Asian Journal of Mycology 2(1): 183–197 (2019) ISSN 2651-1339

www.asianjournalofmycology.org **Article**

Doi 10.5943/ajom/2/1/9

The first report of *Daldinia eschscholtzii* as an endophyte from leaves of *Musa* sp. (Musaceae) in Thailand

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Samarakoon SMBC, Samarakoon MC, Aluthmuhandiram JVS, Wanasinghe DN, Chomnunti P 2019 – The first report of *Daldinia eschscholtzii* as an endophyte from leaves of *Musa* sp. (Musaceae) in Thailand. Asian Journal of Mycology 2(1), 183–197, Doi 10.5943/ajom/2/1/9

Abstract

There has been increasing research interest in the isolation of fungal endophytes from different hosts or tissue types of the same host from many tropical regions. However, there have been few studies conducted on musaceous endophytes in Thailand. In this study, we provide the first report of *Daldinia eschscholtzii* (Hypoxylaceae) as an endophyte isolated from symptomless fresh leaves of *Musa* sp. (Musaceae) in northern Thailand. In addition, this is the first record of *Daldinia* from Musaceae and the second report of *D. eschscholtzii* from monocotyledons. Fungi isolates are illustrated, described and subjected to LSU-ITS-RPB2-BTUB concatenated phylogenies using maximum likelihood and Bayesian analysis with an updated tree of Hypoxylaceae.

Keywords – Banana – Host – Hypoxylaceae – Monocots – Record

Introduction

Fungi exhibit various nutritional modes such as endophytes, saprobes and pathogens in different hosts and habitats (Wang et al. 2005, Hyde et al. 2018, Tibpromma et al. 2018, Jayawardena et al. 2019). Endophytes inhabit living and healthy tissues without causing apparent disease symptoms (Schulz & Boyle 2006). *Musa* sp. (banana) is a tropical monocotyledonous plant in family Musaceae. Several endophytic fungi have been recorded from *Musa* species in Australia (Brown et al. 1998), Brazil (Pereira et al. 1999), Central America (Pocasangre et al. 1999), China (Cao et al. 2002, 2003) and Hong Kong (Brown et al. 1998). *Acremonium* Link (Pocasangre et al. 1999, Cao et al. 2003), *Bipolaris* Shoemaker (Zakaria & Aziz 2018), *Colletotrichum* Corda (Brown et al. 1998, Cao et al. 2003), *Fusarium* Link, *Nigrospora* Zimm. (Brown et al. 1998), *Pestalotiopsis* Steyaert, *Phoma* Fr., *Trichoderma* Pers. and *Verticillium* Nees (Pocasangre et al. 1999, Cao et al. 2003) were the common genera that found as fungal endophytes from *Musa* sp. Cao et al. (2002)

Submitted 16 May 2019, Accepted 25 June 2019, Published 28 June 2019
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recorded *Alternaria* Nees, *Aspergillus* P. Micheli, *Aureobasidium* Viala & G. Boyer, *Cephalosporium* Corda, *Cladosporium* Link, *Deightoniella* S. Hughes, *Gloeosporium* Desm. & Mont., *Myxosporium* Link, *Sarcinella* Sacc., *Sphaceloma* de Bary and *Uncinula* Lév. from *Musa* sp. In addition, there are numerous xylariaceous taxa from Xylariaceae were also recorded as endophytes from *Musa* sp. (Brown et al. 1998, Pereira et al. 1999).

Photita et al. (2001, 2004) documented fungal endophytes of wild *M. acuminata* from northern Thailand including *Guignardia* Viala & Ravaz and *Periconiella* Sacc. The pathogenicity of endophytic *Cladosporium musae* E.W. Mason, *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Deightoniella torulosa* (Syd.) M.B. Ellis, *Guignardia cocoicola* Punith., *Neocordana musae* (Zimm.) Hern.-Restr. & Crous and *Periconiella musae* Stahel ex M.B. Ellis was evaluated and *D. torulosa* was identified to cause disease symptoms in *Musa* leaves *in vitro* (Photita et al. 2004).

The genus *Daldinia* (Hypoxylaceae) was introduced by Cesati & De Notaris (1863). Currently the genus accommodates around 50 taxa and distributed in terrestrial and marine habitats. The genus has frequently recorded from tropics compared to temperate regions with saprobic and endophytic nutritional modes (Johannesson et al. 2001, Guidot et al. 2003, Nugent 2004, Stadler et al. 2014, Wijayawardene et al. 2017).

Traditionally *Daldinia* was accommodated in Xylariaceae. Stadler et al. (2014) provided a detailed comprehensive study on the genus using multiple taxonomic approaches including type material studies, sexual/asexual morphs and the cultural characteristics coupled with Scanning Electron Microscopic (SEM) observations. HPLC chemical profiles, UV visible spectroscopy, mass spectroscopy and 5.8S/ITS nrDNA phylogenetic studies also were used.

Wendt et al. (2018) constructed a multigene phylogeny for stromatic Xylariales species based on ITS, LSU, RPB2 and BTUB sequence data. As a result, Xylariaceae was segregated into many major clades and *Daldinia* was accommodated in Hypoxylaceae. The morphological characteristics such as stromatal pigments and nodulisporium-like asexual morphs further supported the placement of *Daldinia* in Hypoxylaceae by Wendt et al. (2018). After Stadler et al. (2014) the genus was updated with two more new species with *D. korfii* Sir & C. Lambert (Sir et al. 2016) and *D. subvernicosa* Srikit. et al. (Wongkanoun et al. 2019).

Daldinia eschscholtzii was established in 1820 by Ehrenberg as *Sphaeria eschscholtzii*. Apart from the terrestrial plants, *D. eschscholtzii* has also been recorded in marine algae, mangroves, nails, skin and human blood (Karnchanatat et al. 2007, Zhang et al. 2008, Tarman et al. 2012, Kongyen et al. 2015, Ng et al. 2016, Helaly et al. 2018). The sexual morph of *D. eschscholtzii* appears as a turbinate to placentiform shaped stromata in warm and exposed habitats frequently on dead or decaying woody substrates of several angiosperms and few gymnosperms (Stadler et al. 2014). The asexual morph of *D. eschscholtzii* resembles hyaline hyphomycetes and the conidiogenous structures of *D. eschscholtzii* represent a nodulisporium-like branching pattern (Stadler et al. 2014, Wijayawardene et al. 2017).

