC50 (µg/ml)**
	100	**58**	**5.8**	**2.9**	**1.45**	**0.725**	
EA of MFLUCC 14-0151	81.9±6.2%	69.2±2.4%	49.9±9%	37.9±4.6%	42.1±9.7%	50.2±8%	4
MeOH of MFLUCC 14-0151	75.8±5.5%	–	–	–	–	–	–
Doxorubicin	–	77.5±9%	66.5±2.7%	41.4±5.4%	40.4±3.9%	39.9±4.6%	5.1

7.4 Discussion

7.4.1 Xylariaceous Endophyte Associated with *Dendrobium*

From this study, each *Dendrobium* species is associated with two different Xylarialean species on average (exclude the unidentified taxa). The actual numbers would be higher considering the unidentified hosts. *Xylaria* is the most frequently isolated Xylariaceous genera associated with *Dendrobium* followed by *Hypoxylon* and *Nemania. Hypoxylon* and *Xylaria* endophyte mostly occurred on angiosperms and a few gymnosperms mainly distributed in pantropical areas (Davis et al., 2003; Ezra et al., 2004; Bills et al., 2012; Ikeda et al., 2014; Daranagama et al., 2018). *Hypoxylon investiens*, *Nemania diffusa, Nigrospora* species, *Xylaria cubensis*, *Xylaria curta*, *X. feejeensis*, *X. grammica* and *X. venosula* are common fungal endophytes in orchids and most of them were identified by molecular analysis (Bayman et al., 1997; Okane et al., 2008; Yuan et al., 2009; Chen et al., 2011, 2013; Jiang et al., 2011; Sawmya et al., 2013). *Hypoxylon officinalis* and *Xylaria venosula* were isolated from both China and Thailand. Three new hosts were recorded for *Xylaria venosula* in this study. It is noteworthy that eight novel taxa with one cryptic species (Hypoxylaceae sp.) were introduced from forty-seven strains, which implied that fungal endophyte contain many unrecognized or cryptic species. Many xylarialean endophytes from various hosts in the USA have been inferred as novel species with multi-locus (U'Ren et al., 2016). Due to all novel taxa are far away from the species of *Xylaria sensu stricto* (*X. hypoxylon* aggregate, Figure 2), we defined them as *Xylaria sensu lato*.

Although with the limited sample size and scope, we speculate that *Dendrobium* species associated with highly diverse Xylariales, especially species of *Hypoxylon* and *Xylaria*. The result coincides with that of Chen et al. (2013) who investigated 217 xylariaceous strains from seven medical *Dendrobium* species with multi-gene phylogenetic analysis in China. *Xylaria grammica* is the most isolated taxon from the roots of *Dendrobium* in southwestern China (Chen et al., 2013). Our results showed that the penzigioid species *Xylaria berteri* was repeatedly isolated from various *Dendrobium* organs in northern Thailand while most Hypoxylaceae strains were from Chinese *Dendrobium*. As endophyte, *Xylaria berteri* has been often recoded from tropical area such as Brazil, Hawaiian Islands, Mexico and Panama (Carmona et al., 2009; Roger & Zhu, 2012; Chacón & González, 2019). *Hypoxylon officinalis* was introduced based on the traits of three strains from roots of different *Dendrobium* so that we speculated *Hypoxylon officinalis* could be an organ specificity species. However, the organ, climatic or geographic preference in Xylariales endophyte needs further investigation in a wide range (Hsieh et al., 2010).

7.4.2 The Dilemma for *Xylaria* Endophyte Verification

Xylariaceae has been revised for several times (Daranagama et al., 2016; Wendt et al., 2018). The best investigated *Xylaria* way is to combine the traditional morphological concepts based on the type material with multi-gene sequence analysis (Wendt et al., 2018; Lambert et al., 2019). However, many old but well-defined *Xylaria* species lack type material and sequence data (Hsieh et al., 2009; Daranagama et al., 2018). Few reports relate to the *Xylaria* asexual morph. In addition, a considerable number of *Xylaria* species produce sterile mycelia only and reproduction rarely occurs on artificial media (Hsieh et al., 2005; Chen et al., 2013). Altogether these make the *Xylaria* endophyte species resolution very difficult (Petrini, 1985; Rodrigues et al., 1993; U'Ren et al., 2016; Wendt et al., 2018). Therefore, a multi-locus phylogenetic analysis is the most reliable approach to know the taxonomic placement of *Xylaria* endophyte (Chen et al., 2013). Some cryptic *Xylaria* species have been found (Davis et al., 2003; Chen et al., 2013; U'Ren et al., 2016). In this study, most *Xylaria* species identification mainly depends on molecular phylogenetic analysis. We could present only limited morphological characterization with asexual morph sporulated on 4% OA and little available sexual morph record for comparison. Besides type (and ex-type) assignment, sporulation for endophyte such as *Xylaria* is always encouraged (Doilom et al., 2017). More attention should be paid to *Xylaria* asexual morph, which link endophyte with sexual morph of known species and contribute to decipher its genealogy with more novel even cryptic species (Hyde & Soytong, 2008). A breakthrough is needed in *Xylaria* asexual morph study, and any pertaining characteristics should be recorded for recognition, comparison and discussion even though they would be criticized and finally wrong. We recommend use OA with low concentrations under alternative fluorescent light to sporulate *Xylaria* endophyte concerning more and more studies successfully sporulate *Xylaria* species with it (Rogers et al., 1984; Rodrigues et al., 1993; Okane & Nakagiri, 2007). Three new species *Xylaria insolita*, *X. necrophora* and *X. subescharoidea* have been introduced recently with asexual morph sporulated on OA (Hsieh et al., 2020, Garcia-Aroca et al., 2021).

7.4.3 HTS Application Fungal Endophyte

Although multi-gene markers have been employed for further resolving many taxonomic groups, traditional DNA-sequencing method allows only sequencing specimens individually (Stranneheim & Lundeberg, 2012). The next-generation sequencing technologies (NGS) make it possible to sequence mixture environmental bulk samples (Goodwin et al., 2016). The production of tens of thousands genetic reads in parallel contributes to understanding historical, functional and ecological biodiversity (Shokralla et al., 2012; Wu et al., 2019). High-throughput amplicon sequencing (HTS) application to fungal endophyte reveals some sympatric cryptic species, which discovered taxa that could not grow on artificial media (Razgour et al., 2011; Liu et al., 2017; Johnston et al., 2017). The fungal endophyte taxonomic composition test of *Dendrobium catenatum* implemented by high-throughput results (HTS) ITS-rRNA metagenomics analysis showed a very low frequency which perhaps owing to one designed pair of primers were not enough for all endophytes (Liu et al., 2017). Meanwhile, the HTS often produce false negative or erroneous results and its application normally requires separated genomic reads into operational taxonomic units (OTUs), which is feasible to discern environmental DNA to generic levels but loss information on intraspecific diversity (Shokralla et al., 2012). Therefore, the species identification for fungal endophytes is necessary for discovering the real taxonomic placements and ecological strategies (Promputtha et al., 2005; Rodriguez et al., 2009; Ma et al., 2018). Several advanced metabarcoding strategies and platforms have been introduced to achieve species identification in dietary, gut microbiome and wildlife forensic species detection, which is likely to be used for more effective and exact fungal endophytes identification (Staats et al., 2016). According to the results, *Hypoxylon* and *Xylaria* species take accounts of the majority (29% and 51%) of all xylariaceous species. The fungal endophyte taxonomic composition test of *Dendrobium catenatum* implemented by high-throughput results (HTS) ITS-rRNA metagenomics analysis showed a very low Xylariales frequency which perhaps owing to one designed pair of primers were not enough for all endophytes (Liu et al., 2017).

7.4.4 Potential Roles of Xylariaceous Endophyte

In this study, several species were initially found as pathogens. Most grasses and reeds perhaps harbour species of *Apiospora* endophyte (Crous & Groenewald, 2013). *Apiospora* (=*Arthrinium*) sp. was also reported to be associated with *Dendrobium candidum* and *D. nobile* (Chen et al., 2011, 2012). *Nigrospora sphaerica* is an opportunistic pathogen causing onychomycosis in humans and can cause leaf blight on *Camellia sinensis* (Liu et al., 2016; Dutta et al., 2015; Wang et al., 2017). *Nigrospora chinensis* is a common pathogen that has been reported from *Ginkgo biloba* (Lee et al., 2019). *Nigrospora* as a ubiquitous endophyte, plant and human pathogens, have been found in several orchids including *Bulbophyllum neilgherense*, *Dendrobium candidum* and *Vanda testacea* (Chen et al., 2011; Sudheep & Sridhar, 2012). The occurrence of the appressoria-like structure found in the 4% of OA culture of *Xylaria venosula* suggests this species could be invasive in the germination period. These fungal endophytes probably become potential pathogens or saprobes involving senescence during later life (Whalley, 1996; Promputtha et al., 2007; Eaton et al., 2011). It is reported that some volatile organic compounds isolated from Xylariales species can cause pest larval mortality (Ondeyka et al., 1997; Bills et al., 2012; Wang et al., 2018). For further understanding xylarialean endophyte, an optimized methodology is needed on the taxonomy, physiology and functional roles.

7.4.5 *Biscogniauxia* Discussion

This is first time to report that *Dendrobium* orchids associated with *Biscogniauxia* species. *Biscogniauxia* fungi is found to be commonly occurring in natural environment (Ju, 1998, 2001; Raimondo et al., 2016). They are known to be charcoal latent invaders and expected to spread in areas of the drought intensify forests (Nugent et al., 2005; Henriques et al., 2016). The new species *B. dendrobii* is proposed by the largely sequences differences (>4%) and different shape as well as size of conidia according to the recommendations in new fungal species definition by Jeewon and Hyde (2016). The topology of phylogenetic Biscogniauxia tree in current study is congruent to those recovered in other publications although different multi-loci combinations were used (Raimondo et al., 2016; Zhang et al., 2017; Daranagama et al., 2018). There is no contradiction between morphology and phylogenetic analysis in this study.

The *Biscogniauxia* fungi are phylogenetically close to *Obolarina* and *Camillea* but distinguished by the more complex stromatal anatomy, ascospore morphology and the xylocladium-like conidiogenous structures (Wendt et al., 2018). Both endophytic and pathogenic *Biscogniauxia* species were found in oak trees (Collado et al., 2001; Granata & Sidoti, 2004). Their endophytic phase was found to be favored by a decrease in host water potential (Vannini et al., 2009). Reverse transcription polymerase chain reaction (RT-PCR) has been proposed to quickly detect the endophytic (regarded as latent pathogens) Biscogniauxia fungi in inner tissues (Mazzaglia et al., 2001; Luchi et al., 2005; Safaee et al., 2017). As fungal endophytes, *Biscogniauxia* sp. was isolated from *Bulbophyllum exiguum* (Orchidaceae) in Australia (Calvert, 2017).

This is first report of A549 and K562 cytotoxicity of endophytic *Biscogniauxia* petrensis from *Dendrobium* orchids. The results demonstrate that the ethyl acetate of MFLUCC 14-0151 possess moderate and strong cytotoxic activity towards A549 and K562 cell lines respectively. The IC50 in K562 assay surpassed the positive drug (4µg/ml vs. 5.1 µg/ml), which indicated stronger cytotoxic activity. Therefore, it is interesting to investigate the components of the solid fermentation residues of this strain in the future. Some new or bioactive secondary metabolites such as biscogniauxone, biscopyran and dimericbiscognienyne were reported in this group of fungi (Evidente et al., 2005; Wu et al., 2016; Zhou et al., 2016; Nguyen et al., 2017, 2018).

Supplementary tables:

Table 7.7 The morphology of *Biscogniauxia* species

Species	**Conidiophores**	**Conidiogenous cell**	**Conidia**	**Stromata**	**Asci**	**Ascospores**	**Reference**
B. anceps	Hyaline, becoming brown and minutely roughened to coarsely warted, up to at least 700 µm high, 6 µm broad, branching asymmetrically to verticillately	Holoblastic, cylindrical to cylindric-clavate 7–40 × 3–5 µm, becoming distended following abundant conidial production	Hyaline, subglobose to ellipsoidal, smooth, rounded at apices, with flattened bases	Applanate, discoid to widespreading, up to 1 rnm thick, with distinct margins; perithecia 0.2–0.4 mrn diam; ostioles umbilicate, located in grey depressed areas.	8-spored, short stipitate, 110– 140 µm long, 8–11 µm broad, with apical ring bluing in Melzer's reagent, plate-like to almost cuboid	Unequally two-celled, mostly hyaline, smooth, ellipsoid to ellipsoid-inequilateral to obovate, (13–)14–18(–19) µm long	Roger et al. (1996)
B. arima	Pale brownish, roughened, di- or trichotomously branched, 4–5 µm diam with two to three conidiogenous cells arising from each terminus.	Holoblastic, cylindrical, hyaline, smooth, 15–26 × 3–4 µm, bearing denticulate conidial secession scars.	Hyaline, ellipsoid to short cylindrical, smooth, 4.5–8 × 2.5–3 µm, with flattened bases	Applanate, 1.2 cm long × 0.5 cm broad × 0.7 mm thick, distinct margins, internal carbonaceous; perithecia obovoid, 0.2–0.3 mrn diam × 0.5–0.6 mm high; ostioles higher than stromatal surface, with openings papillate	65–75 µm long × 5–5.5 µm broad, with apical ring bluing in Melzer's iodine reagent, discoid, 0.2 µm high × 1.5 µm broad	Light brown, unicellular, ellipsoid, nearly equilateral, with broadly rounded ends, smooth, 7.5– 9 × 3–4 µm, lacking germ slit	Ju and Rogers (1998)
B.atropuncta ta	Hyaline to yellowish, smooth, borne on aerial hyphae, composed of a main axis, 2.5–4 µm broad, one or more major branches, terminating with 2–3 conidiogenous cells	Ovate to short cylindrical, hyaline, smooth, 5–10 × 3.5–4.5 µm, bearing poriod conidical secession scars	Hyaline, smooth, globose to subglobose, 4–5.5 × 3–4.5 µm, with flattened base	Applanate, 2–50 cm long × 2– 25 cm broad × 0.4–0.7 mm thick, internal carbonaceous; perithecia obovoid, 0.2–0.3 mm diam × 0.3–0.5 mm high; ostioles with openings papillate	150–170 µm long × 16–18 µm broad, with apical ring bluning in Melzer's iodine reagent, discoid	Ellipsoid, nearly equilateral, smooth, brown to dark brown, unicellular, (20–)23– 30 × 11.5– 14.5 µm, with narrowly rounded ends and straight germ slit	Ju et al. (2001)

Table 7.7 (continued)

Species	Conidiophores	Conidiogenous cell	Conidia	Stromata	Asci	Ascospores	Reference
B.atropunctata var.*intermedia*	Same with *B atropunctata*	Same with *B. atropunctata*	Hyaline, smooth, globose to subglobose, ellipsoid, 5–11 × 3.5–4.5 µm, with flattened base	Same with *B. atropunctata*	Same with *B. atropunctata*	Ellipsoid, nearly equilateral, smooth, brown to dark brown, unicellular, (20–)23–30 × 11.5– 14.5 µm, with narrowly rounded ends and straight germ slit	Ju and Rogers (1998)
B.bartholomaei	Hyaline, roughed, unbranched or dichotomously branched, 3.5–4.5 µm broad, with 1–2 conidiogenous cell from each terminus	Holoblastic, cylindrical, hyaline, smooth, roughed, 18–27 × 3–3.5 µm, bearing poroid conidial secession scars	Hyaline, smooth, ellipsoid to short cylindrical, 6–10 × 3–4 µm, with flattened base	Applanate, 0.8–3 cm long × 0.4–1.1 cm broad × 0.6–0.8 mm thick, internal carbonaceous; petithecia obovoid; Ostioles with openings punctate	180–200 µm long × 11–13 µm broad, with pical ring bluning in Melzer's iodine reagent, discoid	Dark brown, unicellular, ellipsoid, equilateral to slightly inequilateral, with narrowly rounded ends, smooth, 17.5–22(–25) × (8–)9–11(–12) µm with straight germ slit spore-length	Ju and Rogers (1998)
B.capnodes	Yellowish to brownnish, roughed, composed of a main axis, 3–5 µm, 1 or more major branches	Holoblastic, cylindrical, hyaline to yellowish, roughed, 8–14 × 3.5–5 µm, bearing poroid conidial secession scars	Hyaline, smooth, ellipsoid, 5–7.5 × 2.5–3.3 µm, with flattened base	Applanate, 0.5–13 cm long × 0.5– 5 cm broad × 0.5–1 mm thick, internal carbonaceous; perithecia obovoid to tubular, 0.2–0.4 mm × 0.4–0.8 mm high; ostioles with openings punctate and surrounded by slightly raised rim	70–115 µm long × 7–10 µm broad, with pical ring bluning in Melzer's iodine reagent, discoid	Brown to dark brown, unicellular, ellipsoid, nearly equilateral, with mostly narrowly rounded ends, smooth, 8.5–15 × 5–7.5 µm, with straight germ slit spore-length	Ju and Rogers (1998)