In this study, we collected fresh symptomless leaf samples of *Musa* sp. from Chiang Rai Phu Chi Fah and Phayao in northern Thailand and isolated three endophytic *D. eschscholtzii* strains. We present the first report of *D. eschscholtzii* as a foliicolous endophyte in *Musa* sp. In addition, this is the first endophytic report of genus *Daldinia* in *Musa* sp. and the first molecular and morphological justification of the occurrence of *D. eschscholtzii* in monocotyledons.

Materials and methods

Fungal isolation morphological characterization

Symptomless fresh *Musa* leaves were collected from Chiang Rai, Phu Chi Fah and Phayao in northern Thailand. Small pieces (<5 mm^2) from the leaf tissues were separated using a sterilized scalpel, disinfected in 75% ethanol for 1 min, rinsed three times in sterile distilled water, dried and placed on potato dextrose agar (2% potato dextrose agar PDA). The plates were incubated at 25°C for at least 5 days until fungi developed. Hyphal tips were transferred to fresh PDA plates using a sterilized scalpel and incubated at 25°C temperature and light conditions. Asexual morphs were

observed from the cultures after 14 days using a Motic SMZ 168 Stereo Zoom Microscope. Observed conidia were used for single spore isolations and incubated at 25°C. Growth rates and cultural characteristics were documented. Living and dry cultures were deposited in the Mae Fah Luang University Culture Collection (MFLUCC) and Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand respectively.

DNA extraction and PCR amplification

Fungal isolates were grown on potato dextrose agar (PDA) for 4 weeks at 25°C and total genomic DNA was extracted from 50 to 100 mg of axenic mycelium of the growing culture (Wanasinghe et al. 2018). Mycelium was ground to a fine powder with liquid nitrogen and fungal DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, P.R. China) according to the instructions of the manufacturer. Four gene regions as ITS, partial 28S large subunit (LSU), partial beta-tubulin (BTUB) and partial second largest subunit of the DNA-directed RNA polymerase II (RPB2) were amplified using ITS5/ITS4 (White et al. 1990), LR0R/LR5 (Vilgalys & Hester 1990), T1/T2 (O'Donnell & Cigelnik 1997) and fRPB2-5f/fRPB2-7cR (Liu et al. 1999) primers respectively.

A total volume of 26.5 µl PCR mixture contained TaKaRa E-Taq DNA polymerase 0.3 µl, 12.5 µl of 2 × PCR buffer with 2.5 µl of dNTPs, 1 µl of each primer, 9.2 µl of double-distilled water and 100–500 ng of DNA template followed by thermal cycle programs described by Wanasinghe et al. (2014) and Wendt et al. (2018). All the PCR products were visualized by staining with ethidium bromide (EtBr) on 1.2 % agarose gels. Successful PCR products were purified according to the manufacturer's instructions of a Qiagen purification kit (Qiagen, USA) and sequenced in Sunbiotech Company, Beijing, China.

Sequencing and sequence alignment

Obtained sequences were subjected to BLAST search in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). BLAST search results and initial morphological studies have supported that our isolates have belonged to Hypoxylaceae. Other sequences used in the analyses were obtained from GenBank based on recently published data (Stadler et al. 2014, Wendt et al. 2018) (Table 1). The single gene alignments were automatically done by MAFFT v. 7.036 (http://mafft.cbrc.jp/alignment/server/index.html, Katoh et al. 2017) using the default settings and later refined where necessary, using BioEdit v. 7.0.5.2 (Hall 1999).

Table 1 Taxa that used in the phylogenetic analysis of *D. eschscholtzii* with the corresponding GenBank Accession numbers. Type strains are superscripted with T and newly generated strains are indicated bold.

Species	**Strain number**	**GenBank accession numbers**				**References**
		ITS	**LSU**	**RPB2**	**BTUB**	
Annulohypoxylon atroroseum	ATCC 76081	AJ390397	KY610422	KY624233	DQ840083	Kuhnert et al. (2014), Wendt et al. (2018)
A. michelianum	CBS 119993	KX376320	KY610423	KY624234	KX271239	Kuhnert et al. (2014), Wendt et al. (2018)
A. moriforme	CBS 123579	KX376321	KY610425	KY624289	KX271261	Kuhnert et al. (2014), Wendt et al. (2018)
A. nitens	MFLUCC 12-0823	KJ934991	KJ934992	KJ934994	KJ934993	Daranagama et al. (2015)

Table 1 Continued.

Species	Strain number	GenBank accession numbers				References
		ITS	LSU	RPB2	BTUB	
A. stygium	MUCL 54601	KY610409	KY610475	KY624292	KX271263	Wendt et al. (2018)
Daldinia andina^{T}	CBS 114736	-	KY610430	KY624239	KC977259	Kuhnert et al. (2014), Wendt et al. (2018)
D. bambusicola^{T}	CBS 122872	KY610385	KY610431	KY624241	AY951688	Hsieh et al. (2005), Wendt et al. (2018)
D. caldariorum	MUCL 49211	AM749934	KY610433	KY624242	KC977282	Bitzer et al. (2008), Kuhnert et al. (2014), Wendt et al. (2018)
D. concentrica	CBS 113277	AY616683	KY610434	KY624243	KC977274	Triebel et al. (2005), Kuhnert et al. (2014), Wendt et al. (2018)
D. dennisii^{T}	CBS 114741	JX658477	KY610435	KY624244	KC977262	Stadler et al. (2014), Kuhnert et al. (2014), Wendt et al. (2018)
D. eschscholtzii	MUCL 45435	JX658484	KY610437	KY624246	KC977266	Stadler et al. (2014)
D. eschscholtzii	CBS 113042	JX658497	-	-	-	Stadler et al. (2014)
D. eschscholtzii	CBS 113047	AY616684	-	-	-	Stadler et al. (2014)
D. eschscholtzii	CBS 116032	JX658500	-	-	-	Stadler et al. (2014)
D. eschscholtzii	CBS 116035	JX658498	-	-	-	Stadler et al. (2014)
D. eschscholtzii	CBS 116037	JX658492	-	-	-	Stadler et al. (2014)
D. eschscholtzii	CBS 116037(2)	JX658499	-	-	-	Stadler et al. (2014)
D. eschscholtzii	CBS 117735	JX658480	-	-	-	Stadler et al. (2014)
D. eschscholtzii	CBS 117740	JX658481	-	-	-	Stadler et al. (2014)
D. eschscholtzii	CBS 117741	JX658491	-	-	-	Stadler et al. (2014)
D. eschscholtzii	CBS 122876	JX658438	-	-	-	Stadler et al. (2014)

Table 1 Continued.