Table 7.7 (continued)

Species	Conidiophores	Conidiogenous cell	Conidia	Stromata	Asci	Ascospores	Reference
B.citriformis	Brownish, roughed, composed of a main axis, 4–5 µm broad, one or more major branches	Holoblastic, cylindrical, hyaline to yellowish, roughed, 8–16 × 3–5 µm, bearing poroid conidial secession scars	Hyaline smooth, ellipsoid, 5.7–7.5 × 1.5–2 µm, with flattened base	Applanate, 0.5–6 cm long × 0.5–5 cm broad × 0.7–1.2 mm thick, internal carbonaceous; perithecis obovoid to tubular; ostioles with openings umbilicate	100–125 µm long × 6–7 µm broad, with pical ring bluning in Melzer's iodine reagent, discoid	Dark brown, unicellular, ellipsoid, inequilateral, C-shape, with pinched ends, smooth, 10.5–12.5 × 5.5–7.5 µm with straight broad, faint germ slit spore-length	Ju and Rogers (1998)
B.cylindrispora	Hyaline to yellowish, roughened, composed of a main axis, 3.5–5 µm broad, one or more major branches, mostly terminating with 3–5 conidiogenous cells	Holoblastic, cylindrical, hyaline to yellowish, smooth to roughened, 8– 19 × 3.5–4.5 µm, bearing poroid conidial secession scars	Smooth, ellipsoid, obovoid to clavate, 5.5–9 × 2.5–3(–3.5) µm, with flattened base	Applanate, 0.3–2 cm long × 0.3–1.5 cm broad × 0.6–0.8 mm thick ; outer dehiscing layer dull brown, thin. Perithecia obovoid to tubular; ostioles lower than stromatal surface, with openings punctate	170–205 µm length × 15–18 µm broad, the spore-bearing parts 160–185 µm long, with apical ring bluing in Melzer's iodine reagent	Blackish brown, unicellular, cylindrical to ellipsoid, with broadly rounded ends, smooth, 20–26 × 10– 14(–16) µm, with straight germ slit spore-length or nearly so. Paraphyses and allophyses present	Ju et al. (2001)
B.dendrobii	Hyaline to brown, 2.4–4.2 µm diam,aseptate, cylindrical, thick-walled, composed of one main axis, one or more major branches, with conidiogenous cell arising terminally or laterally	Holoblastic, cylindrical with guttules, integrated, smooth, thick-walled, bearing poroid conidial secession scars	Hyaline, 11–45 × 2–4.5 µm, hyaline, cylindrical to clavate, with round or truncate apex, thick-walled, aseptate.	—	—	—	This study

Table 7.7 (continued)

Species	Conidiophores	Conidiogenous cell	Conidia	Stromata	Asci	Ascospores	Reference
B. formosana	Yellowish to brownish, roughened, composed of a main axis, 3.5–5 µm broad, one or more major branches, mostly terminating with 3–5 conidiogenous cells	Holoblastic, cylindrical to barrel-shaped, yellowish to brownish, roughened, 5–10(–13) × 3–5 µm, bearing poroid conidial secession scars	Hyaline, smooth, obovoid, 4.5–5.5 × 2.5–3.2 µm, with flattened base	Applanate, 0.4–3 cm long × 0.4–2.5 cm broad × 0.6–0.7 mm thick, internal carbonaceous, outer dehiscing layer dull brown, thin; perithecia obovoid to tubular, ostioles lower than stromatal surface, with openings punctate	160–200 µm length × 9–12 µm broad, the spore-bearing parts 150–170 µm long, with apical ring bluing in Melzer's iodine reagent.	Dark brown, unicellular, fusoid-ellipsoid-inequilateral, with narrowly rounded ends frequently pinched, smooth, (18–)19–24(–25) × 8.5–10 µm , with straight or slightly oblique germ slit spore-length or nearly so on less convex side	Ju et al. (2001)
B. granmoi	Similar to *B. nummularia*	Similar to *B. nummularia*	Similar to *B. nummularia*	Erumpent, 4 × 3 mm to 27 × 12 mm, typically ca. 10 × 9 mm, 1 mm thick, circular to irregular, internal carbonaceous, ostioles inconspicuous to conspicuous, Perithecia narrowly ovoid to cylindrical	8-spored, cylindrical, short stipitate, 119–142 × 7–8 5 µm, with apical ring dark bluing in Melzer's reagent, paraphyses filiform, unbranched	Slightly inequilateral-ellipsoid with broadly rounded ends, often more rounded at one end, translucent brown, uniseriate within ascus, 0-1–2 guttulate, germ slit full spore-length, conspicuous, typically on the more convex side, (10–)l1–13.5(–15) × (4.5–)5–6.5(–7) µm	Thomas et al. (1999)

Table 7.7 (continued)

Species	Conidiophores	Conidiogenous cell	Conidia	Stromata	Asci	Ascospores	Reference
B.latirima	Pale brownish, roughened, composed of a main axis, 3.5–5.5 µm broad, one or more major branches, mostly terminating with 3–5 conidiogenous cells	Holoblastic, cylindrical, hyaline to yellowish, smooth to roughened, 8–18 × 3.5–5 µm, bearing poroid conidial secession scars	Hyaline, smooth, ellipsoid, 7–9(–10.5) × 4–5 µm, with flattened base	Applanate, 0.3–2.5 cm diam × 0.6–0.8 mm thick, internal carbonaceous, outer dehiscing layer brown, thin; perithecia tubular; ostioles lower than stromatal surface, with openings punctate	155–180 µm length × 12–14 µm broad, the spore-bearing parts 140–160 µm long, with apical ring bluing in Melzer's iodine reagent	Dark brown to blackish brown, unicellular, ellipsoid-inequilateral, with one end broadly rounded and the other narrowly rounded and beaked, smooth, (16–)18–21.5 × 10–12 µm, with straight, 2 µm-broad germ slit slightly less than spore-length on more convex side	Ju et al. (2001)
B. marginata	Mononematous, with subverticillately arranged branches	Holoblastic, cylindrical, borne in loose whorls of 3–8, (18–)23 –25(–30) × 3.0–4.0 µm	Hyaline, smooth, ovoid to ellipsoid, (5–)5.5–7.0(–8) × 3–4 µm	Raised-discoid, with concave surface, 3.5–7 mm, diam × 2–3 mm thick, dark brown, internal carbonaceous; perithecia obovoid; ostioles with openings smooth to slightly punctate	170–200 µm total length × 12–14 µm broad, with apical ring bluing in Melzer's iodine reagent,discoid	Brown to dark brown, unicellular, subglobose, equilateral, with broadly rounded ends, smooth, 13–16.5 × 9.5–13.5 µm, with sigmoid germ slit spore-length	Callan and Rogers (1986); Vasilyeva et al. (2007)
B.mediterranea	Macro-nematous, mononematous, roughened, main axis erect, straight, cylindrical, septate, dominant towards the apex of the conidiophore	Polyblastic, terminal, 8–14.5 × 3.5–6µm, cylindrical, hyaline to pale brown, roughened, conidiogenous regions swollen at the apex and with conidial secession scar	Sympodially, 4.0–7.5 × 2–3.5 µm, ellipsoid to obovoid, hyaline, smooth, dry, rounded at the apex, base truncated with a small frill when detached	Applanate, 1.8–7 cm long × 0.5–4 cm broad × 0.6–1 mm thick, internal carbonaceous; perithecia obovoid to tubular, laterally flattened; ostioles with openings coarsely papillate	160–185 µm, long × 9–12.5 µm broad, with apical ring discoid, with staining light blue	Brown to dark brown, unicellular, ellipsoid, nearly equilateral, with narrowly to broadly rounded ends, smooth, 15.5–21 × (6.6–)7–10 µm, with straight germ slit spore-length	Collado et al. (2001); Ju et al. (1998)

Table 7.7 (continued)

Species	**Conidiophores**	**Conidiogenous cell**	**Conidia**	**Stromata**	**Asci**	**Ascospores**	**Reference**
B.nummularia	Inserted on a fawn coloured cortical layer, about 160 µm thick	—	—	With or without carbonaceous material, 0.3–2 cm long × 0.3–1.5 cm broad × 0.6–0.8 mm thick ; perithecia obovoid to tubular; ostioles lower than stromatal surface, with openings punctate	8-spored, cylindrical, short stipitate, persisten, with apical ring discoid, with staining light blue	Ellipsoid, or short fusoid, inequilateral, with acute narrowly rounded, or broadly rounded, ends, with straight or sigmoid germ slit spore pale brown to blackish brown, unicellular.	Ju and Rogers (1996, 1998)
B.petrensis	Hyaline to slightly yellowish, rough-walled, composed of main axis, 3–4.5 µm diam, one or more major branches, with conidiogenous cells arising terminally or laterally	Holoblastic, hyaline, swollen at the apex and with conidial secession scars, thin and rough-walled, cylindrical to oblong, 7–13 × 3–4.5 µm	Unicellular, hyaline, smooth, ovoid to clavate, 4.5–7.5 × 2.5–4.5 µm, with obtuse tip and acute truncated base	—	—	—	Zhang et al. (2017)
B.petrensis	Hyaline to slightly brownish, finely roughed, composed of main axis, one or more major branches, with conidiogenous cell arising terminally or laterally, 2.3–4.5 µm diam	Holoblastic, hyaline to light brownish, finely roughed, 11 ± 1.5 × 3.5 ± 0.8 µm, determinate, integrated, finely roughed, cylindrical to oblong, swollen at the apex with conidia secession scars	Hyaline to light brown, obovoid to clavate with obtuse or acute truncated apex, smooth-walled, 4 ± 0.8 × 2.5 ± 0.4 µm	—	—	—	This study

Table 7.7 (continued)

Species	Conidiophores	Conidiogenous cell	Conidia	Stromata	Asci	Ascospores	Reference
B.philippinensis var.*microspora*	Yellowish to brownish, roughened, composed of a main axis, 6–7.5 µm broad, one or more major branches, mostly terminating with 3–5 conidiogenous cells	Holoblastic, cylindrical, yellowish to brownish, roughened, 7–15 × 3–4.5 µm, bearing poroid conidial secession scars	Hyaline, smooth, obovoid, 5.5–7 × 3–4 µm, with flattened base indicating former point of attachment to conidiogenous cell	Applanate, 5–10 cm long × 2–5 cm broad × 1.5–2.5 mm thick, internal carbonaceous; perithecia tubular; ostioles with openings punctate, usually surrounded by slightly raised rim	190–220 µm long × 13.5–14.5 µm broad, with apical ring bluing in Melzer's iodine reagent	Dark brown to blackish brown, ellipsoid-inequilateral, with one end narrowly rounded and the other bearing a hyaline cellular appendage 2–3 µm long × 3.5–4 µm broad, 13.5-19 × 8-10 µm, with straight, broad faint germ slit spore-length	Ju and Rogers (1998, 2001)
B.repanda	Mononematous, with subverticillately arranged branches	Holoblastic, cylindrical, borne in loose whorls of 3 or more, (21–)23–28(–32) × 2.5–3.0 µm, sympodial	Hyaline, smooth, subglobose to ellipsoid, 4.0–6.0 × 2.0–2.5(–3.0)µm, with small, flat basal scars	Discrete, Internal carbonaceous, with blackened outer layer; papillate, black perithecial ostioles; and raised, armillate margins	Extremely reduced apical rings	Ellipsoid, with germ slits completely circumscribing the spore	Jong and Benjamin (1971); Callan and Rogers (1986)
B.rosacearum	Hyaline to brownish, smooth to roughened, dichotomously branched	Holoblastic, cylindrical, hyaline to broownish, smooth-roughed, (3.64–)6.39–5.30(–9.27) × (1.90–)2.49–2.69(–3.08) µm, bearing denticular conidial secession scars.	Hyaline, smooth, obovoid to clavate, (3.31–)5.72–6.26(–8.23) × (1.66–)2.17–2.32(–2.89) µm, with flattened base	Erumpent, internal carbonaceous, applanate, irregularly ovate, 10–90 mm long, 10–20mm wide, 0.5–1.5mm thick, perithecia dark brown, tubular, monostichous, minute, ostioles papillate with punctate ostiolar openings, paraphyses filiform, tapering, sparsely septate.	8-spored, cylindrical, uniseriate, short stipitate, with oily contents, (78.9–)88.8–94.2(–109.1) × (5.1–) 6.4–6.9(–8.6) µm, with apical ring bluing in Lugol's iodine reagent, discoid	Dark brown, unicellular, ascospores list equilateral with broadly rounded ends, smooth, (8.0–)9.2–9.8(–11.2) × (4.0–)5.0–5.4(–6.3) µm, envoloped by a hyaline sheath, germ slit straight, parallel-long axis of the spore	Raimondo et al. (2016)

Table 7.7 (continued)

Species	Conidiophores	Conidiogenous cell	Conidia	Stromata	Asci	Ascospores	Reference
B.simplicior	Pale brown, smooth or roughed, dichotomously branched, 3–3.5 µm, diam, with 2–3 conidiogenous cells arising from each terminus	Holoblastic, cylindrical, hyaline, smooth, 15–30 × 3–3.5 µm diam, bearing poroid conidial secession scars	Hyaline, smooth, ellipsoid to short cylindrical, 8–13 × 2.5–3 µm, with flattened base	Raised-discoid, with concave surface, 1–1.2 cm diam × 2.5–3 mm thick, dark brown, internal carbonaceous; perithecia tubular; ostioles with openings punctate	120–155 µm, long × 7-8 µm broad, with apical ring bluing in Melzer's iodine reagent, discoid	Brown, unicellular, ellipsoid to fusoid, nearly equilateral, with narrowly rounded ends, smooth, 13–17.5 × 5–6.5 µm, with straight germ slit spore-length	Ju and Rogers (1998)
B. uniapiculata	Brownish, roughed, composed of a main axis, 3.5–5.5 µm broad, one or more major branches	Holoblastic, cylindrical, hyaline to yellowish, roughed, 8–18 × 2.5–3.5 µm, bearing poroid conidial secession scars	Hyaline, smooth, ellipsoid to short cylindrical, (5.5–)6.5–7.5(–8) × (2–)2.5–3 µm, with flattened base	Applanate, 0.5–6 cm long × 0.5-4 cm broad × 0.4–0.7 mm thick, internal carbonaceous; perithecis obovoid; ostioles with openings punctate	75–130 µm long × 5.5–7 µm broad, with apical ring bluing in Melzer's iodine reagent, discoid	Brown, ellipsoid, inequilateral, with one end narrowly to broadly rounded and bearing a hyaline or pale brown cellular appendage 2–2.5 µm long × 2–3 µm broad, smooth, with straight erm slit spore-length	Ju and Rogers (1998)

Table 7.8 Strains used for phylogenetic tree construction in Apiosporaceae (*Arthrinium* and *Nigrospora*)

Species	Strain	GenBank Accession No.				Ref.
		ITS	LSU	TUB2	TEF1-α	
Apiospora aquaticum	**MFLU 18-1628**	MK828608	MK835806	–	–	Luo et al. (2019)
Apiospora arundinis	CBS 124788	KF144885	KF144929	KF144975	KF145017	Crous and Groenewald (2013)
	LC4951	KY494698	KY494774	KY705168	KY705097	Wang et al. (2018)
Apiospora aureum	**CBS 244.83**	AB220251	KF144935	KF144981	KF145023	Crous and Groenewald (2013)
Apiospora balearicum	**CBS 145129**	MK014869	MK014836	MK017975	–	Pintos et al. (2019)
Apiospora bambusae	**CGMCC 3.18335**	KY494718	KY494794	KY705186	KY806204	Wang et al. (2018)
	LC7107	KY494719	KY494795	KY705187	KY705117	Wang et al. (2018)
Apiospora bambusicola	**MFLUCC 20-0144**	MW173030	MW173087	–	MW183262	Tang et al. (2020)
Apiospora camelliae-sinensis	**CGMCC 3.18333**	KY494704	KY494780	KY705173	KY705103	Wang et al. (2018)
	LC8181	KY494761	KY494837	KY705229	KY705157	Wang et al. (2018)
Apiospora chinense	**CFCC 53036**	MK819291	–	MK818547	MK818545	Jiang et al. (2020)
Apiospora chromolaenae	**MFLUCC 17-1505**	MT214342	MT214436	–	–	Mapook et al. (2020)
Apiospora dendrobii	**MFLUCC 14-0152**	MZ463151	MZ463192			This study

Table 7.8 (continued)

Species	Strain	GenBank Accession No.				Ref.
		ITS	LSU	TUB2	TEF1-α	
Apiospora descalsii	**CBS 145130**	MK014870	MK014837	MK017976	–	Pintos et al. (2019)
Apiospora dichotomanthi	**CGMCC 3.18332**	KY494697	KY494773	KY705167	KY705096	Wang et al. (2018)
	LC8175	KY494755	KY494831	KY705223	KY705151	Wang et al. (2018)
Apiospora esporlense	**CBS 145136**	MK014878	MK014845	MK017983	MK017954	Pintos et al. (2019)
Apiospora euphorbiae	IMI 285638b	AB220241	AB220335	AB220288	–	Ogawa et al. (2005)
Apiospora gaoyouense	**CFCC 52301**	MH197124	–	MH236789	MH236793	Jiang et al. (2020)
Apiospora garethjonesii	KUMCC 16-0202	KY356086	KY356091	–	–	Dai et al. (2016)
Apiospora guizhouense	**CGMCC3.18334**	KY494709	KY494785	KY705178	KY705108	Wang et al. (2018)
	LC5318	KY494708	KY494784	KY705177	KY705107	Wang et al. (2018)
Arthrinium gutiae	**CBS 135835**	KR011352	MH877577	KR011350	KR011351	Kajale et al. (2015)
Apiospora hispanicum	**IMI 326877**	AB220242	AB220336	AB220289	–	Ogawa et al. (2005)
Apiospora hydei	**CBS 114990**	KF144890	KF144936	KF144982	KF145024	Crous and Groenewald (2013)
	LC7103	KY494715	KY494791	KY705183	KY705114	Crous and Groenewald (2013)
Apiospora hyphopodii	**MFLUCC 15-003**	KR069110	KY356093	–	–	Dai et al. (2016)
Apiospora hysterinum	ICMP 6889	MK014874	MK014841	MK017980	MK017951	Pintos et al. (2019)
Apiospora ibericum	**CBS 145137**	MK014879	MK014846	MK017984	–	Pintos et al. (2019)
Apiospora italicum	**AP221017**	MK014880	MK014847	MK017985	MK017956	Pintos et al. (2019)
Apiospora jatrophae	**MMI 00052**	JQ246355	–	–	–	Sharma et al. (2012)
Apiospora jiangxiense	**CGMCC 3.18381**	KY494693	KY494769	KY705163	KY705092	Wang et al. (2018)
	LC4578	KY494694	KY494770	KY705164	KY705093	Wang et al. (2018)
Apiospora kogelbergense	**CBS 113333**	KF144892	KF144938	KF144984	KF145026	Crous and Groenewald (2013)
	CBS 113332	KF144891	KF144937	KF144983	KF145025	Crous and Groenewald (2013)
Apiospora locuta-pollinis	**LC11683**	MF939595	–	MF939622	MF939616	Zhao et al. (2017)
Apiospora longistromum	**MFLUCC 11-0481**	KU940141	KU863129	–	–	Dai et al. (2016)
	MFLUCC 11-0479	KU940142	KU863130	–	–	Dai et al. (2016)
Apiospora malaysianum	**CBS 102053**	KF144896	KF144942	KF144988	KF145030	Crous and Groenewald (2013)
Apiospora marii	**CBS 497.90**	AB220252	KF144947	KF144993	KF145035	Ogawa et al. (2005)
Apiospora mediterranei	**IMI 326875**	AB220243	AB220337	AB220290	–	Ogawa et al. (2005)
Apiospora mytilomorphum	**DAOM 214595**	KY494685	–	–	–	Wang et al. (2018)
Apiospora neogarethjonesii	**KUMCC 18-0192**	MK070897	MK070898	–	–	Dai et al. (2019)
Apiospora neosubglobosum	**KUMCC 16-0203**	KY356090	KY356095	–	–	Dai et al. (2016)
	JHB006	KY356089	KY356094	–	–	Dai et al. (2016)
Apiospora obovatum	**CGMCC 3.18331**		–	–	–	Wang et al. (2018)
	LC8177	KY494757	KY494833	KY705226	KY705154	Wang et al. (2018)
Apiospora ovatum	**CBS 115042**	KF144903	KF144950	KF144995	KF145037	Crous and Groenewald (2013)
Apiospora paraphaeospermum	MFLUCC 13-0644	KX822128	KX822124	–	–	Senanayakeet al. (2016)
Apiospora phaeospermum	CBS 114314	KF144904	KF144951	KF144996	KF145038	Crous and Groenewald (2013)
Apiospora phaeospermum	CBS 114315	KF144905	KF144952	KF144997	KF145039	Crous and Groenewald (2013)
Apiospora phragmits	**CPC 18900**	KF144909	KF144956	KF145001	KF145043	Crous and Groenewald (2013)
Apiospora piptatheri	**CBS 145149**	MK014893	MK014860	–	–	Pintos et al. (2019)
Apiospora phyllostachium	**MFLUCC 18-1101**	MK351842	MH368077	MK291949	MK340918	Yang et al. (2019)

Table 7.8 (continued)

Species	Strain	GenBank Accession No.				Ref.
		ITS	**LSU**	**TUB2**	**TEF1-α**	
Apiospora pseudoparenchymaticum	**LC7234**	KY494743	KY494819	KY705211	KY705139	Wang et al. (2018)
	LC8173	KY494753	KY494829	KY705221	KY705149	Wang et al. (2018)
Apiospora pseudosinense	**CPC 21546**	KF144910	KF144957	–	KF145044	Crous and Groenewald (2013)
Apiospora pseudospegazzinii	**CBS 102052**	KF144911	KF144958	KF145002	KF145045	Crous and Groenewald (2013)
Apiospora pterospermum	**CPC 20193**	KF144913	KF144960	KF145004	KF145046	Crous and Groenewald (2013)
Apiospora qinlingense	**CFCC 52303**	MH197120	–	MH236791	MH236795	Jiang et al. (2020)
Apiospora rasikravindrae	**NFCCI 2144**	JF326454	–	–	–	Schoch et al. (2014)
	LC5449	KY494713	KY494789	KY705182	KY705112	Wang et al. (2018)
Apiospora sacchari	CBS 212.30	KF144916	AB220351	KF145005	KF145047	Crous and Groenewald (2013)
	CBS 301.49	KF144917	AB220352	KF145006	KF145048	Crous and Groenewald (2013)
Apiospora saccharicola	CBS 334.86	AB220257	KF144967	KF145010	KF145052	Crous and Groenewald (2013)
	CBS 463.83	KF144921	KF144968	KF145011	KF145053	Crous and Groenewald (2013)
Apiospora serenense	**IMI 326869**	AB220250	AB220344	AB220297	–	Ogawa et al. (2005)
Apiospora setostromum	**KUMCC 19-0217**	MN528012	MN528011	–	MN527357	Jiang et al. (2019)
Apiospora subglobosum	**MFLUCC 11-0397**	KR069112	KR069113	–	–	Senanayake et al. (2015)
Apiospora subroseum	**CGMCC3.18337**	KY494752	KY494828	KY705220	KY705148	Wang et al. (2018)
	LC7291	KY494751	KY494827	KY705219	KY705147	Wang et al. (2018)
Apiospora thailandicum	**MFLUCC 15-0202**	KU940145	KU863133	–	–	Dai et al. (2016)
	LC5630	KY494714	KY494790	KY806200	KY705113	Wang et al. (2018)
Apiospora vietnamensis	**IMI 99670**	KX986096	KX986111	KY019466	–	Wang et al. (2018)
Apiospora xenocordella	**CBS 478.86**	KF144925	–	KF145013	KF145055	Crous and Groenewald (2013)
Apiospora xenocordella	CBS 595.66	KF144926	KF144971	–	–	Crous and Groenewald (2013)
Apiospora yunnanum	**MFLUCC 15-0002**	KU940147	KU863135	–	–	Dai et al. (2016)
Arthrinium austriacum	GZU 345006	MW208929	MW208860	–	–	Pintos and Alvarado (2021)
Arthrinium caricicola	AP23518	MK014871	MK014838	MK017977	MK017948	Pintos et al. (2019)
Arthrinium crenatum	**CBS 146353**	MW208931	MW208861	MW221923	MW221917	Pintos and Alvarado (2021)
Arthrinium curvatum var. *curvatum*	AG191036	MW208935	MW208862	MW221924	–	Pintos and Alvarado (2021)
Arthrinium curvatum var. *minus*	AP25418	MK014872	MK014839	MK017978	MK017949	Pintos et al. (2019)
Arthrinium japonicum	IFO 31098	AB220264	AB220358	AB220311	–	Ogawa et al. (2005
Arthrinium luzulae	AP7619-3	MW208937	MW208863	MW221925	MW221919	Pintos and Alvarado (2021)
Arthrinium morthieri	GZU 345043	MW208938	MW208864	MW221926	MW221920	Pintos and Alvarado (2021)
Arthrinium sphaerospermum	AP25619	MW208943	MW208865	–	–	Pintos and Alvarado (2021)
Arthrinium sporophleoides	GZU 345102	MW208944	MW208866	–	MW208866	Pintos and Alvarado (2021)
Arthrinium sporophleum	AG19067	MW208945	–	MW221928	MW221921	Pintos and Alvarado (2021)
Arthrinium trachycarpum	**CFCC 53038**	MK301098	–	MK303394	MK303396	Jiang et al. (2020)
Arthrinium urticae	IMI 326344	AB220245	AB220339	AB220292	–	Ogawa et al. (2005)
Nigrospora aurantiaca	**CGMCC 3.18130**	KX986064	KX986098	KY019465	KY019295	Wang et al. (2018)
	LC7034	KX986093	–	KY019598	KY019394	Wang et al. (2018)

Table 7.8 (continued)

Species	Strain	GenBank Accession No.				Ref.
		ITS	LSU	TUB2	TEF1-α	
Nigrospora bambusae	**CGMCC 3.18327**	KY385307	–	KY385319	KY385313	Dai et al. (2016)
	LC7244	KY385306	–	KY385320	KY385314	Wang et al. (2017)
Nigrospora camelliae-sinensis	**CGMCC 3.18125**	KX985986	KX986103	KY019460	KY019293	Wang et al. (2017)
	LC4460	KX986015	–	KY019538	KY019353	Wang et al. (2017)
Nigrospora chinensis	**CGMCC 3.18127**	KX986023	KX986107	KY019462	KY019422	Wang et al. (2017)
	LC4593	KX986024	–	KY019546	KY019443	Wang et al. (2017)
	MFLUCC 14-0109	MZ463152	MZ463193	–		This study
	MFLUCC 18-1215	MZ463150	MZ463191	–		This study
Nigrospora gorlenkoana	**CBS 480.73**	KX986048	KX986109	KY019456	KY019420	Wang et al. (2017)
Nigrospora guilinensis	LC7301	KX986063	–	KY019608	KY019404	Wang et al. (2017)
Nigrospora hainanensis	**CGMCC 3.18129**	KX986091	KX986112	KY019464	KY019415	Wang et al. (2017)
	LC6979	KX986079	–	KY019586	KY019416	Wang et al. (2017)
Nigrospora lacticolonia	**CGMCC 3.18123**	KX985978	KX986105	KY019458	KY019291	Wang et al. (2017)
	LC7009	KX986087	–	KY019594	KY019454	Wang et al. (2017)
Nigrospora musae	**CBS 319.34**	KX986076	KX986110	KY019455	KY019419	Wang et al. (2017)
	LC6385	KX986042	–	KY019567	KY019371	Wang et al. (2017)
Nigrospora oryzae	LC6759	KX986054	–	KY019572	KY019374	Wang et al. (2017)
	LC6760	KX986055	–	KY019573	KY019375	Wang et al. (2017)
Nigrospora osmanthi	**CGMCC 3.18126**	KX986010	KX986106	KY019461	KY019421	Wang et al. (2017)
	LC4487	KX986017	–	KY019540	KY019438	Wang et al. (2017)
Nigrospora pyriformis	**CGMCC 3.18122**	KX985940	KX986100	KY019457	KY019290	Wang et al. (2017)
	LC2688	KX985941	–	KY019468	KY019297	Wang et al. (2017)
Nigrospora rubi	**CGMCC 3.18326**	KX985948	KX986102	KY019475	KY019302	Wang et al. (2017)
Nigrospora sphaerica	**LC13523**	MN215807	–	MN329970	MN264046	Wang et al. (2017)
	LC7295	KX985933	–	KY019603	KY019398	Wang et al. (2017)
	GZAC O37S13	MZ463153	–	–	–	
Nigrospora sp. 1	LC2725	KX985960	KX986104	KY019487	KY019313	Wang et al. (2017)
Nigrospora sp. 1	LC4566	KX986022	–	KY019545	KY019354	Raza et al. (2019)
Nigrospora sp. 2	LC6704	KX986047	KX986108	KY019571	KY019373	Wang et al. (2017)
Nigrospora vesicularis	**CGMCC 3.18128**	KX986088	KX986099	KY019463	KY019294	Wang et al. (2017)
	LC0322	KX985939	–	KY019467	KY019296	Wang et al. (2017)
Nigrospora zimmermanii	**CBS 290.62**	KY385309	–	KY385317	KY385311	Wang et al. (2017)
	CBS 984.69	KY385310	–	KY385322	KY385316	Wang et al. (2017)
Pseudomassaria corni	MFLUCC 14-0544	KR092791	–	–	–	Wang et al. (2017)
Pseudomassaria chondrospora	MFLUCC 14-0545	KR092790	KR092779	–	–	Wang et al. (2017)
Pseudomassaria sepincoliformis	CBS 129022	JF440984	–	–	–	Wang et al. (2017)

Table 7.9 Strains used for phylogenetic tree construction in other genera of Xylariales fungi (mainly *Hypoxylon, Nemania* and *Xylaria*)