Species	Strain number	GenBank accession numbers				References
		ITS	LSU	RPB2	BTUB	
D. eschscholtzii	CBS 122877	JX658439	-	-	-	Stadler et al. (2014)
D. eschscholtzii	CBS 122878	JX658440	-	-	-	Stadler et al. (2014)
D. eschscholtzii	KC 1616	JX658496	-	-	-	Stadler et al. (2014)
D. eschscholtzii	KC1699	JX658490	-	-	-	Stadler et al. (2014)
D. eschscholtzii	MUCL 38740	JX658493	-	-	-	Stadler et al. (2014)
D. eschscholtzii	MUCL 41777	JX658486	-	-	-	Stadler et al. (2014)
D. eschscholtzii	MUCL 41778	JX658494	-	-	-	Stadler et al. (2014)
D. eschscholtzii	MUCL 43508	JX658495	-	-	-	Stadler et al. (2014)
D. eschscholtzii	MUCL 45434	JX658484	-	-	-	Stadler et al. (2014)
D. eschscholtzii	MUCL 47965	JX658482	-	-	-	Stadler et al. (2014)
D. eschscholtzii	**MFLUCC18-0177**	**MK587659**	**MK587746**	**MK625010**	**MK636689**	This study
D. eschscholtzii	**MFLUCC19-0154**	**MK587660**	**MK587747**	**MK625011**	**MK636690**	This study
D. eschscholtzii	**MFLUCC19-0153**	**MK587661**	**MK587748**	**MK625012**	**MK636691**	This study
D. korfii^{T}	STMA14089	KY204020	-	-	-	Sir et al. (2016)
D. loculatoides^{T}	CBS 113279	MH862918	KY610438	KY624247	KX271246	Johannesson et al. (2000), Stadler et al. (2014)
D. macaronesica^{T}	CBS 113040	KY610398	KY610477	KY624294	KX271266	Wendt et al. (2018)
D. petriniae^{T}	MUCL 49214	-	KY610439	KY624248	KC977261	Bitzer et al. (2008), Kuhnert et al. (2014), Wendt et al. (2018)
D. placentiformis	MUCL 47603	AM749921	KY610440	KY624249	KC977278	Bitzer et al. (2008), Kuhnert et al. (2014), Wendt et al. (2018)
D. pyrenaica^{T}	MUCL 53969	KY610413	-	KY624274	KY624312	Wendt et al. (2018)
D. steglichii^{T}	MUCL 43512	KY610399	KY610479	KY624250	KX271269	Wendt et al. (2018)

Table 1 Continued.

Species	Strain number	GenBank accession numbers				References
		ITS	LSU	RPB2	BTUB	
D. theissenii[T]	CBS 113044	KY610388	KY610441	KY624251	KX271247	Wendt et al. (2018)
D. vernicosa[T]	CBS 119316	KY610395	KY610442	KY624252	KC977260	Kuhnert et al. (2014), Wendt et al. (2018)
Entonaema liquescens	ATCC 46302	KY610389	KY610443	KY624253	KX271248	Wendt et al. (2018)
Hypoxylon carneum	MUCL 54177	KY610400	KY610480	KY624297	KX271270	Kuhnert et al. (2014), Wendt et al. (2018)
H. cercidicola	CBS 119009	KC968908	KY610444	KY624254	KC977263	Kuhnert et al. (2014), Wendt et al. (2018)
H. crocopeplum	CBS 119004	KC968907	KY610445	KY624255	KC977268	Kuhnert et al. (2014), Wendt et al. (2018)
H. fendleri	MUCL 54792	KF234421	KY610481	KY624298	KF300547	Kuhnert et al. (2014), Wendt et al. (2018)
H. fragiforme[T]	MUCL 51264	KC477229	KM186295	KM186296	KX271282	Stadler et al. (2014), Daranagama et al. (2015) ,Wendt et al. (2018)
H. fuscum[T]	CBS 113049	KY610401	KY610482	KY624299	KX271271	Wendt et al. (2018)
H. griseobrunneum[T]	CBS 331.73	KY610402	KY610483	KY624300	KC977303	Kuhnert et al. (2014), Wendt et al. (2018)
H. haematostroma[T]	MUCL 53301	KC968911	KY610484	KY624301	KC977291	Kuhnert et al. (2014), Wendt et al. (2018)
H. howeanum	MUCL 47599	AM749928	KY610448	KY624258	KC977277	Bitzer et al. (2008), Kuhnert et al. (2014), Wendt et al. (2018)
H. hypomiltum	MUCL 51845	KY610403	KY610449	KY624302	KX271249	Wendt et al. (2018)

Table 1 Continued.

Species	Strain number	GenBank accession numbers				References
		ITS	LSU	RPB2	BTUB	
H. investiens	CBS 118183	KC968925	KY610450	KY624259	KC977270	Kuhnert et al. (2014), Wendt et al. (2018)
H. lateripigmentum^{T}	MUCL 53304	KC968933	KY610486	KY624304	KC977290	Kuhnert et al. (2014), Wendt et al. (2018)
H. lenormandii	CBS 119003	KC968943	KY610452	KY624261	KC977273	Kuhnert et al. (2014), Wendt et al. (2018)
H. monticulosum^{T}	MUCL 54604	KY610404	KY610487	KY624305	KX271273	Wendt et al. (2018)
H. musceum	MUCL 53765	KC968926	KY610488	KY624306	KC977280	Kuhnert et al. (2014), Wendt et al. (2018)
H. papillatum^{T}	ATCC 58729	KC968919	KY610454	KY624223	KC977258	Kuhnert et al. (2014), Wendt et al. (2018)
H. perforatum	CBS 115281	KY610391	KY610455	KY624224	KX271250	Wendt et al. (2018)
H. petriniae^{T}	CBS 114746	KY610405	KY610491	KY624279	KX271274	Kuhnert et al. (2014), Wendt et al. (2018)
H. porphyreum	CBS 119022	KC968921	KY610456	KY624225	KC977264	Kuhnert et al. (2014), Wendt et al. (2018)
H. pulicicidum^{T}	CBS 122622	JX183075	KY610492	KY624280	JX183072	Bills et al. (2012), Wendt et al. (2018)
H. rubiginosum^{T}	MUCL 52887	KC477232	KY610469	KY624266	KY624311	Stadler et al. (2013), Wendt et al. (2018)
H. samuelsii^{T}	MUCL 51843	KC968916	KY610466	KY624269	KC977286	Kuhnert et al. (2014), Wendt et al. (2018)
H. submonticulosum^{T}	CBS 115280	KC968923	KY610457	KY624226	KC977267	Kuhnert et al. (2014), Wendt et al. (2018)

Table 1 Continued.

Species	Strain number	GenBank accession numbers				References
		ITS	LSU	RPB2	BTUB	
H ticinense	CBS 115271	JQ009317	KY610471	KY624272	AY951757	Hsieh et al. (2005), Wendt et al. (2018)
H. trugodes[T]	MUCL 54794	KF234422	KY610493	KY624282	KF300548	Kuhnert et al. (2014), Wendt et al. (2018)
Jackrogersella multiformis[T]	CBS 119016	KC477234	KY610473	KY624290	KX271262	Kuhnert et al. (2014), Kuhnert et al. (2016), Wendt et al. (2018)
Pyrenopolyporus nicaraguensis	CBS 117739	AM749922	KY610489	KY624307	KC977272	Bitzer et al. (2008), Wendt et al. (2018)
Rhopalostroma angolense	CBS 126414	KY610420	KY610459	KY624228	KX271277	Wendt et al. (2018)
Rostrohypoxylon terebratum[T]	CBS 119137	DQ631943	DQ840069	DQ631954	DQ840097	Tang et al. (2007), Fournier et al. (2010)
Ruwenzoria pseudoannulata[T]	MUCL 51394	KY610406	KY610494	KY624286	KX271278	Wendt et al. (2018)
Thamnomyces dendroidea	CBS 123578	FN428831	KY610467	KY624232	KY624313	Stadler et al. (2010), Wendt et al. (2018)
Xylaria hypoxylon[T]	CBS 122620	KY610407	KY610495	KY624231	KX271279	Wendt et al. (2018)
X. polymorpha	MUCL 49884	KY610408	KY610464	KY624288	KX271280	Wendt et al. (2018)

*ATCC: American Type Culture Collection, Virginia, USA: CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, KC: Kew Culture Collection, United Kingdom, MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand, MUCL: Université Catholique de Louvain, Belgium, STMA: Personal Herbarium and Culture Collection of M. Stadler.

Phylogenetic analysis

Maximum likelihood (ML) trees were generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using GTR+I+G model of evolution.

A Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronqvist 2001) to evaluate Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). Two parallel runs were conducted, using the default settings, but with the following adjustments: Four simultaneous Markov chains were run for 2,000,000 generations and trees were sampled every 100th generation and 20,000 trees were

obtained. The first 4,000 trees, representing the burnin phase of the analyses and discarded. The remaining 16,000 trees were used for calculating PP in the majority rule consensus tree.

Phylograms were visualized with FigTree v1.4.0 program (Rambaut 2011) and reorganized in Microsoft power point.

Results

Phylogenetic analysis of *D. eschscholtzii*

The combined LSU, ITS, RPB2 and BTUB matrix comprised 76 sequences including selected genera in Hypoxylaceae, *D. eschscholtzii* (MFLUCC18-0177, MFLUCC19-0154, MFLUCC19-0153) strains and *Xylaria hypoxylon* (CBS 122620) and *X. polymorpha* (MUCL 49884) (Xylariaceae) as out group taxa (Fig. 1). Four different alignments of each individual gene and a combined alignment of four genes were analyzed in this study. A best scoring RAxML tree is shown in Fig. 1 with a final ML optimization likelihood value of -35661.5741. The matrix had 1340 distinct alignment patterns, with 28.10% of undetermined characters or gaps. Estimated base frequencies were as follows; A=0.246955, C=0.244469, G=0.266113, T=0.242464; substitution rates AC=1.597317, AG=4.770781, AT=1.560157, CG=1.203822, CT=7.713893, GT=1.000000; proportion of invariable sites I=0.466559; gamma distribution shape parameter α=0.816750. All trees (ML and BYPP) were similar in topology and did not differ significantly (data not shown) at the generic relationships which is in agreement with previous study based on multi-gene phylogeny of Stadler et al. (2014) and Wendt et al. (2018). Bootstrap support (BS) values of ML (equal or greater than 60 % based on 1000 replicates) are shown on the upper branches with blue. Branches with Bayesian posterior probabilities (PP) greater than 0.95 from MCMC analyses are given in blue. Our isolates (MFLUCC18-0177, MFLUCC19-0154, MFLUCC19-0153) were clustered with in *D. eschscholtzii* with a significant bootstrap support (ML=100%, PP=1.00).

Taxonomy

***Daldinia eschscholtzii* (Ehrenb.) Rehm, Annls mycol. 2(2): 175 (1904)** Fig. 2

Index Fungorum Number: IF544992; Facesoffungi Number: FoF02990

Endophytic on fresh leaves of *Musa* sp. *Colonies* on PDA at 25°C temperature, light, reaching 7 cm in two weeks, initially white with a diffuse margin. Colonies become gray, with olive green and later become dull green spots. When mature purple pigmentation occurs from the center of the colony. Sexual morph: *Stromatic structures* arising from the surface after 8 weeks, convexly curved, swelled, sterile. Asexual morph: Sporulation after two to three weeks, under 25°C, entire surface after three weeks, becomes gray after four weeks, reverse black at the center and whitish gray at the periphery after two weeks. *Mycelium* 1–3.5 µm ($\bar{x}$=2.75 µm) wide, superficial, composed of septate, branched, rough, inflated, often have melanized hyphae with brownish exudates in old cultures. *Conidiophores* 0.8–2 µm long × 0.7–1.5 µm diam. ($\bar{x}$=1.5 × 1.3 µm, n=10) hyaline, mononematous synonymous, short, small, conidiogenous structure dichotomous or trichotomous with nodulisporium-like branching pattern, 1–3 conidiogenous cells arise from each terminus. *Conidiogenous cells* 2.8–4 ×1.7–5 µm ($\bar{x}$=3.4 × 2.1 µm, n=10), hyaline, holoblastic, terminal or intercalary, cylindrical, having rounded apices, texture, collaret or opening width. *Conidia* 3–5.5 µm × 2–3.5 µm ($\bar{x}$=4.6 × 2.5 µm, n=40), obovoid to ellipsoid, aseptate, hyaline, smooth often flat at the base.