Species name	Strain/Specimens code	Strain accession number				Ref.
		ITS	LSU	TUB2	RPB2	
Alloanthostomella rubicola	MFLUCC 16-0479	KX533455	KX533456	KX789494	KX789493	Bitzer et al. (2008)
Amphirosellinia fushanensis	**91111209 (HAST)**	NR153514	—	GQ495950	GQ848339	Hsieh et al. (2010)
Amphirosellinia nigrospora	**91092308 (HAST)**	NR153513	—	GQ495951	GQ848340	Hsieh et al. (2010)
Annulohypoxylon annulatum	**CBS 140775**	NR153579	KY610418	KX376353	KY624263	Sir et al. (2016)
Annulohypoxylon areolatum	**MFLUCC 14-1233**	KX376327	—	KX376344	—	Kuhnert et al. (2017)
Annulohypoxylon atroroseum	ATCC 76081	AF201712	KY610422	DQ840083	KY624233	Wendt et al. (2018)
Annulohypoxylon bahnphadengense	STMA 13115	KX376338	—	KX376347	—	Kuhnert et al. (2017)
Annulohypoxylon cf. *truncatum*	EKTX140201	KX376331	—	KX376351	—	Kuhnert et al. (2017)
Annulohypoxylon elevatidiscus	**BCRC34014**	—	—	AY951656	—	Hsieh et al. (2005)
Annulohypoxylon fulvum	**MUCL 54617**	KX376336	—	KX376355	—	Kuhnert et al. (2017)
Annulohypoxylon ilanense	YMJ 37	—	—	AY951657	—	Hsieh et al. (2005)
Annulohypoxylon leptascum	MFLUCC 13-0587	KU604576	—	KU604580	—	Sir et al. (2016)
Annulohypoxylon massivum	**MUCL 47218**	AM749938	—	KC977276	—	Bitzer et al. (2008)
Annulohypoxylon michelianum	CBS 119993	KX376320	KY610423	KX271239	KY624234	Wendt et al. (2018)
Annulohypoxylon microdiscum	BCRC34018	EF026137	—	—	AY951660	Kuhnert et al. (2017)
Annulohypoxylon moniliformis	**MFLUCC 18-1214**	MZ463121	—	MZ998970	—	This study
Annulohypoxylon moriforme	CBS 123579	KU683751	KY610425	KX271261	KU684279	U'Ren et al. (2016)
Annulohypoxylon nitens	**MFLUCC 12-0823**	KJ934991	KJ934992	KJ934993	KJ934994	Daranagama et al. (2015)
Annulohypoxylon nouraguense	**MUCL 54607**	KX376335	—	KX376348	—	Kuhnert et al. (2017)
Annulohypoxylon purpureopigmentum	MUCL 54616	KC968942	—	KC977306	—	Kuhnert et al. (2014)
Annulohypoxylon squamulosum	**BCRC34022**	EF026139	—	AY951665	—	Hsieh et al. (2005)
Annulohypoxylon stygium	MUCL 54601	KY610409	KY610475	KX271263	KY624292	Wendt et al. (2018)
Annulohypoxylon substygium	STMA 14066	KU604575	—	KU159526	—	Sir et al. (2016)
Annulohypoxylon thailandicum	**MFLUCC 13-0118**	KP744434	KP744476	KX376349	—	Liu et al. (2015)
Annulohypoxylon truncatum	**CBS 140778**	NR153580	KY610419	KX376352	KY624277	Wendt et al. (2018)
	EKTX14006	KX376329	—	KX376352	—	Kuhnert et al. (2017)
Annulohypoxylon urceolatum	YMJ 92090413	—	—	AY951670	—	Hsieh et al. (2005)
Annulohypoxylon violaceopigmentum	**MFLUCC 14-1225**	KX376326	—	KX376343	—	Kuhnert et al. (2017)
Annulohypoxylon viridistratum	**MFLUCC 14-1224**	KX376325	—	KX376342	—	Kuhnert et al. (2017)
Annulohypoxylon yungensis	STMA 14049	KX376324	—	KX376341	—	Kuhnert et al. (2017)
Anthocanalis sparti	**MFLUCC 14-0557**	KP297395	KP340537	KP406606	KP340523	Daranagama et al. (2015)
Anthostomella eucalyptorum	CBS 120036	DQ890026	—	—	—	Crous et al. (2006)
Anthostomella helicofissa	**MFLUCC 14-0173**	KP297406	—	KP406617	KP340534	Daranagama et al. (2015)
Anthostomelloides krabiensis	**MFLUCC 15-0678**	KX305927	KX305928	—	KX305929	Tibpromma et al. (2017)
Anthostomelloides leucospermi	**CBS 110126**	NR153510	—	—	—	Marincowitz et al. (2008)
Astrocystis concavispora	**MFLUCC 14-0174**	KP297404	KP340545	KP406615	KP340532	Daranagama et al. (2015)
Barrmaelia macrospora	BM	KC774566	—	MF489014	MF488995	Voglmayr et al. (2017)
Biscogniauxia arima	**WSP 122**	EF026150	—	AY951672	GQ304736	Hsieh et al. (2010)
Biscogniauxia atropunctata	YMJ 128	JX507799	—	AY951673	JX507778	Hsieh et al. (2012)
Biscogniauxia marginata	**MFLUCC 12-0740**	KJ958407	KJ958408	KJ958406	KJ958409	Daranagama et al. (2015)
Biscogniauxia nummularia	**MUCL 51395**	KY610382	KY610427	KX271241	KY624236	Wendt et al. (2018)
Biscogniauxia repanda	ATCC 62606	KY610383	KY610428	KX271242	KY624237	Wendt et al. (2018)
Brunnelperidium gracilentum	**MFLUCC 14-0011**	KP297400	KP340542	KP406611	KP340528	Daranagama et al. (2015)
Brunneiperidium involucratum	**MFLUCC 14-0009**	KP297399	KP340541	KP406610	KP340527	Daranagama et al. (2015)

Table 7.9 (continued)

Species name	Strain/Specimens code	Strain accession number				Ref.
		ITS	LSU	TUB2	RPB2	
Camillea obularia	ATCC 28093	KY610384	KY610429	KX271243	KY624238	Wendt et al. (2018)
Camillea tinctor	YMJ 363	JX507806	—	JX507795	JX507790	Wendt et al. (2018)
Clypeosphaeria mamillana	**CBS 140735**	KT949897	MH554225	MH704637	MF489001	Jaklitsch et al. (2016)
Collodiscula bambusae	**GZU H0102**	KP054279	KP054280	KP276674	KP276675	Li et al. (2015)
Collodiscula fangjingshanensis	**GZUH0109**	KR002590	NG058915	KR002589	KR002592	Li et al. (2015)
Collodiscula japonica	CBS 124266	JF440974	MH874889	KY624316	KY624273	Wendt et al. (2018)
Daldinia andina	**CBS 114736**	KU684017	KY610430	KC977259	KY624239	Wendt et al. (2018)
Daldinia bambusicola	**CBS 122872**	NR152464	KY610431	AY951688	KY624241	Wendt et al. (2018)
	MFLUCC 11-0605	KU940155	KU863143	—	KU940181	Dai et al. (2016)
Daldinia brachysperma	BCC 33676	MN153854	MN153871	MN172205	—	Wongkanoun et al. (2019)
Daldinia caldariorum	MUCL 49211	AM749934	KY610433	KC977282	KY624242	Wendt et al. (2018)
Daldinia chiangdaoensis	BCC 88221	MN153851	MN153868	MN172198	MN172209	Wongkanoun et al. (2019)
	BCC 88220	MN153850	MN153867	MN172197	MN172208	Wongkanoun et al. (2019)
Daldinia childiae	**CBS 122881**	MH863249	MH874773	KU684129	KU684290	U'Ren et al. (2016)
Daldinia concentrica	CBS 113277	AY616683	KY610434	KC977274	KY624243	Wendt et al. (2018)
Daldinia decipiens	**CBS 122879**	MH863247	MH874771	AY951694	—	Vu et al. (2019)
Daldinia dennisii	**CBS 114741**	MH862968	MH874530	KC977262	KY624244	Wendt et al. (2018)
Daldinia eschscholtzii	MUCL 45435	JX658484	KY610437	KC977266	KY624246	Wendt et al. (2018)
Daldinia flavogranulata	BCC 89365	MN153857	MN153874	MN172201	MN172212	Wongkanoun et al. (2019)
Daldinia flavogranulata	**BCC 89363**	MN153856	MN153873	MN172200	MN172211	Wongkanoun et al. (2019)
Daldinia loculatoides	**CBS 113279**	MH862918	MH874491	KX271246	KY624247	Wendt et al. (2018)
Daldinia macaronesica	**CBS 113040**	JX658504	KY610477	KX271266	KY624294	Wendt et al. (2018)
Daldinia petriniae	**MUCL 49214**	AM749937	KY610439	KC977261	KY624248	Wendt et al. (2018)
Daldinia phadaengensis	BCC 89350	MN153853	MN153870	MN172196	MN172207	Wongkanoun et al. (2019)
	BCC 89349	MN153852	MN153869	MN172195	MN172206	Wongkanoun et al. (2019)
Daldinia placentiformis	MUCL 47603	AM749921	KY610440	KC977278	KY624249	Wendt et al. (2018)
Daldinia pyrenaica	MUCL 53969	KY610413	KY610413	KY624312	KY624274	Wendt et al. (2018)
Daldinia singularis	YMJ 120	—	—	AY951700	—	Hsieh et al. (2005)
Daldinia steglichii	**MUCL 43512**	KY610399	KY610479	KX271269	KY624250	Wendt et al. (2018)
Daldinia theissenii	**CBS 113044**	KY610388	KY610441	KX271247	KY624251	Wendt et al. (2018)
Daldinia vernicosa	**CBS 119316**	NR152501	KY610442	KC977260	KY624252	Wendt et al. (2018)
Diatrype disciformis	**MFLUCC 15-0538**	AJ390410	KR092784	—	—	Senanayake et al. (2015)
Durotheca comedens	**YMJ 90071615**	EF026128	—	EF025613	JX507793	Ju et al. (2003)
Durotheca crateriformis	**GMBC0205**	MH645426	MH645425	MH049441	MH645427	Long et al. (2019)
Durotheca depressa	**BCC23016**	—	—	GQ160491	—	Læssøe et al. (2013)
Durotheca eurima	**GMBC0060**	MH645419	MH645421	MH049437	MH645422	Long et al. (2019)
Durotheca guizhouensis	GMBC0206	MH645429	MH645430	MH049446	MH645431	Long et al. (2019)
Durotheca rogersii	**GMBC0204**	MH645433	MH645434	MH049449	MH645435	Long et al. (2019)
Euepixylon sphaeriostomum	**JDR 261**	GU292821	—	GQ470224	GQ844774.1	Hsieh et al. (2010)
Entoleuca mammata	JDR 100	GU300072	—	GQ470230	GQ844782	Hsieh et al. (2010)
Entosordaria perfidiosa	BW3	MF488992	—	MF489020	MF489002	Voglmayr et al. (2017)
Graphostroma platystoma	**CBS 270.87**	JX658535	DQ836906	HG934108	KY624296	Wendt et al. (2018)
Hypocreodendron sanguineum	**JDR 169**	GU322433	—	GQ487710	GQ844819	Hsieh et al. (2010)
Hypomontagnella barbarensis	**STMA 14081**	MK131720	MK131718	MK135891	MK135893	Lambert et al. (2019)

Table 7.9 (continued)

Species name	Strain/Specimens code	Strain accession number				Ref.
		ITS	LSU	TUB2	RPB2	
Hypomontagnella monticulosum	**MUCL 54604**	KY610404	KY610487	KX271273	KY624305	Wendt et al. (2018)
Hypomontagnella submonticulosum	CBS 115280	JX658449	KY610457	KC977267	KY624226	Wendt et al. (2018)
Hypoxylon addis	**MUCL 52797**	KC968931	—	KC977287	—	Kuhnert et al. (2017)
Hypoxylon aeruginosum	**MUCL 54620**	KC968941	—	KC977305	—	Kuhnert et al. (2014)
Hypoxylon argillaceum	CBS 527.63	KU683764	—	KU684187	KU684283	U'Ren et al. (2016)
Hypoxylon aff. rubiginosum	**MUCL 57724**	MT214999	MT214994	MT212241	MT212237	Pourmoghaddam et al. (2020)
Hypoxylon anthochroum	BCRC34050	JN660819	—	AY951703	—	Hsieh et al. (2005)
Hypoxylon baihualingense	**H14**	MG490190	—	MH790276	—	Ma et al. (2018)
Hypoxylon baruense	**UCH9545**	MN056428	—	MK908142.	—	Cedeño–Sanchez et al. (2019)
Hypoxylon begae	BCRC 34051	JN660820	—	AY951704	—	Hsieh et al. (2005)
Hypoxylon bellicolor	**UCH9543**	MN056425	—	—	MK908139	Cedeno-Sanchez et al. (2019)
Hypoxylon brevisporum	BCRC33809	JN660821	—	AY951705	—	Hsieh et al. (2005)
Hypoxylon calileguense	**STMA 14059**	KU604566	—	KU604579	—	Sir et al. (2016)
Hypoxylon carneum	MUCL 54177	KY610400	KY610480	KX271270	KY624297	Wendt et al. (2018)
Hypoxylon cercidicola	CBS 119009	KU683766	KY610444	KU684189	KY624254	Wendt et al. (2018)
Hypoxylon cf. *subticinense*	MUCL 53752	KC968913	—	KC977297	—	Kuhnert et al. (2014)
Hypoxylon chionostomum	STMA 14060	KU604563	—	—	—	Sir et al. (2016)
Hypoxylon cinnabarinum	YMJ 43	JN979409	—	AY951709	—	Hsieh et al. (2005)
Hypoxylon crocopeplum	CBS 119004	KC968907	KY610445	KC977268	KY624255	Wendt et al. (2018)
Hypoxylon dieckmannii	BCRC34058	JN979413	—	AY951712	—	Hsieh et al. (2005)
Hypoxylon duranii	BCRC34060	JN979414	—	AY951714	—	Hsieh et al. (2005)
Hypoxylon endophyticum	**MFLUCC 18-1206**	MZ463115	MZ463160	MZ998938	MZ613967	This study
	MFLUCC 18-1209	MZ463134	MZ463177	MZ998953	MZ970698	This study
	MFLUCC 18-1211	MZ463118	MZ463163	MZ998941	MZ690490	This study
	MFLUCC 18-1208	MZ463131	MZ463174	MZ998950	MZ970703	This study
	MFLUCC 18-1210	MZ463137	MZ463180	MZ998956	MZ970705	This study
	MUCL 53759	KC968910	—	KC977296	—	Wendt et al. (2018)
Hypoxylon fendleri	MUCL 54792	KF234421	KY610481	KF300547	KY624298	Wendt et al. (2018)
Hypoxylon ferrugineum	CBS 141259	KX090079	—	KX090080	—	Friebes et al. (2016)
Hypoxylon flavoargillaceum	STMA 14062	KU604577	—	KU159532	—	Sir et al. (2016)
Hypoxylon fragiforme	**MUCL 51264**	KM186294	KM186295	KM186293	KM186296	Wendt et al. (2018)
	YMJ 387	JN979419	—	AY951719	—	Hsieh et al. (2005)
Hypoxylon fraxinophilum	**STMA 12011**	KC968938	—	KC977301	—	Kuhnert et al. (2014)
Hypoxylon fulvosulphureum	**MFLUCC 13-0589**	KP401576	—	KP401584	—	Sir et al. (2016)
Hypoxylon fuscopurpureum	BCRC34067	JN979421	—	AY951721	—	Hsieh et al. (2005)
Hypoxylon fuscum	**CBS 113049**	KY610401	KY610482	KX271271	KY624299	Wendt et al. (2018)
Hypoxylon gibriacense	**MUCL 52698**	KC968930	—	AY951739	—	Kuhnert et al. (2014)
Hypoxylon griseobrunneum	**CBS 331.73**	KY610402	MH872399	KC977303	KY624300	Wendt et al. (2018)
Hypoxylon guilanense	**MUCL 57726**	MT214997	MT214992	MT212239	MT212235	Pourmoghaddam et al. (2020)
Hypoxylon haematostroma	MUCL 47600	AM749924	KY610447	KC977279	KY624257	Wendt et al. (2018)
Hypoxylon hercoglossi	**MFLUCC 18-1207**	MZ463125	MZ463168	—		This study
Hypoxylon hinnuleum	**CBS 286.62**	MH858152	MH869746	MK287575	MK287562	Sir et al. (2019)
Hypoxylon howeanum	MUCL 47599	AM749928	KY610448	KC977277	KY624258	Wendt et al. (2018)

Table 7.9 (continued)