Materials examined **–** THAILAND, Chiang Rai, in symptomless fresh leaves of *Musa* sp., 15 October 2017, BC. Samarakoon, BN021 (5) (MFLU 19–0409), living culture MFLUCC 18–0177. Phayao, in symptomless fresh leaves of *Musa* sp., 23 January 2018, BC. Samarakoon, BNE003 (MFLU 19–0408), living culture MFLUCC 19–0154. Phu Chi Fah, in symptomless fresh leaves of *Musa* sp. 17 March 2018, BC. Samarakoon, BNE002 (MFLU 19–0407), living culture MFLUCC 19–0153.

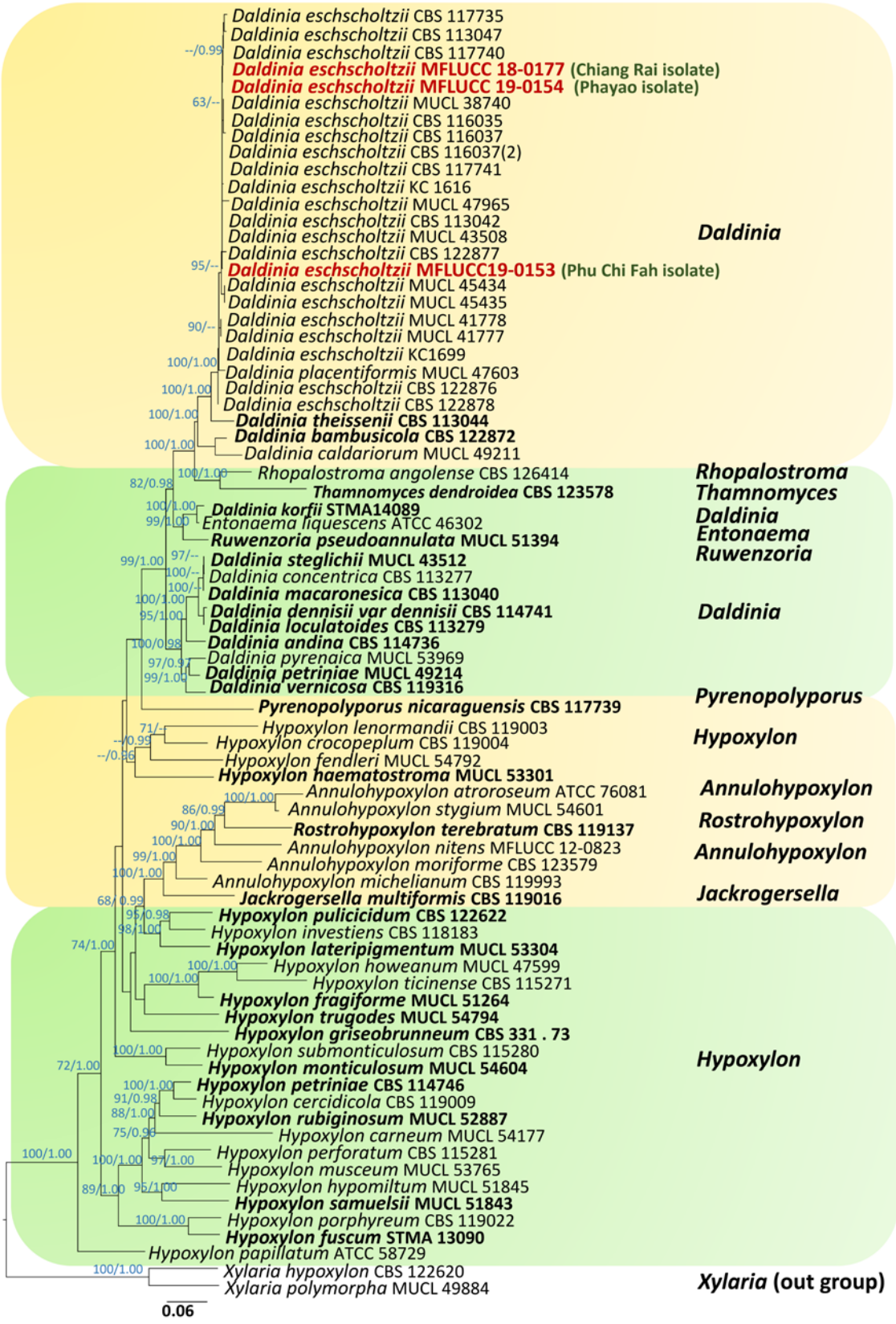

Fig. 1 – Maximum likelihood tree revealed by RAxML from an analysis of combined LSU-ITS-RPB2 and BTUB rDNA matrix of *Daldinia*, showing the phylogenetic position of *D. eschscholtzii*. ML bootstrap supports (≥60 %) and Bayesian posterior probabilities (≥ 0.95 PP) are given above in the branches respectively. The tree was rooted with *Xylaria polymorpha* and *X. hypoxylon* (Xylariaceae). Strain generated in this study is indicated in red-boldface, and type strains are in black-boldface. The scale bar represents the expected number of nucleotide substitutions per site.

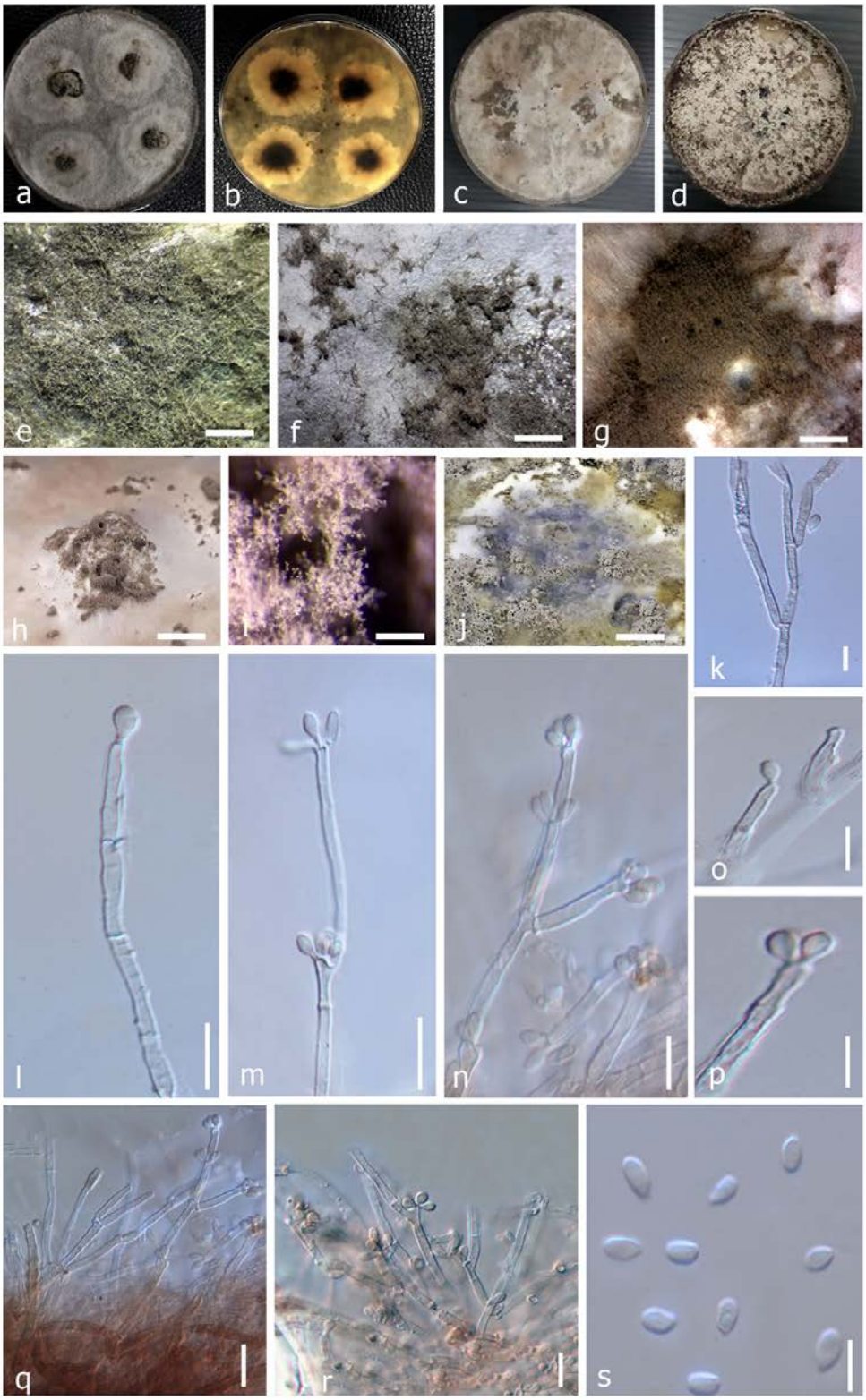

Fig. 2 – *Daldinia eschscholtzii*. a, b immature colony on PDA after 3 weeks. c Immature colony on PDA after 2 weeks. d Colonies on PDA after 8 weeks bearing sterile stromatic structures showing sporulation in mouse gray. e greenish patches on PDA. f–i Sporulation of the colony showing conidiomata on PDA. j Pigmentation on PDA and sterile stromatic structures. k Mycelium with nodulisporium-like branching pattern. l, m Conidial attachments and conidiogenous cells showing sporothrix-like branching pattern in the mycelium. n–r Conidial attachments and conidiogenous cells showing nodulisporium-like branching pattern in the mycelium s Conidia. a, b, e, f, j, k, l–r from MFLUCC 18–0177; c, g, h, i from MFLUCC 19–0154; d from MFLUCC 19–0153. Scale bars: e–j = 200 μm, k–r = 10 μm, s = 2μm

Discussion

Studies about musaceous endophytes in Thailand were conducted by Photita et al. (2001, 2004) who isolated 61 fungal taxa from Doi Suthep Pui National Park, but did not conduct any molecular justification. To address this research gap, we are reinvestigating the fungi on *Musa* sp. in different geographic regions around the country, isolate them into a culture, and describe their morphology and phylogenetic relationships. During our study so far, we have isolated many taxa that have previously been recorded in Photita et al. (2001, 2004) with confirmed molecular data (not shown).

Few studies found that nonpathogenic endophytic strains of *Fusarium oxysporum* Schltdl. that have been isolated from healthy banana rhizomes can induce systemic resistance of the plant against *Radopholus similis* (pathogenic root nematode) in *Musa* sp. (Vu et al. 2006). In addition, endophytic *F. oxysporum* strains have controlled the nematode reproduction and damage (Sikora et al. 2008) and acted as potential growth promoters in *Musa* sp. (Ting et al. 2008). Therefore, the role of endophytic fungi in *Musa* sp. should be further investigated.

Daldinia graminis Dargan & K.S. Thind and *D. sacchari* Dargan & K.S. Thind was recorded from sugarcane plant in India. In addition *D. bambusicola* Y.M. Ju, J.D. Rogers & F. San Martín was found from bamboo in Thailand (Stadler et al. 2014). As a conclusion, four *Daldinia* species have so far been found on monocots including *D. eschscholtzii*.

Production of stromata is a characteristic feature of all the daldinoid clades in Hypoxylaceae. Generally, in natural habitats, the stromatic structures support the fungi to survive in harsh environmental conditions (Stadler et al. 2014). The stromata of *D. eschscholtzii* produce bioactive compounds (secondary metabolites) such as binaphthalene derivatives (BNT), cytochalasins and concentricols (Stadler et al. 2014, Zhang et al. 2008, 2011). In addition Helaly et al. (2018) have documented, a mantis-associated culture of *D. eschscholtzii* produces dalesconols and spirodalesol, which have strong immunomodulatory effects. Interestingly our asexual cultures of *D. eschscholtzii* isolates had a limited lifespan compared to the other endophytic isolates. This can be due to the self-poisoning of the cultures as a result of releasing antibiotic compounds (Stadler et al. 2014). Therefore, future studies will be rather warranted to extract the bioactive compounds from our *D. eschscholtzii* isolates and investigate the functions of the compounds on the host or commercial industry.

Acknowledgements

The authors like to acknowledge the National Research Council of Thailand (Grant No. 256108A3070006) and Mae Fah Luang University (Grant No. DR256201012003) for the financial support. Dhanushka Wanasinghe would like to thank CAS President's International Fellowship Initiative (PIFI) for funding his postdoctoral research (number 2019PC0008), the National Science Foundation of China and the Chinese Academy of Sciences for financial support under the following grants: 41761144055, 41771063 and Y4ZK111B01. We would like to thank Kevin D. Hyde, Q. Tian, Chuangen Lin, W.A.E. Yasanthika, C.S. Bhunjun and G. Samarakoon for the valuable suggestions they have made.