Species name	Strain/Specimens code	Strain accession number				Ref.
		ITS	LSU	TUB2	RPB2	
Hypoxylon hypomiltum	MUCL 51845	KY610403	KY610449	KX271249	KY624302	Wendt et al. (2018)
Hypoxylon investiens	CBS 118185	FJ185308	KY610451	FJ185299	KY624260	Platas et al. (2009)
Hypoxylon investiens	CBS 118183	FJ185307	KY610450	FJ185298	KY624259	Platas et al. (2009)
Hypoxylon investiens	YMJ 89062905	JN979428	—	AY951730	—	Hsieh et al. (2005)
Hypoxylon isabellinum	**MUCL 53308**	KC968935	—	KC977295	—	Kuhnert et al. (2014)
Hypoxylon jaklitschii	**CBS 138916**	KM610290	—	KM610304	—	Kuhnert et al. (2015)
Hypoxylon jecorinum	YMJ 39	JN979429	—	AY951731	—	Hsieh et al. (2005)
Hypoxylon lateripigmentum	**MUCL 53304**	NR155156	KY610486	KC977290	KY624304	Wendt et al. (2018)
Hypoxylon lenormandii	CBS 119003	KC968943	KY610452	KC977273	KY624261	Wendt et al. (2018)
Hypoxylon lienhwacheense	MFLUCC 14-1231	KU604558	—	KU159522	—	Sir et al. (2016)
Hypoxylon lilloi	**STMA 14142**	KU604574	—	KU159537	—	Sir et al. (2016)
Hypoxylon liviae	**CBS 115282**	KC968922	—	KC977265	—	Kuhnert et al. (2017)
Hypoxylon lividicolor	**BCRC34076**	JN979432	—	AY951734	—	Hsieh et al. (2005)
Hypoxylon lividipigmentum	**BCRC34077**	JN979433	MG685777	AY951735	—	Sir et al. (2016)
Hypoxylon macrosporum	YMJ 47	JN979434	—	AY951736	—	Hsieh et al. (2005)
Hypoxylon moschaticum	**MFLUCC 15-1155**	MZ463149	—	MZ998968		This study
Hypoxylon munkii	MUCL 53315	KC968912	—	KC977294	—	Kuhnert et al. (2014)
Hypoxylon musceum	MUCL 53765	KC968926	KY610488	KC977280	KY624306	Wendt et al. (2018)
Hypoxylon notatum	YJM 250	JQ009305	—	AY951739	—	Hsieh et al. (2010)
Hypoxylon ochraceum	**MUCL 54625**	NR155158	—	KC977300	KY624271	Wendt et al. (2018)
Hypoxylon officinalis	MFLUCC 14-0078	MZ463109	MZ463156	MZ998933	—	This study
	MFLUCC 14-0075	MZ463108	MZ463155	MZ613966	—	This study
	MFLUCC 21-0060	MZ463123	—	MZ998943	MZ690492	This study
Hypoxylon olivaceopigmentum	**DSM 107924**	MK287530	MK287542	MK287568	MK287555	Sir et al. (2019)
Hypoxylon papillatum	**ATCC 58729**	NR155153	KY610454	KC977258	KY624223	Wendt et al. (2018)
Hypoxylon perforatum	CBS 115281	AM749935	KY610455	KX271250	KY624224	Wendt et al. (2018)
Hypoxylon petriniae	**CBS 114746**	NR155185	KY610491	KX271274	KY624279	Wendt et al. (2018)
Hypoxylon pilgerianum	YMJ 92042505	JQ009310	—	AY951744	KY624308	Hsieh et al. (2005)
Hypoxylon polyporoideum	BCRC34088	JQ009311	—	AY951747	—	Hsieh et al. (2005)
Hypoxylon polyporus	STMA 14090	KU604570	—	KU159530	—	Sir et al. (2016)
Hypoxylon porphyreum	CBS 119022	KC968921	KY610456	KC977264	KY624225	Kuhnert et al. (2014)
Hypoxylon pulicicidum	**MUCL 49879**	JX183075	—	JX183072	—	Bills et al. (2012)
	MUCL 53764	JX183077	—	JX183073	—	Bills et al. (2012)
	CBS 122622	NG066188	KY610492	JX183074	KY624280	Wendt et al. (2018)
	GZAC O37L14	MZ463124	MZ463167	MZ998944	MZ690493	This study
Hypoxylon rickii	**MUCL 53309**	NR137115	KY610416	KC977288	KY624281	Wendt et al. (2018)
Hypoxylon rubiginosum	**MUCL 52887**	NR155152	KY610469	KY624311	KY624266	Wendt et al. (2018)
Hypoxylon rutilum	YMJ 181	—	—	AY951752	—	Hsieh et al. (2005)
Hypoxylon samuelsii	**MUCL 51843**	NR137159	KY610466	KC977286	KY624269	Wendt et al. (2018)
Hypoxylon shearii var. *minor*	**BCRC34093**	EF026142	—	AY951753	—	Hsieh et al. (2005)
Hypoxylon spegazzinianum	**STMA 14082**	KU604573	—	KU604582	—	Sir et al. (2016)
Hypoxylon sporistriatatunicum	**UCH9542**	MN056426	—	MK908140	—	Cedeno-Sanchez et al. (2019
Hypoxylon subgilvum	YMJ 88113007	JQ009314	—	AY951754	—	Hsieh et al. (2005)
Hypoxylon sublenormandii	JF13026	KM610291	—	KM610303	—	Kuhnert et al. (2015)
Hypoxylon subrutiloides	F202416	FJ185304	—	FJ185281	—	Platas et al. (2009)

Table 7.9 (continued)

Species name	Strain/Specimens code	Strain accession number				Ref.
		ITS	LSU	TUB2	RPB2	
Hypoxylon texense	**DSM 107933**	MK287536	MK287548	MK287574	MK287561	Kuhnert et al. (2017)
Hypoxylon ticinense	CBS 115271	JQ009317	KY610471	AY951757	KY610471	Wendt et al. (2018)
Hypoxylon trugodes	**MUCL 54794**	KF234422	KY610493	KF300548	KY624282	Wendt et al. (2018)
Hypoxylon ulmophilum	**YMJ 350**	JQ009320	—	AY951760		Hsieh et al. (2005)
Hypoxylon vinosopulvinatum	**BCRC34101**	JQ009321	—	AY951761	—	Hsieh et al. (2005)
Hypoxylon vogesiacum	CBS 115273	KY610417	KY610417	KX271275	KY624283	Wendt et al. (2018)
Induratia alba	**MONT 620**	AF324336	—	—	—	Worapong et al. (2001)
Induratia brasiliensis	**LGMF 1256**	KY924494	—	—	MF510171	Pena et al. (2019)
Induratia camphorae	**NFCCI 3236**	KC481681	—	—	—	Meshram et al. (2017)
Induratia cinnamomi	**BCC 38842**	GQ848369	—	—	—	Suwannarach et al. (2010)
Induratia coffeana	**COAD 1842**	KM514680	—	KM514681	—	Hongsanan et al. (2015)
Induratia crispans	**MONT 2347**	EU195297	—	—	—	Suwannarach et al. (2010)
Induratia equiseti	**JCM 18233**	JX089322	—	—	—	Suwannarach et al. (2013)
Induratia darjeelingensis	**NFCCI 3095**	NFCCI 3095	—	—	—	Saxena et al. (2014)
Induratia fengyangensis	ZJLQ024	HM034855	HM034861	HM034842	HM034851	Zhang et al. (2010)
Induratia ghoomensis	**NFCCI 3234**	KF537625	—	—	—	Meshram et al. (2015)
Induratia indica	**NFCCI 3235**	KF537626	—	—	—	Meshram et al. (2015)
Induratia kashayum	**NFCCI 2947**	KC481680	—	—	—	Meshram et al. (2013)
Induratia musae	**JCM 18230**	JX089323	—	—	—	Suwannarach et al. (2010)
Induratia oryzae	**JCM 18231**	JX089321	—	—	—	Suwannarach et al. (2010)
Induratia rosea	**MONT 2098**	AY034665	—	—	—	Worapong et al. (2002)
Induratia sp.	MFLUCC 15-1218	MZ463110	—	—	—	This study
Induratia suthepensis	**JCM 18232**	JN558830	—	—	—	Suwannarach et al. (2010)
Induratia sutura	**MSUB 2380**	JF938595	—	—	—	Kudalkar et al. (2012)
Induratia thailandica	**MFLUCC 17-2669**	MK762707	MK762714	MK791283	MK776960	Samarakoon et al. (2020)
Induratia tigerensis	**NFCCI 3172**	JQ409998	—	—	—	Saxena et al. (2015)
Induratia vitigena	**MONT P-15**	AY100022	—	—	—	Daisy et al. (2002)
Induratia yucatanensis	**MEXU 25511**	FJ917287	—	—	—	González et al. (2009)
Induratia yunnanensis	**CGMCC 3.18908**	MG866046	MG866038	MG866059	MG866066	Chen et al. (2019)
Induratia ziziphi	**MFLUCC 17-2662**	MK762705	MK762712	MK791281	MK776958	Samarakoon et al. (2020)
Jackrogersella cohaerens	CBS 119126	KC477233	KY610497	KY624314	KY624270	Wendt et al. (2018)
Jackrogersella minutella	CBS 119015	KY610381	KY610424	KX271240	KY624235	Wendt et al. (2018)
Jackrogersella multiformis	**CBS 119016**	NR154784	KT281893	KX271262	KY624290	Wendt et al. (2018)
Kretzschmaria clavus	YMJ 114	EF026126	—	EF025611	GQ844789	Hsieh et al. (2010)
Kretzschmaria deusta	CBS 163.93	KC477237	KY610458	KX271251	KY624227	Wendt et al. (2018)
Kretzschmaria guyanensis	89062903 (HAST)	GU300079	—	GQ478214	GQ844792	Hsieh et al. (2010)
Kretzschmaria lucidula	YMJ 112	EF026125	—	EF025610	GQ844790	Hsieh et al. (2010)
Kretzschmaria neocaledonica	94031003 (HAST)	GU300078	—	GQ478213	GQ844788	Hsieh et al. (2010)
Kretzschmaria pavimentosa	YMJ 109	GU300077	—	GQ478212	GQ844787	Hsieh et al. (2010)
Kretzschmaria sandvicensis	YMJ 113	GU300076	—	GQ478211	GQ844786	Hsieh et al. (2010)
Kretzschmariella culmorum	JDR 88	KX430043	—	KX430046	KX430045	Fournier et al. (2018)
Lopadostoma gastrinum	**CBS 134632**	NR132030	MH877560	—	—	Vu et al. (2019)

Table 7.9 (continued)

Species name	Strain/Specimens code	Strain accession number				Ref.
		ITS	LSU	TUB2	RPB2	
Lopadostoma insulare	**CBS 133214**	NR132031	—	—	—	Jaklitsch et al. (2014)
Lunatiannulus irregularis	**MFLUCC 14-0014**	KP297398	KP340540	KP406609	KP340526	Daranagama et al. (2015)
Natonodosa speciosa	**CLM-RV86**	MF380435	MF380435	—	MH745150	Heredia et al. (2020)
Nemania abortiva	**BISH467**	GU292816	—	GQ470219	KU684251	Hsieh et al. (2010)
Nemania aenea	CBS 680.86	AJ390427	—	—	—	Sanchez-Ballesteros et al. (2000)
Nemania aenea var. *aureolutea*	ATCC 60819	AF201704	—	—	—	Pinto-Sherer et al. (2000)
Nemania beaumontii	405 (HAST)	GU292819	—	GQ470222	GQ844772	Hsieh et al. (2010)
Nemania bipapillata	5336	JQ862691	JQ862649	JX868546	—	Chen et al. (2013)
	90080610 (HAST)	GU292818	—	GQ470221	GQ844771	Hsieh et al. (2010)
Nemania chestersii	ATCC 38988	AJ390430	—	DQ631949	—	Sanchez-Ballesteros et al. (2000)
Nemania dendrobii	**MFLUCC 18-1213**	MZ463137	MZ463181	MZ998957	MZ970708	This study
	MFLUCC 18-1212	MZ463135	MZ463178	MZ998954	MZ970699	This study
Nemania diffusa	**91020401 (HAST)**	GU292817	—	GQ470220	GQ844769	Hsieh et al. (2010)
	JZB3370003	MT509577	—	—	MT512901	Manawasinghe et al. (2021)
	MFLUCC 14-0139	MZ463143	MZ463185	MZ998962	—	This study
Nemania illita	YMJ 236	EF026122	—	EF025608	—	Hsieh et al. (2010)
Nemania macrocarpa	**CBS 109567**	MH862830	MH874423	GQ470226	GQ844776	Vu et al. (2019)
Nemania maritima	**89120401 (HAST)**	GU292822	—	GQ470225	GQ844775	Hsieh et al. (2010)
Nemania orchidacearum	**MFLUCC 14-0138**	MZ463141	MZ463183	MZ998960	MZ970706	This study
	MFLUCC 14-0105	MZ463114	MZ463159	MZ998937	—	This study
Nemania plumbea	JF TH-04-01	DQ641634	—	DQ631952	—	Tang et al. (2007)
Nemania primolutea	**91102001 (HAST)**	EF026121	—	EF025607	GQ844767	Tang et al. (2007)
Nemania serpens	ATCC 16078	AJ390431	—	—	—	Sanchez-Ballesteros et al. (2000)
Nemania serpens var. *macrospora*	ATCC 60822	AJ390433	—	—	—	Sanchez-Ballesteros et al. (2000)
Neoxylaria arengae	**MFLUCC 15-0292**	MT496747	—	—	MF459058	Konta et al. (2020)
Neoxylaria juruensis	92042501 (HAST)	GU322439	—	GQ495932	GQ844825	Konta et al. (2020)
Obolarina dryophila	MUCL 49882	GQ428316	GQ428316	GQ428322	KY624284	Wendt et al. (2018)
Podosordaria mexicana	WSP176	GU324762	—	GQ844840	GQ853039	Hsieh et al. (2010)
Podosordaria muli	**WSP167**	GU324761	—	GQ844839	GQ853038	Hsieh et al. (2010)
Poronia pileiformis	**WSP 88113001**	GU324760	—	GQ502720	GQ853037	Hsieh et al. (2010)
Poronia punctata	**CBS 656.78**	KT281904	KY610496	KX271281	KY624278	Wendt et al. (2018)
Pyrenopolyporus hunteri	**MUCL 52673**	KY610421	KY610472	KU159530	KY624309	Wendt et al. (2018)
Pyrenopolyporus laminosus	**MUCL 53305**	NR154296	KY610485	KC977292	KY624303	Wendt et al. (2018)
Pyrenopolyporus nicaraguensis	CBS 117739	AM749922	KY610489	AM749922	KY624307	Wendt et al. (2018)
Rhopalostroma angolense	CBS 126414	KY610420	KY610459	KX271277	KY624228	Wendt et al. (2018)
Rosellinia aquila	MUCL 51703	KY610392	KY610460	KX271253	KY624285	Wendt et al. (2018)
Rosellinia buxi	JDR 99	GU300070	—	GQ470228	GQ844780	Hsieh et al. (2010)
Rosellinia corticium	MUCL 51693	KY610393	KY610461	KX271254	KY624229	Wendt et al. (2018)
Rosellinia lamprostoma	YMJ 89112602	EF026118	—	EF025604	GQ844778	Hsieh et al. (2010)
Rosellinia necatrix	CBS 349.36	AY909001	KF719204	KY624310	—	Vu et al. (2019)
Rosellinia sanctae-cruciana	90072903(HAST)	GU292824	—	GQ470227	GQ844777	Hsieh et al. (2010)

Table 7.9 (continued)