References

Bills GF, Gonzalez-MenendezV MJ, Platas G, Fournier J et al. 2012 – *Hypoxylon pulicicidum* sp. nov. (Ascomycota, Xylariales), a pantropical insecticide-producing endophyte. PLoS One 7:e46687

Bitzer J, Læssøe T, Fournier J, Kummer V et al. 2008 – Affinities of *Phylacia* and the daldinoid Xylariaceae, inferred from chemotypes of cultures and ribosomal DNA sequences. Mycological Research 112:251–270.

Brown KB, Hyde KD, Guest DI. 1998 – Preliminary studies on endophytic fungal communities of *Musa acuminata* species complex in Hong Kong and Australia. Fungal Diversity 1, 27–51.

Cao LX, Tian XL, Zhou SN. 2003 – Isolation of endophytic fungi and Actinomycetes from banana (*Musa paradisiaca*) plants. Acta Scientiarum Naturalium Universitatis Sunyatseni 2, 20–23.
Cao LX, You JL, Zhou SN. 2002 – Endophytic fungi from *Musa acuminata* leaves and roots in South China. World Journal of Microbiology and Biotechnology 18, 169–171. https://doi.org/10.1023/A:1014491528811
Cesati V, De Notaris G. 1863 – Schema di classificazione degle sferiacei italici aschigeri piu' o meno appartenenti al genere Sphaeria nell'antico significato attribuitoglide Persono. Commentario della Società Crittogamologica Italiana 1, 177–420.
Daranagama DA, Camporesi E, Tian Q, Liu X et al. 2015 – *Anthostomella* is polyphyletic comprising several genera in Xylariaceae. Fungal Diversity 73, 203–238.
Fournier J, Stadler M, Hyde KD, Duong LM. 2010 – The new genus *Rostrohypoxylon* and two new *Annulohypoxylon* species from Northern Thailand. Fungal Diversity 40, 23–36.
Guidot A, Johannesson H, Dahlberg A, Stenlid J. 2003 – Parental tracking in the postfire wood decay ascomycete *Daldinia loculata* using highly variable nuclear gene loci. Molecular Ecology 12, 1717–1730. https://doi.org/10.1046/j.1365-294X.2003.01858.x
Hall TA. 1999 – BioEdit: a user–friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98. Available from: http://www.mbio.-ncsu.edu/bioedit/bioedit.html
Helaly SE, Thongbai B, Stadler M. 2018 – Diversity of biologically active secondary metabolites from endophytic and saprotrophic fungi of the ascomycete order Xylariales. Natural product reports 35, 992–1014.
Hsieh HM, Ju YM, Rogers JD. 2005 – Molecular phylogeny of *Hypoxylon* and closely related genera. Mycologia 97, 844–865.
Huelsenbeck JP, Ronquist F. 2001 – MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755. https://doi.org/10.1093/bioinformatics/17.8.754
Hyde KD, Norphanphoun C, Chen J, Dissanayake AJ et al. 2018 – Thailand's amazing diversity: up to 96% of fungi in northern Thailand may be novel. Fungal Diversity 93, 215–239. https://doi.org/10.1007/s13225-018-0415-7
Jayawardena RS, Hyde KD, Jeewon R, Ghobad–Nejhad M et al. 2019 – One stop shop II: taxonomic update with molecular phylogeny for important phytopathogenic genera: 26–50. Fungal Diversity 94, 41–129. https://doi.org/10.1007/s13225-019-00418-5
Johannesson H, Laessøe T, Stenlid J. 2000 – Molecular and morphological investigation of the genus *Daldinia* in Northern Europe. Mycological Research 104, 275–280.
Johannesson H, Vasiliauskas R, Dahlberg A, Penttilä R, Stenlid J. 2001 – Genetic differentiation in Eurasian populations of the postfire ascomycete *Daldinia loculata*. Molecular Ecology 10, 1665–1677. https://doi.org/10.1046/j.1365-294X.2001.01317.x
Karnchanatat A, Petsom A, Sangvanich P, Piaphukiew J et al. 2007 – Purification and biochemical characterization of an extracellular beta–glucosidase from the wood–decaying fungus *Daldinia eschscholzii* (Ehrenb.:Fr.) Rehm. FEMS Microbiology Letters 270, 162–70. https://doi.org/10.1111/j.1574-6968.2007.00662.x
Katoh K, Rozewicki J, Yamada KD. 2017 – MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics. bbx108. https://doi.org/10.1093/bib/bbx108
Kongyen W, Rukachaisirikul V, Phongpaichit S, Sakayaroj J. 2015 – A new hydronaphthalenone from the mangrove–derived *Daldinia eschscholtzii* PSU–STD57. Natural Product Research 29, 1995–1999. https://doi.org/10.1080/14786419.2015.1022542
Kuhnert E, Fournier J, Peršoh D, Luangsa-ard JJD, Stadler M. 2014 – New *Hypoxylon* species from Martinique and new evidence on the molecular phylogeny of *Hypoxylon* based on ITS rDNA and β-tubulin data. Fungal Diversity 64,181–203.
Liu YJ, Whelen S, Hall BD. 1999 – Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Molecular Biology and Evolution 16, 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Miller MA, Pfeiffer W, Schwartz T. 2010 – Creating the CIPRES science gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), November 14, 2010, New Orleans, Louisiana 1–8. https://doi.org/10.1109/GCE.2010.5676129

Ng KP, Chan CL, Yew SM, Yeo SK et al. 2016 – Identification and characterization of *Daldinia eschscholtzii* isolated from skin scrapings, nails, and blood. PeerJ 4, e2637. https://doi.org/10.7717/peerj.2637

Nugent LK. 2004 – Latent invasion by Xylariaceous fungi. PhD Thesis. Liverpool John Moores University, Liverpool, UK.

O'Donnell K, Cigelnik E. 1997 – Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Molecular Phylogenetics and Evolution 7, 103–116. https://doi.org/10.1006/mpev.1996.0376

Pereira JO, Vieira MC, Azevedo JL. 1999 – Endophytic fungi from *Musa acuminata* and their reintroduction into axenic plants. World Journal of Microbiology and Biotechnology 15, 37–40. https://doi.org/10.1023/A:1008859823806

Photita W, Lumyong S, Lumyong P. 2001 – Endophytic fungi of wild banana (*Musa acuminata*) at Doi Suthep Pui National Park, Thailand. Mycological Research 105, 1508–1513. https://doi.org/10.1017/S0953756201004968

Photita W, Lumyong S, Lumyong P, McKenzie EHC, Hyde KD. 2004 – Are some endophytes of *Musa acuminata* latent pathogens? Fungal Diversity 16, 131–140.

Pocasangre L, Sikora RA, Vilich V, Schuster RP. 1999 – Survey of banana endophytic fungi from Central America and screening for biological control of *Radopholus similis*. In II ISHS Conference on Fruit Production in the Tropics and Subtropics 531, 283–290. https://doi.org/10.17660/ActaHortic.2000.531.47

Rambaut A. 2011 – FigTree. Tree figure drawing tool version 1.3.1, Institute of Evolutionary Biology, University of Edinburgh. Available from: http://tree.bio.ed.ac.uk/software/figtree/ (accessed 20 June 2019)

Rannala B, Yang Z. 1996 – Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. Journal of Molecular Evolution 43, 304–311. https://doi.org/10.1007/BF02338839

Schulz B, Boyle C. 2006 – What are endophytes? In Microbial root endophytes pp. 1–13. Springer, Berlin, Heidelberg. https://doi.org/10.1007/3-540-33526-9_1

Sikora RA, Pocasangre L, zum Felde A, Niere B et al. 2008 – Mutualistic endophytic fungi and in–planta suppressiveness to plant parasitic nematodes. Biological Control 46, 15–23. https://doi.org/10.1016/j.biocontrol.2008.02.011

Sir EB, Lambert C, Wendt L, Hladki AI et al. 2016 – A new species of *Daldinia* (Xylariaceae) from the Argentine subtropical montane forest. Mycosphere 7, 1378–1388. https://doi.org/10.5943/mycosphere/7/9/11

Stadler M, Laessoe T, Fournier J, Decock C et al. 2014 – A polyphasic taxonomy of *Daldinia* (Xylariaceae). Studies in Mycology 77, 1–143. https://doi.org/10.3114/sim0016

Stamatakis A. 2014 – RAxML version 8: a tool for phylogenetic analysis and post–analysis of large phylogenies. Bioinformatics 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stamatakis A, Hoover P, Rougemont J. 2008 – A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57, 758–771. https://doi.org/10.1080/10635150802429642

Tang AM, Jeewon R, Hyde KD. 2007 – Phylogenetic relationships of *Nemania plumbea* sp. nov. and related taxa based on ribosomal ITS and RPB2 sequences. Mycological Research 111, 392–402.

Tibpromma S, Hyde KD, Bhat JD, Mortimer PE et al. 2018 – Identification of endophytic fungi from leaves of Pandanaceae based on their morphotypes and DNA sequence data from southern Thailand. MycoKeys 33, 25–67. https://doi.org/10.3897/mycokeys.33.23670

Ting AS, Meon S, Kadir J, Radu S, Singh G. 2008 – Endophytic microorganisms as potential growth promoters of banana. BioControl 53, 541–553. https://doi.org/10.1007/s10526-007-9093-1

Triebel D, Peršoh D, Wollweber H, Stadler M. 2005 – Phylogenetic relationships among *Daldinia*, *Entonaema* and *Hypoxylon* as inferred from ITS nrDNA sequences. Nova Hedwigia 80, 25–43.

Vilgalys R, Hester M. 1990 – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172, 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Vu T, Sikora R, Hauschild R. 2006 – *Fusarium oxysporum* endophytes induced systemic resistance against *Radopholus similis* on banana. Nematology 8, 847–852. https://doi.org/10.1163/156854106779799259

Wanasinghe DN, Jeewon R, Jones EG, Boonmee S et al. 2018 – Novel palmicolous taxa within Pleosporales: multigene phylogeny and taxonomic circumscription. Mycological Progress 17, 571–590. https://doi.org/10.1007/s11557-018-1379-4

Wanasinghe DN, Jones EBG, Camporesi E, Boonmee S et al. 2014 – An exciting novel member of Lentitheciaceae in Italy from *Clematis vitalba*. Cryptogamie Mycologie 35, 323–337. https://doi.org/10.7872/crym.v35.iss4.2014.323

Wang YU, Guo LD, Hyde KD. 2005 – Taxonomic placement of sterile morphotypes of endophytic fungi from *Pinus tabulaeformis* (Pinaceae) in northeast China based on rDNA sequences. Fungal Diversity 20, 235–260.

Wendt L, Sir EB, Kuhnert E, Heitkämper S et al. 2018 – Resurrection and emendation of the Hypoxylaceae, recognised from a multigene phylogeny of the Xylariales. Mycological Progress 17, 115–154. https://doi.org/10.1007/s11557-017-1311-3

White TJ, Bruns T, Lee SJWT, Taylor JL. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18, 315–322.

Wijayawardene NN, Hyde KD, Rajeshkumar KC, Hawksworth DL et al. 2017 – Notes for genera: Ascomycota. Fungal Diversity 86, 1–594. https://doi.org/10.1007/s13225-017-0386-0

Wongkanoun S, Wendt L, Stadler M, Luangsa–ard J, Srikitikulchai P. 2019 – A novel species and a new combination of *Daldinia* from Ban Hua Thung community forest in the northern part of Thailand. Mycological Progress 18, 553–564. https://doi.org/10.1007/s11557-019-01469-3

Zakaria L, Aziz W. 2018 – Molecular identification of endophytic fungi from banana leaves (*Musa* spp.). Tropical Life Sciences Research 29, 201–211. https://doi.org/10.21315/tlsr2018.29.2.14

Zhang YL, Ge HM, Zhao W, Dong H et al. 2008 – Unprecedented immunosuppressive polyketides from *Daldinia eschscholzii*, a mantis–associated fungus. Angewandte Chemie International Edition in English 47, 5823–5826. https://doi.org/10.1002/anie.200801284

Zhang YL, Zhang J, Jiang N, Lu YH et al. 2011 – Immunosuppressive polyketides from mantis–associated *Daldinia eschscholzii*. Journal of the American Chemical Society 133, 5931–5940. https://doi.org/ 10.1021/ja110932p

Zhaxybayeva O, Gogarten JP. 2002 – Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. BMC genomics 3, 4. https://doi.org/10.1186/1471-2164-3-4