Species name	Strain/Specimens code	Strain accession number				Ref.
		ITS	LSU	TUB2	RPB2	
Rostrohypoxylon terebratum	**CBS 119137**	NR137677	NG057759	DQ840097	DQ631954	Wendt et al. (2018)
Ruwenzoria pseudoannulata	**MUCL 51394**	NR137733	NG059787	KX271278	KY624286	Stadler et al. (2009)
Sarcoxylon compunctum	CBS 359.61	KT281903	KY610462	KX271255	KY624230	Wendt et al. (2018)
Stilbohypoxylon elaeicola	YMJ 173	EF026148	—	EF025616	GQ844826	Hsieh et al. (2010)
Stilbohypoxylon quisquiliarum	YMJ 172	EF026119	—	EF025605	GQ853020	Hsieh et al. (2010)
Thamnomyces dendroidea	**CBS 123578**	NR154472	KY610467	KY624313	KY624232	Stadler et al. (2010)
Xylaria acuminatilongissima	**95060506 (HAST)**	EU178738	—	GQ502711	GQ853028	Ju and Hsieh (2007)
Xylaria acuta	AFTOL-ID 63	DQ491493	AY544676	—	DQ247797	Lutzoni et al. (2004)
Xylaria adscendens	570 (HAST)	GU300101	—	GQ487708	GQ844817	Hsieh et al. (2010)
Xylaria aethiopica	YMJ 1136	MH790445	—	MH785221	MH785222	Fournier et al. (2018)
Xylaria allantoidea	94042903 (HAST)	GU324743	—	GQ502692	GQ848356	Hsieh et al. (2010)
Xylaria amphithele	529 (HAST)	GU300083	—	GQ478218	GQ844796	Hsieh et al. (2010)
Xylaria apiculata	EF6	MF927538	—	—	—	Poornima et al. (2017)
Xylaria aphylli	**MFLUCC 21-0059**	MZ463116	MZ463161	MZ998939	MZ613968	This study
Xylaria apoda	90080804 (HAST)	GU322437	—	GQ495930	GQ844823.	Hsieh et al. (2010)
Xylaria arbuscula	CBS 126415	KY610394	KY610463	KX271257	KY624287	Wendt et al. (2018)
	CBS 126416	MH864102	MH875561	—	—	Vu et al. (2019)
Xylaria arbuscula var. *plenofissura*	93082814 (HAST)	GU339495	—	GQ478225	GQ844804	Hsieh et al. (2010)
Xylaria areolata	543 (HAST)	GU300080	—	GQ478215	GQ844793	Hsieh et al. (2010)
Xylaria atrodivaricata	**95052001 (HAST)**	EU178739	—	GQ502713	GQ853030	Ju and Hsieh (2007)
Xylaria atrosphaerica	91111214 (HAST)	GU322459	—	GQ495953	GQ848342	Hsieh et al. (2010)
Xylaria badia	5256	JQ862687	JQ862643	JX868543	—	Chen et al. (2013)
Xylaria bambusicola	MFLUCC 11-0606	KU940160	KU863148	—	KU940183	Dai et al. (2016)
	WSP 205	NR153200	—	AY951762	GQ844802	Hsieh et al. (2010)
Xylaria berteri	90112623 (HAST)	GU324749	—	—	—	Hsieh et al. (2010)
	JDR 256	GU324750	—	GQ502698	GQ848363	Hsieh et al. (2010)
	YMJ 95101511	KC473562	—	KC473561	—	Fu et al. (2013)
	MFLUCC 14-0095	MZ463112	MZ463157	MZ998935	MZ490538	This study
	MFLUCC 14-0102	MZ463113	MZ463158	MZ998936	MZ490539	This study
	MFLUCC 14-0110	MZ463111	—	MZ998934	—	This study
	MFLUCC 14-0117	MZ463130	MZ463173	MZ998949	—	This study
	MFLUCC 14-0126	MZ463128	MZ463171	MZ998947	MZ970695	This study
	MFLUCC 14-0143	MZ463144	MZ463186	MZ998963	—	This study
	MFLUCC 14-0150	MZ463147	MZ463189	MZ998966	MZ970707	This study
	MFLUCC 14-0158	MZ463146	MZ463188	MZ998965	—	This study
	MFLUCC 21-0061	MZ463127	MZ463170	MZ998946	—	This study
Xylaria brunneovinosa	**720 (HAST)**	NR153201	—	GQ502706	GQ853023	Hsieh et al. (2010)
Xylaria castorea	**ATCC 76020**	AF163030	—	—	—	Lee et al. (2000)
	PDD 600	GU324751	—	GQ502703	GQ853018	Hsieh et al. (2010)
Xylaria cf. *castorea*	91092303 (HAST)	GU324752	—	GQ502704	GQ853019	Hsieh et al. (2010)
Xylaria cf. *heliscus*	88113010 (HAST)	GU324742	—	GQ502691	GQ848355	Hsieh et al. (2010)
Xylaria chaiyaphumensis	**SWUF17-49.2**	MT622775	—	—	—	Wangsawat et al. (2021)
Xylaria chrysanthi	**MFLUCC 21-0014**	MZ463133	MZ463176	MZ998952	MZ970697	This study
Xylaria cirrata	**664 (HAST)**	EU179863	—	GQ502707	GQ853024	Hsieh et al. (2010)

Table 7.9 (continued)

Species name	Strain/Speciemens code	Strain accession number				Ref.
		ITS	LSU	TUB2	RPB2	
Xylaria coccophora	786 (HAST)	GU300093	—	GQ487701	GQ844809	Hsieh et al. (2010)
Xylaria cranioides	226 (HAST)	GU300075	—	GQ478210	GQ844785	Ju and Hsieh (2007)
Xylaria crozonensis	398 (HAST)	GU324748	—	GQ502697	GQ848361	Hsieh et al. (2010)
Xylaria cubensis	GENT 159	—	—	GQ502702	GQ853017	Hsieh et al. (2010)
	477 (HAST)	—	—	GQ502699	GQ848364	Hsieh et al. (2010)
	JDR 860	GU991523	—	GQ502700	GQ848365	Hsieh et al. (2010)
Xylaria culleniae	JDR 189	GU322442	—	GQ495935	GQ844829	Hsieh et al. (2010)
Xylaria curta	494 (HAST)	GU322444	—	GQ495937	GQ844831	Hsieh et al. (2010)
Xylaria curta	92092022 (HAST)	GU322443	—	GQ495936	GQ844830	Hsieh et al. (2010)
Xylaria cf. *curta*	GZAC O36L23	MZ463122	MZ463166	MZ998942	MZ690491	This study
Xylaria cf. *glebusa*	431 (HAST)	GU322462	—	GQ495956	GQ848345	Hsieh et al. (2010)
Xylaria dendrobii	**MFLUCC 14-0137**	MZ463140	—	MZ998959	—	This study
	GZAC O6LA2	MZ463148	MZ463190	MZ998967	—	This study
Xylaria digitata	919 (HAST)	GU322456	—	GQ495949	GQ848338	Hsieh et al. (2010)
Xylaria discolor	**YMJ 1280**	JQ087405	—	JQ087414	JQ087411	Ju et al. (2012)
Xylaria enterogena	785 (HAST)	GU324736	—	GQ502685	GQ848349	Hsieh et al. (2010)
Xylaria escharoidea	**658 (HAST)**	EU179864	—	GQ502709	GQ853026	Hsieh et al. (2010)
Xylaria feejeensis	JDR 180	GU322453	—	GQ495946	GQ848335	Hsieh et al. (2010)
	GZAC O30S21	MZ463117	MZ463162	MZ998940	MZ690489	This study
Xylaria fimbriata	491 (HAST)	GU324753	—	GQ502705	GQ853022	Hsieh et al. (2010)
Xylaria fissilis	367 (HAST)	GU300073		GQ470231	GQ844783	Hsieh et al. (2010)
Xylaria frustulosa	92092010 (HAST)	GU322451	—	GQ495944	GQ844838	Hsieh et al. (2010)
Xylaria globosa	775 (HAST)	GU324735	—	GQ502684	GQ848348	Hsieh et al. (2010)
Xylaria grammica	5228	JQ862677	JQ862638	JX868538	—	Chen et al. (2013)
	479 (HAST)	GU300097	—	GQ487704	GQ844813	Hsieh et al. (2010)
	5151	JQ862665	JQ862626	JX868535	—	Chen et al. (2013)
	BCC1002	AB625411	AB376679	—	—	Okane et al. (2012)
	MFLUCC 14-0093	MZ463107	MZ463154	MZ613965	—	This study
	MFLUCC 14-0146	MZ463139	MZ463182	MZ998958	MZ970700	This study
Hypoxylon griseobrunneum	**CBS 331.73**	KY610402	MH872399	KC977303	KY624300	Wendt et al. (2018)
Xylaria haemorrhoidalis	89041207 (HAST)	GU322464	—	GQ502683	GQ848347	Hsieh et al. (2010)
Xylaria hongkongensis	**GDGM 40058**	NR154905	—	—	—	Tang et al. (2013)
	GZAC O32S24	MZ463119	MZ463164	—	MZ970702	This study
Xylaria hypoxylon	**CBS 122620**	AM993141	KY610495	KX271279	KY624231	Persoh et al. (2009)
Xylaria ianthinovelutina	553 (HAST)	GU322441		GQ495934	GQ844828	Hsieh et al. (2010)
Xylaria insolita	YMJ 99090301-1251	MN655979	—	MN656983	MN656981	Hsieh et al. (2020)
Xylaria intracolorata	90080402 (HAST)	GU324741	—	GQ502690	GQ848354	Hsieh et al. (2010)
Xylaria intraflava	725 (HAST)	EU179866	—	GQ502718	GQ853035	Ju and Hsieh (2007)
Xylaria ischnostroma	SWUF18-22.1	MT622788	—	MW459244	—	Wangsawat et al. (2021)
Xylaria karyophthora	DRH059	KY564220	—	—	KY564216	Husbands et al. (2017)
Xylaria laevis	95072910 (HAST)	GU324747	—	GQ502696	GQ848360	Hsieh et al. (2010)
	419 (HAST)	GU324746	—	GQ502695	GQ848359	Hsieh et al. (2010)
	GZAC O33L12	MZ463120	MZ463165	MZ998969		This study
Xylaria liquidambaris	93090701 (HAST)	GU300094	—	GQ487702	GQ844810	Hsieh et al. (2010)
Xylaria longipes	**CBS 148.73**	KU683768	—	KU684204	KU684280	Vu et al. (2019)
Xylaria longissima	**IRAN 2268C**	NR147567	—	—	—	Hashemi et al. (2015)
Xylaria luteostromata	508 (HAST)	GU324739	—	GQ502688	GQ848352	Hsieh et al. (2010)

Table 7.9 (continued)

Species name	Strain/Speciemens code	Strain accession number				Ref.
		ITS	**LSU**	**TUB2**	**RPB2**	
Xylaria mali	CBS 385.35	AF163040	MH867225	KU684205	KU684286	Hsieh et al. (2010)
Xylaria meliacearum	JDR 148	GU300084	—	GQ478219	GQ844797	Hsieh et al. (2010)
Xylaria melitensis	CS1223	MW513723	MW51376	—	—	Fournier et al. (2021)
Xylaria microceras	414 (HAST)	GU300086	—	GQ478221	GQ844799	Hsieh et al. (2010)
Xylaria minima	**SWUF18-3.2**	MT622789	—	MW459245	—	Wangsawat et al. (2021)
Xylaria montagnei	495 (HAST)	GU322455	—	GQ495948	GQ848337	Hsieh et al. (2010)
Xylaria multiplex	580 (HAST)	GU300098	—	GQ487705	GQ844814	Hsieh et al. (2010)
Xylaria muscula	520 (HAST)	GU300087	—	GQ478222	GQ844800	Hsieh et al. (2010)
Xylaria necrophora	DMCC3829	MT808616	—	MT812966	MT812957	Garcia-Aroca et al. (2021)
Xylaria nigripes	653 (HAST)	GU324755	—	GQ502710	GQ853027	Hsieh et al. (2010)
Xylaria ochraceostroma	**401(HAST)**	EU179869	—	GQ502717	GQ853034	Ju and Hsieh (2007)
Xylaria oligotoma	784 (HAST)	GU300092	—	GQ487700	GQ844808	Hsieh et al. (2010)
Xylaria ophiopoda	93082805 (HAST)	GU322461	—	GQ495955	GQ848344	Hsieh et al. (2010)
Xylaria oxyacanthae	JDR 859	GU322434	—	GQ495927	GQ844820	Hsieh et al. (2010)
Xylaria palmicola	604 (PDD)	GU322436	—	GQ495929	GQ844822	Hsieh et al. (2010)
Xylaria papulis (=*Xylaria hongkongensis*)	5118	JX868517	JQ862616	JX868529	—	Chen et al. (2013)
Xylaria phyllocharis	528 (HAST)	GU322445	—	GQ495938	GQ844832	Hsieh et al. (2010)
Xylaria plebeja	91122401 (HAST)	GU324740	—	GQ502689	GQ848353	Hsieh et al. (2010)
Xylaria polymorpha	MUCL 49884	KY610408	KY610464	KX271280	KY624288	Wendt et al. (2018)
Xylaria reevesiae	HMH-2010g	GU322435	—	GQ495928	GQ844821	Hsieh et al. (2010)
Xylaria regalis	920 (HAST)	GU324745	—	GQ502694	GQ848358	Hsieh et al. (2010)
X. reinkingii var.*microspora*	**SWUF17-19.1**	MT622769	—	MW459234	—	Wangsawat et al. (2021)
Xylaria ripicola	**KH KA11-0060-1**	NR153251	—	—	KU554696	Kim et al. (2016)
Xylaria schweinitzii	92092023 (HAST)	GU322463	—	GQ495957	GQ848346	Hsieh et al. (2010)
Xylaria scuposa	497 (HAST)	GU322458	—	GQ495952	GQ848341	Hsieh et al. (2010)
Xylaria sicula f. *major*	90071613 (HAST)	GU300081	MH869355	GQ478216	GQ844794	Vu et al. (2019)
Xylaria sihanonthii	**SWUF18-1.3**	MT622785	—	MW459242	—	Wangsawat et al. (2021)
Xylaria spinulosa	**GZUCC13016**	—	—	KM236099	KM236098	Li et al. (2017)
Xylaria striata	304 (HAST)	GU300089	—	GQ478224	GQ844803	Hsieh et al. (2010)
Xylaria subescharoidea	**YMJ 99060401-1188**	MN655980	—	MN656984	MN656982	Hsieh et al.2020)
Xylaria subintraflava	**SWUF16-4.3**	MT622762	—	MW459230	—	Wangsawat et al. (2021)
Xylaria subtropicalis	**X1**	MG013556	MG013566	—	MG013547	Chacón et al. (2019)
Xylaria telfairii	421 (HAST)	GU324737	—	GQ502686	GQ848350	Hsieh et al. (2010)
Xylaria tentaculata	KA13-1325	KM077164	—	—	—	Kim et al. (2016)
Xylaria thienhirunae	**SWUF17-44.1**	MT622771	—	—	—	Wangsawat et al. (2021)
Xylaria tuberoides	475 (HAST)	GU300074	—	GQ478209	GQ844784	Hsieh et al. (2010)
Xylaria venosula	94080508 (HAST)	EF026149	—	EF025617	GQ844806	Ju et al. (2007)
	MFLUCC 14-0114	MZ463129	MZ463172	MZ998948	—	This study
	MFLUCC 21-0013	MZ463126	MZ463169	MZ998945	MZ970694	This study
	MFLUCC 21-0015	MZ463136	MZ463179	MZ998955	MZ970704	This study
	MFLUCC 21-0016	MZ463132	MZ463175	MZ998951	MZ970696	This study
	MFLUCC 21-0017	MZ463145	MZ463187	MZ998964	MZ970701	This study
Xylaria venustula	88113002 (HAST)	GU300091	—	GQ487699	GQ844807	Hsieh et al. (2010)
Xylaria vinacea	**SWUF18-2.1**	MT622781	—	MW459239	—	Wangsawat et al. (2021)
Xylaria vivantii	HMH-2010h	GU322438	—	GQ495931	GQ844824	Hsieh et al. (2010)
Xylariaceae sp.	MFLUCC 14-0141	MZ463142	MZ463184	MZ998961	—	This study

Table 7.10 Strains involved for *Biscogniauxia* phylogenetic analysis

Species name	**Strain/Speciemens code**	**ITS**	**LSU**	**TUB2**	**RPB2**
Annulohypoxylon cohaerens	YMJ310	EF026140	AY951655	GQ844766	—
Biscogniauxia anceps	YMJ 123	EF026132	AY951671	JX507777	—
Biscogniauxia arima	WSP122	EF026150	—	GQ304736	—
Biscogniauxia atropunctata	YMJ 128	JX507799	AY951673	JX507778	—
Biscogniauxia atropunctata var.*intermedia*	ATCC 38987	AF201705	—	—	—
Biscogniauxia bartholomaei	ATCC38992	AF201719	—	—	—
Biscogniauxia capnodes	YMJ 138	EF026131	AY951675	JX507779	—
Biscogniauxia citriformis	YMJ 88113012	JX507800	AY951677	JX507780	—
Biscogniauxia cylindrispora	YMJ 89092701*	EF026133	AY951679	JX507782	—
Biscogniauxia dendrobii	MFLUCC 17-2607*	MK944282	MK975826	—	MK951679
Biscogniauxia formosana	YMJ 89032201*	JX507802	AY951680	JX507783	—
Biscogniauxia granmoi	YMJ 135	JX507803	AY951681	JX507784	—
Biscogniauxia latirima	YMJ 90080703	EF026135	AY951683	JX507786	—
Biscogniauxia marginata	MFLUCC 12-0740	KJ958407	KJ958406	KJ958409	KJ958408
Biscogniauxia mediterranea	YMJ 147	EF026134	AY951684	—	—
Biscogniauxia nummularia	MUCL 51395*	NR153649	KX271241	KY624236	KY610427
Biscogniauxia petrensis	CGMCC3.17912*	KU746667	KU746763	—	KU746716
	CGMCC3.17949	KU746671	KU746762	—	KU746717
	MFLUCC 14-0151	MK944283	MK975827	MK975825	MK951680
Biscogniauxia philippinensis var.*microspora*	YMJ 89041101	EF026136	AY951685	JX507787	—
Biscogniauxia repanda	ATCC 62606	—	KX271242	KY624237	KY610428
Biscogniauxia rosacearum	BX1	KT253495	KT253529	—	—
Biscogniauxia simplicior	YMJ 136	EF026130	AY951686	JX507788	—
Biscogniauxia sp.	BRO-2013	KF367566	—	—	KF428799
Biscogniauxia uniapiculata	YMJ 90080608	JX507805	AY951687	JX507789	—
Camillea obularia	ATCC 28093	AJ390423	KX271243	KY624238	KY610429
Camillea tinctor	YMJ 363	JX507806	JX507795	JX507790	—
Daldinia concentrica	CBS 113277	AY616683	KC977274	KY624243	KY610434
Hypoxylon fragiforme	MUCL 51264	KM186294	KM186293	KM186296	KM186295
Jackrogersella multiformis	CBS 119016	KC477234	KX271262	KY624290	KY610473
Kretzschmaria deusta	CBS 163.93*	KC477237	KX271251	—	KY610458
Lunatiannulus irregularis	MFLUCC 14-0014*	KP297398	KP406609	KP340526	KP340540
Obolarina dryophila	MUCL 49882*	—	—	KY624284	—
Podosordaria mexicana	WSP 176*	GU324762	GQ844840	GQ853039	—
Podosordaria muli	WSP 167*	GU324761	GQ844839	GQ853038	—
Poronia pileiformis	WSP 88113001*	GU324760	GQ502720	GQ853037	—
Poronia punctata	CBS 656.78*	KT281904	KX271281	—	KY610496
Xylaria acuminatilongissima	HAST 95060506*	EU178738	—	—	—
Xylaria arbuscula	CBS 126415*	—	KX271257	KY624287	KY610463
Xylaria bambusicola	WSP 205*	NR153200	—	GQ844802	—

Table 7.10 (continued)

Species name	**Strain/Speciemens code**	**ITS**	**LSU**	**TUB2**	**RPB2**
Xylaria brunneovinosa	HAST 720*	NR153201	GQ502706	GQ853023	—
Xylaria hypoxylon	CBS 122620*	AM993141	KX271279	KY624231	KY610495
Xylaria multiplex	HAST 580*	GU300098	GQ487705	GQ844814	—

Note*Ex-type or ex-epitype strains were labeled with asterisk

CHAPTER 8

OVERALL CONCLUSIONS

8.1 The Current Taxonomic Dilemma for Fungal Endophyte

The taxonomy of fungal endophytes based on ITS sequence only was out of consideration when many researchers review fungal categories on account of lacking clear morphology and uninformative gene sequences. The results of the fungal endophyte study are subjected to the surface-sterilized method. The surface-sterilization reagents, time and environment could affect the final fungal isolation. The checking for surface sterilization method is a key process during the whole study. Some fungal endophytes may present cryptic groups that need more protein genes and morphological traits for solving their real taxonomic placements. The method nowadays limited our inspection for its occurrence and functions.

8.2 Fungal Endophyte Diversity of *Dendrobium* Species

A total of 750 culturable fungal endophytic strains from 65 *Dendrobium* samples collected from southwestern China and northern Thailand were identified to 110 species with 25 novel taxa and 50 new host records were identified. These endophytic species are distributed in 70 genera of 52 families belonging to 24 orders of 8 classes mainly in Ascomycota (94.6%) and less in Basidiomycota (5.4%). In Sordariomycetes, 20 novel species (8 in *Colletotrichum*, 3 in *Xylaria*, 2 in *Diaporthe*, 2 in *Fusarium*, 2 in *Hypoxylon*, 1 in *Annulohypoxylon*, 1 in *Neopestalotiopsis*, 1 in *Nemania*) and 40 new host records were reported. Four and one novel species were discovered from Dothideomycetes (*Aureobasidium*, *Epicoccum*, *Mycosphaerella*, *Phyllosticta*) and Leotiomycetes (*Pezicula*), respectively. The rest isolates were categorized to Agaricomycetes (3.4%), Eurotiomycetes (3.2%), Pezizomycetes (1.4%), Saccharomycetes (5.2%) and Ustilagiomycetes (0.1%). *Colletotrichum*, *Diaporthe* and *Fusarium* were the most three frequently isolated genera in Sordariomycetes also in this phylum. *Colletotrichum siamense* is the most separated species. *Phyllosticta* species are the most isolated group in Dothideomycetes. *Meyerozyma* and *Penicillium* take the majority of Saccharomycetes and Eurotiomycetes, respectively. In Basidiomycota, 13 genera were discovered in Agaricomycetes. *Pseudozyma tsukubaensis* is the solo species identified in Ustilaginomycetes. Both leave and stems have higher species richness than that of roots followed by flowers. The host, geographic and organ specificity between fungal endophytes and *Dendrobium* species were rarely observed. *Phyllosticta capitalensis* was mostly separated from the *Dendrobium* leaves.

8.3 Potential Roles of Fungal Endophyte in *Dendrobium* Species

In this study, most species identified are phylogenetically nested within known pathogens that have been reported from other plant hosts including some orchids. A few of them have been reported as saprobes in other orchids. Therefore, we speculated that most fungal endophytes in this study could be latent pathogens. Meanwhile, some species may play roles in orchid germination and seedling development even in adulthood like mycorrhizal fungi. The uncultured *Tulasnella* sp. was regarded as mycorrhizal fungi. Further evidence with time-scale study is needed for evaluating their functional roles.

8.4 Bioactivities of Fungal Endophyte in *Dendrobium* Species

In this study, cytotoxic assays were carried out with 153 crude extracts including some further fractions. The crude extracts of ten species were found to possess cytotoxic potentials that vary from medium to strong degrees. The crude extracts of *Hypoxylon investiens* (MFLUCC 15-1188), *Neopestalotiopsis dendrobii* (MFLUCC 14-0106) and *Xylaria venosula* (MFLUCC 21-0013) performed the highest inhibitory rates towards A549 (64.8%), K562 (89%) and PC3 (55.8%) cell lines, respectively. Several endophytic xylarialean strains stand out of other species which perhaps indicate that they contain natural compounds for inhibiting tumor cell growth.

8.5 Research Advantages & Future Work

This study provides the taxonomic placement for many fungal endophytes of *Dendrobium* species, which lays a foundation for endophytic life mode changing, physiology and functions. The results indicate that *Dendrobium* possesses a highly diverse fungal endophyte and could have a close relationship with fungal endophytes during their lives. The fungal obtained in this study have different growth rates and sporulation conditions on artificial media. With these available cultures, we could improve their cultivation via trying different substrates and finding the possible limiting factors. Meanwhile, much more gene sequences for these endophytes were recorded in this study, which showed some cryptic taxa and facilitate the phylogenetic stability for some fungal groups. The results also indicate these fungal assemblages can be a huge repertoire to produce interesting bioactive compounds.

Some interdisciplinary subjects such as whole-genome sequencing, systematic biology and synthetic biology perhaps facilitate more accurate and controllable endophytic research. Then, we may break through the dilemma caused by method limitations and be free from the laborious and low effective endophyte identification method. In the future study, more type or epitype specimens of fungal endophytes with more protein annotations are needed to strengthen the taxonomic results. A time-course observation is recommended for endophytic research in the future.

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APPENDICES

APPENDIX A

CHEMICAL REAGENTS AND MEDIA

1. **Lactoglycerol** used for maintaining semipermanent slides

Lactic acid	10 ml
Glycerol	10 ml
Distilled water	10 ml

Mix 10 ml lactic acid, 10 ml glycerol, and add 10 ml distilled water

2. Lactophenol-Cotton Blue used to stain for observe fungal structures such as pseudoparaphyses, septate or ascus walled, viewed by the light compound microscope.

Phenol (crystals)	20 g
Lactic acid	16 ml
Glycerol	31 ml
Cotton Blue	0.05 g
Distilled water	20 ml

Dissolve phenol in distilled water, add lactic acid, glycerol and 0.05 g of Poirrier's (cotton) blue or acid fuchsin.

3. Corn Meal Agar (CMA) used for fungal cultivation and sporulation

Corn Meal Infusion	20 g
Peptione	20 g
D. glucose	20 g
Agar	15 g
Distilled water	1000 ml

Suspend 20 g of malt extract agar in 1000 ml of distilled water and mix thoroughly. Heat with frequent agitation and boil to completely dissolve the power. Autoclave at 121 °C for 20 minutes.

4. Malt Extract Agar (MEA) used for fungal cultivation and sporulation

Agar	20 g
Malt extract	20 g
D-glucose	20 g
Peptone	6 g
Distilled water	1000 ml

Suspend 66 g of malt extract agar in 1000 ml of distilled water and mix thoroughly. Heat with frequent agitation and boil to completely dissolve the power. Autoclave at 121 °C for 20 minutes.

5. Oat Agar (OA) used for fungal sporulation and sporulation

Oat extract	39g
Agar	15 g
Distilled water	1000 ml

Suspend 39 g of oat extract agar in 1000 ml of distilled water and mix thoroughly. Heat with frequent agitation and boil to completely dissolve the power. Autoclave at 121 °C for 20 minutes.

6. Oat Malt Agar (OMA) used for fungal cultivation and sporulation

Oat Malt extract	60 g
Agar	12.5 g
Distilled water	1000 ml

Suspend 60 g of malt extract agar in 1000 ml of distilled water and mix thoroughly. Heat with frequent agitation and boil to completely dissolve the power. Autoclave at 121 °C for 20 minutes.

7. Potato Dextrose Agar (PDA) used for fungal cultivation and sporulation

Potato Infusion	4g
Dextrose	20g
Agar	15g
Distilled water	1000 ml

Suspend 39 g of potato dextrose agar in 1000 ml of distilled water and mix thoroughly. Heat with frequent agitation and boil to completely dissolve the power. Autoclave at 121 °C for 20 minutes.

8. Sabouraud Dextrose Agar (SDA) used for fungal cultivation and sporulation

Peptone	10 g
Glucose	40 g
Agar	15 g

Suspend 65 g of potato dextrose agar in 1000 ml of distilled water and mix thoroughly. Heat with frequent agitation and boil to completely dissolve the power. Autoclave at 121 °C for 20 minutes.

9. Synthetic low-Nutrient Agar (SNA) used for fungal cultivation and sporulation

KH_2PO_4	1 g
KNO_3	1 g
$MgSO_4\cdot 7H_2O$	0.5 g
KCl	0.5 g
Glucose	0.2 g
Sucrose	0.2 g
Agar	15.0 g
Distilled water	1000 ml

Suspend 18 g of potato dextrose agar in 1000 ml of distilled water and mix thoroughly. Heat with frequent agitation and boil to completely dissolve the power. Autoclave at 121 °C for 20 minutes.

10. Water agar (WA) used for fungal cultivation and sporulation

Agar	8–16 g
Distilled water	1000 ml

Thoroughly mix agar powder in 1000 ml of distilled water. Heat with frequent agitation and boil to completely dissolve. Autoclave at 121 °C for 20 minutes.

11. Ethyl acetate (Fuyu Chemical Co., Ltd. Tian Jin, China)

12. Methanol (Fuyu Chemical Co., Ltd. Tian Jin, China)

13. Cell Counting Kit-8 (CCK8, Dojindo, Kumamoto, Japan)

14. RPMI 1640 Media (500ml, Gibco Laboratories, Grand Island, NY, America)

15. Cell Culture Antibiotics (100ml, Penicillin-Streptomycin (10,000 U/mL), Gibco Laboratories, Grand Island, NY, America)

16. Fetal Bovine Serum (FBS, AusGeneX, Pty Ltd, Brisbane, Australia)

17. Vitamin C (Enzyme Link Biotechnology Co., Ltd. Shang Hai, China)

18. 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH, He Fei Bo Mei Biotechnology co. Ltd, An Hui, China)

19. Dimethyl Sulfoxide (DMSO, Komiou Chemical Reagent Co., Ltd., Tian Jin, China)

20. Doxorubicin hydrochloride (Aladdin Biochemical Technology Co., Ltd., Shang Hai, China)

APPENDIX B

ABSTRACT OF PUBLICATIONS

SPECIAL SECTION: FUNGAL ENDOPHYTES

Non-mycorrhizal endophytic fungi from orchids

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Orchidaceae is one of the largest flowering plant families of the plant kingdom. The habitats of orchids are highly diverse, ranging from tree bark and damp forest floors to rock crevices, sandy dunes and semi-arid deserts. The diversity of endophytes (internal symbiotic fungi) associated with orchids is enormous. Most studies of endophytic fungi from orchids in the past have focused on mycorrhizal endophytes (internal symbiotic fungi associated with plant roots). There has, however, been an increasing trend to study non-mycorrhizal endophytes from orchids because of their physiological roles and their potential as sources of novel bioactive compounds. This review discusses the methods used in the isolation and identification of endophytic fungi from orchids, their diversity and host-specificity, their significance in orchid conservation and cultivation, and their potential application in the discovery of bioactive compounds.

Keywords: Bioactive compounds, diversity, non-mycorrhizal endophytic fungi, orchids.

Introduction

ORCHIDACEAE is one of the largest flowering plant families of the plant kingdom, which comprises more than 899 genera and 27,801 species (The Plant List 2013). Of these, over 200 genera have been studied for their endophytic fungal diversity (see Appendix 1), which is less than 30% of the total orchid genera. Orchids with horticultural, ornamental, medical and commercial importance have been studied for the presence of endophytes[1,2]. Rare or endangered orchids, including species in *Cypripedium*, *Holcoglossum* and *Paphiopedilum* have also received attention[3–5]. Terrestrial orchids which make up nearly one-third of all orchid species occupy approximately half of the endangered orchid list (The World Conservation Union 1999)[6]. Many of them have also been subjected to endophyte research (Table 1). Orchid species in the genera *Aa*, *Hadrolaelia*, *Gavilea* and *Satyrium* have been poorly studied and can be regarded as new topics for research[7–10].

Research on endophytic fungi in orchids has been carried out in all trophic groups (i.e. photosynthetic, mixotrophic and mycoheterotrophic) of all growth habits (i.e. terrestrial, epiphytic and lithophytic), from highly diverse habitats (e.g. rainforests, evergreen forests, coniferous forests, bamboo forests, ectomycorrhizal forests, wetlands, swamps, calcareous coastal plains, botanical gardens and greenhouses) in all continents except Antarctica (Table 1). Some orchids occur in a wide range of habitats, while others are endemic to certain regions. For example, *Platanthera minor* grows in forests on slopes and alpine meadows at elevations 90–3000 m in China, Japan and Korea[11,12]. *Satyrium nepalense* was reported to be distributed from grassy hill slopes at varying altitudes (600–4600 m) in India[10]. *Ophrys benacensis* occurs only in northern Italy[13] and *Piperia yadonii* only in North America[14]. The epiphytic orchid *Sarcochilus parviflorus* survives only with its main host *Backhousia myrtifolia*[15].

The purpose of this article is to review the studies on non-mycorrhizal endophytic fungi of orchids and present the main conclusions from the research.

Isolation and identification of fungal endophytes from orchids

Isolation

Orchid mycorrhizal fungi are known to be associated with roots of orchids[16,17]. Therefore, most endophyte studies on orchids have investigated orchid roots for mycorrhizal and endophytic diversity[18]. Other orchid parts, including leaves, rhizomes, mature bulbs, tubers, stems and stem-collars have also been studied for endophytes[19,20]. Since endophytes are commonly defined as 'all organisms inhabiting plant organs that at some time in their life, can colonize internal plant tissues without causing apparent harm to the host'[21,22], only healthy organs were used in these studies[23].

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RESEARCH ARTICLE

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Endophytic *Colletotrichum* species from *Dendrobium* spp. in China and Northern Thailand

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Abstract

Species of *Colletotrichum* are commonly found in many plant hosts as pathogens, endophytes and occasionally saprobes. Twenty-two *Colletotrichum* strains were isolated from three *Dendrobium* species – *D. cariniferum*, *D. catenatum* and *D. harveyanum*, as well as three unidentified species. The taxa were identified using morphological characterisation and phylogenetic analyses of ITS, GAPDH, ACT and ß–tubulin sequence data. This is the first time to identify endophytic fungi from *Dendrobium* orchids using the above method. The known species, *Colletotrichum boninense*, *C. camelliae-japonicae*, *C. fructicola*, *C. jiangxiense* and *C. orchidophilum* were identified as fungal endophytes of *Dendrobium* spp., along with the new species, *C. cariniferi*, *C. chiangraiense*, *C. doitungense*, *C. parallelophorum* and *C. watphraense*, which are introduced in this paper. One strain is recorded as an unidentified species. Corn meal agar is recommended as a good sporulation medium for *Colletotrichum* species. This is the first report of fungal endophytes associated with *Dendrobium cariniferum* and *D. harveyanum*. *Colletotrichum camelliae-japonicae*, *C. jiangxiense*, and *C. orchidophilum* are new host records for Thailand.

Keywords

Colletotrichum, *Dendrobium*, Endophytic fungi, multi-loci, new species

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Endophytic pestalotiod taxa in *Dendrobium* orchids

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Abstract

Pestalotiod taxa commonly occur in plants as endophytes, pathogens or saprobes. Endophytic *Pestalotiopsis* and *Neopestalotiopsis* species were isolated from *Dendrobium cariniferum, D. loddigesii* and two unidentified orchid species sampled in southwestern China and northern Thailand. Morphological and molecular comparison identified the isolates as two new species; *Neopestalotiopsis dendrobii,* and *Pestalotiopsis doitungensis*; two existing species *P. lushanensis* and *P. trachicarpicola*. This is the first report of *Neopestalotiopsis* isolated from the orchid genus *Dendrobium* and is the first report of pestalotiod fungi in *D. careniferum. Pestalotiopsis lushanensis* and *P. trachicarpicola* are new recorded fungal endophytes in *Dendrobium* orchids.

Keywords: 2 new taxa, 2 new records, Sporocadaceae, multi-loci

Introduction

Pestalotiopsis and *Neopestalotiopsis* (*Sporocadaceae*, Amphisphaeriales) are endophytes in various plants (Kang *et al.* 1998, 1999; Senanayake *et al.* 2015; Jaklitsch *et al.* 2016; Wijayawardene *et al.* 2017). Taxa in these genera have distinctive appendage-bearing conidia and they are known as pestalotiod asexual morphs (Maharachchikumbura *et al.* 2011, 2012, 2014; Senanayake *et al.* 2015). Pestalotiod species are rich producers of bioactive compounds (Strobel *et al.* 1996; Chen *et al.* 2018). They are widely distributed in tropical and temperature areas (Bate-Smith and Metcalfe 1957; Maharachchikumbura *et al.* 2012; 2017). Pestalotiod taxa have been found as endophytes in many plants, such as *Camellia*, *Fragraea*, *Podocarpus* and *Taxus* species (Strobel *et al.* 2000; Maharachchikumbura *et al.* 2012; Liu *et al.* 2017). *Pestalotiopsis* spp. have been reported to be associated with *Dendrobium falconeri*. and they were ubiquitous in the roots of *D. chrysanthum* and *D. nobile* (Chen *et al.* 2011; 2012). *Neopestalotiopsis* and *Pseudopestalotiopsis* were proposed as independent pestalotiod genera as they split from *Pestalotiopsis* in molecular analysis, but share similar, although differing morphological characters (Maharachchikumbura *et al.* 2014). *Neopestalotiopsis* species have been reported to cause fruit rot in Africa, Asia, Europe and South America (Ayoubi *et al.* 2016; Chamorro *et al.* 2016; Jayawardena *et al.* 2016; Maharachchikumbura *et al.* 2017; Solarte *et al.* 2018), but they are rarely reported as endophytes (Chen *et al.* 2011; 2012).

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Dendrobium is one of the largest genera in *Orchidaceae* and some species possess important medicinal and ornamental values (Ma *et al.* 2015). For example, moscatilin (4,4'-dihydroxy-3,3',5-trimethoxybibenzyl) isolated from many *Dendrobium* spp. possesses anti-inflammatory, anti-platelet aggregation, anti-tumor and antioxidant bioactivities (Fan *et al.* 2001; Zhang *et al.* 2008; Hu *et al.* 2009; Hwang *et al.* 2010; Sritularak *et al.* 2011; Zhao *et al.* 2015). Many *Dendrobium* orchids have been listed as endangered species due to habitat destruction and low seed germination

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Biscogniauxia dendrobii sp. nov. and *B. petrensis* from *Dendrobium* orchids and the first report of cytotoxicity (towards A549 and K562) of *B. petrensis* (MFLUCC 14-0151) in vitro

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ABSTRACT

Biscogniauxia belongs to the family Graphostromataceae, and species have been found mainly as pathogens in dicotyledonous plants worldwide. Two endophytic strains, *Biscogniauxia dendrobii* sp. nov. and *B. petrensis* were isolated from *Dendrobium aphyllum* and *D. harveyanum* sampled from southwestern China and northern Thailand respectively. The conidiophores and conidia were used for morphological characteristic estimation. The maximum likelihood and Bayesian analysis with ITS, TUB2, *RPB*2 and LSU sequence data were combined for phylogenctic identification. *Biscogniauxia dendrobii* sp. nov. is introduced based on the large discrepancy of both sequences (5.2%) and size of conidia. Another known species *B. petrensis* is also illustrated and described in this study to facilitate species identification on *D. harveyanum*. The ethyl acetate crude extract of the isolate *B. petrensis* showed moderate and strong cytotoxic activity against A549 and K562 using the CCK8 assay (IC_{50} of it 13.5 and 3.5 ug/ml respectively). This is the first report of endophytic *Biscogniauxia* in *Dendrobium* species and of their cytotoxicity to A549 and K562, all of which enhances the current knowledge on the genus *Biscogniauxia*.

Introduction

Endophytes inhabited in plants with no obvious symptoms during some times (Hyde and Soytong 2008) and species of *Xylariaceae* such as *Xylaria* and *Hypoxylon* are common endophytes which have been found in many flowering plants (Davis et al. 2003; Promputtha et al. 2005; Chen et al. 2013; Jeewon et al. 2013; Rampadarath et al. 2018). The roles of fungal endophyte have always been subject to debate amongst mycologists (Liu et al. 2012; Doilom et al. 2017; Jeewon et al. 2017; 2018). Some studies have documented their roles as saprobes that become active when plants die (Hyde et al. 2001; Okane et al. 2008; Kumar et al. 2018) while others postulated that they are dormant or latent pathogens (Choi et al. 2005; Chareprasert et al. 2012 Promputtha et al. 2007; Sieber 2007). There have been several studies on endophytes from *Dendrobium* orchids (Yuan et al. 2009; Chen et al. 2013; Ma et al. 2018).

Dendrobium is one of the largest genera in the family Orchidaceae and comprises many endangered and medicinal orchids (Ma et al. 2015). To document *Dendrobium* mycorrhizal and non-mycorrhizal endophytes is very important because they can impact the growth, development and the production of secondary metabolites (Pant et al. 2017; Ma et al. 2018). Six endophytic xylariaceous species were reported from *Dendrobium aphyllum* in China (Chen et al. 2013). *Colletotrichum orchidophilum* has been the only endophytic fungus found in *Dendrobium harveyanum* collected in northern Thailand so far (Ma et al. 2018). There has been no report on endophytic *Biscogniauxia* species associated with *Dendrobium* orchids.

The classification and phylogeny of *Biscogniauxia* has been discussed in depth (e.g. Tang et al. 2009) and currently it belongs to the family Graphostromataceae (Daranagama et al. 2018; Wendt et al. 2018; Wijayawardene et al. 2018). *Biscogniauxia* species are characterized by bipartite stromata that do not release KOH extractable pigments, 8-spored and unitunicate, cylindrical, short-pedicellate asci, with J+ apical ring, overlapping uniseriate ascospores

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Three new host records of endophytic *Neofusicoccum* species reported from *Dendrobium* orchids

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Abstract

Neofusicoccum species are endophytes, saprobes and opportunistic pathogens of many economic and ornamental plants. There are few reports of *Neofusicoccum* species are available to be associated with orchids worldwide. In this study, five endophytic *Neofusicoccum* strains were isolated from *Dendrobium* orchid leaves and stems in southwestern China and northern Thailand. Morphological characteristics and phylogenetic analysis of the combined partial nuclear rDNA internal transcribed spacer (ITS), partial RNA polymerase Ⅱ second largest subunit (*RPB2*), part of the translation elongation factor 1 alpha (EF-1α) and beta-tubulin (*TUB2*) dataset revealed that these isolates belong to two species *Neofusicoccum occulatum* and *N. parvum*. This is the first record of *Neofusicoccum occulatum* associated with *Dendrobium chrysanthum* while *N. parvum* associated with *D. harveyanum* and *D. moschatum*.

Keywords: *Botryosphaeriaceae*, endophytes, multi-genes, new host records, *Orchidaceae*

Introduction

Dendrobium Sw. is an important medicinal and ornamental genus in *Orchidaceae* (Bulpitt *et al.* 2007). Many species in this genus have been endangered according to The International Union for Conservation of Nature red list of threatened species (https://www.iucnredlist.org/search?query=Dendrobium&searchType=species). A considerable amount of the research on *Dendrobium* has focused on their cultivation and associated mycorrhizae (Cameron *et al.* 2007; Nontachaiyapoom *et al.* 2010; Shao *et al.* 2020).

Endophytes reside in plants asymptomatically (Hyde and Soytong 2008; Huang *et al.* 2009; DeMers and May 2020). Many species are endophytes and pathogens in the fungal order Botryosphaeriales on a wide range of woody plants (Yang *et al.* 2017; Jami *et al.* 2018). Especially, *Neofusicoccum* and *Phyllosticta* species in Botryosphaeriales are common endophytes in orchids (Yuan *et al.* 2009; Sawmya *et al.* 2013).

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ORIGINAL RESEARCH

Notes for genera: *Ascomycota*

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Abstract Knowledge of the relationships and thus the classification of fungi, has developed rapidly with increasingly widespread use of molecular techniques, over the past 10–15 years, and continues to accelerate. Several genera have been found to be polyphyletic, and their generic concepts have subsequently been emended. New names have thus been introduced for species which are phylogenetically distinct from the type species of particular genera. The ending of the separate naming of morphs of the same species in 2011, has also caused

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This paper is dedicated to the memory of K. Walter Gams, Hubertus Antonius van der Aa, Larissa N. Vasilyeva, E. Punithalingam and Vadim A. Mel'nik whose efforts contributed greatly to mycology.

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A new species of *Monilochaetes* from *Nothapodytes pittosporoides*

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Abstract

Monilochaetes nothapodytis sp. nov. was isolated from healthy *Nothapodytes pittosporoides* in Tongren City, Guizhou Province, China. Molecular analysis based on combined ITS and LSU sequence data showed that *M. nothapodytis* formed a distinct clade with the type species *M. infuscans* and other species in this genus. Morphological examination showed that *M. nothapodytis* differs from other *Monilochaetes* species in its host and in having aseptate, ellipsoidal conidia, the micro-conidiophores are shorter and the micro-conidia are smaller than in other species. The new species is described and illustrated and an updated phylogenetic tree is presented for Glomerellales.

Key words: ascomycetes, Australiascaceae, endophytic fungi, Glomerellales, taxonomy

Introduction

Nothapodytes pittosporoides (Oliv.) Sleum (Icacinaceae) is used in Traditional Chinese Medicine (TCM) and is distributed mainly in southern China (Fang 1981). There has been a wide, but unsubstantiated belief, in the scientific community, that endophytes in medicinal plants may be responsible for producing their bioactive compounds (Nisa *et al.* 2015, Hao *et al.* 2014, Heinig *et al.* 2013). This study was therefore initiated to isolate endophytes from *N. pittosporoides* in order to establish if the isolates could produce bioactive metabolites. Endophytes are an endosymbiotic group of microorganisms, often bacteria or fungi, that colonize the inter- and/or intracellular organs of plants (Hyde & Soytong 2008, Pimentel *et al.* 2011, Luis *et al.* 2013, Zambell & White 2015, Singh & Dubey 2015). In this study, endophytes from *N. pittosporoides* were isolated and identified. The paper focuses on an endophytic fungal isolate from a leaf of *N. pittosporoides* which produced its asexual morph in culture and belongs in Australiascaceae.

The family Australiascaceae accommodates the sexual genus *Australiasca* and the asexual genus *Monilochaetes* (Sivanesan & Alcorn 2002, Réblová *et al.* 2011a, Maharachchikumbura *et al.* 2015, 2016). The dematiaceous hyphomycete genus *Monilochaetes* was described and illustrated for a single species, *M. infuscans* Harter (Halsted 1890, Harter 1916, Lawrence 1981) that causes sweet-potato scurf. Réblová *et al.* (2011a) showed that *Dischloridium* was a synonym of *Monilochaetes* using phylogenies from analyses of ITS and LSU sequence data. Therefore, *Dischloridium laeënse* (Matsush.) B. Sutton was transferred to *Monilochaetes* and *Dischloridium* became a generic synonym of *Monilochaetes* (Réblová *et al.* 2011a). *Monilochaetes* presently contains *M. basicurvata* (Matsush.) Réblová & Seifert, *M. camelliae* (Alcorn & Sivan.) Réblová, W. Gams & Seifert, *M. dimorphospora* Réblová & W. Gams, *M. guadalcanalensis* (Matsush.) I.H. Rong & W. Gams, *M. infuscans* Harter, *M. regenerans* (Bhat & W.B. Kendr.) Réblová & Seifert and *M. laeënsis* (Matsush.) Réblová, W. Gams & Seifert (Réblová *et al.* 2011a, 2011b, Rong & Gams 2000, Harter 1916, Halsted 1890, Matsushima 1995, Bhat & Kendrick 1993).

The aim of the present paper is to introduce a new species of *Monilochaetes*, which was isolated as an endophyte of *Nothapodytes pittosporoides*. The new species is described and illustrated and evidence for its novelty is provided by a morphological comparison and analysis of ITS and LSU sequence data.

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RESEARCH ARTICLE

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Two new endophytic *Colletotrichum* species from *Nothapodytes pittosporoides* in China

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Abstract

Two new endophytic species, *Colletotrichum jishouense* **sp. nov.** and. *C. tongrenense* **sp. nov.** were isolated from *Nothapodytes pittosporoides* in Guizhou and Hunan provinces, China. Detailed descriptions and illustrations of these new taxa are provided and morphological comparisons with similar taxa are explored. Phylogenetic analysis with combined sequence data (ITS, GAPDH, ACT and TUB2) demonstrated that both species formed distinct clades in this genus. This is the first record of *Colletotrichum* species from *N. pittosporoides* in China.

Keywords

Ascomycota, Multi-loci, Phylogeny, Morphology, Taxonomy

Introduction

Nothapodytes pittosporoides (Oliv.) Sleum (Icacinacceae) has been used as Traditional Chinese Medicine (TCM) and is mainly distributed in southern China (Fang 1981). It is quickly gaining attention as the characteristic compounds of camptothecin and its derivatives (CIDs) in *N. pittosporoides* (Dong et al. 2015) are used as anti-cancer drugs in the world market (Demain and Vaishnav 2011). It is recognised that endophytes reside in the internal tissues of living plants and potentially have the capability to produce the same functional compounds as their hosts (Stierle et al. 1993, 1995; Kusari et al.

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WORK EXPERIENCE

2013–Present	Postgraduate studentship sponsored by Prof. Ji-Chuan Kang's grants of National Natural Science Foundation of China (Grants Nos. 31670027 & 31460011 & 30870009) and the Agricultural Science and Technology Foundation of Guizhou Province, China (Grant No. NY [2013]3042), studying in the Center of Excellence in Fungal Research International, Mae Fah Luang University
2018	Studied isolation and identification of secondary metabolites in Institute of Microbiology, Chinese Academy of Sciences 2017 –2018 1. Trained the skills for cell lines cultivation, storage and Recovery 2. Attended the International conference hold in the Center of Excellence in Fungal Research, Mae Fah Luang University

	3. Attended the Mycological conference in China and give an oral presentation on Endophytic Colletotrichum in Dendrobium orchdis
2016	1. Trained evolutionary molecular clock theory and phylogenetic analysis 2. Attended the Mycological conference in China.
2013 – 2015	Learned fungal endophytes isolation and identification skills
2013	Excellent Bachelor graduate thesis titled ‘The biodiversity of mushrooms on the Yun-Tai Mountain in Gui Zhou Province’ Gui Zhou University
2012	Awarded the second scholarship, Excellent Committee Leader, Academic Excellence student Gui Zhou University
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PUBLICATIONS (Totally 8 first-author papers and 5 co-author papers)

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