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The first record of *Daldinia eschscholtzii* on *Phegopteris* species from Northern Thailand

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Abstract

There is a scarcity of fungal documentation associated with seedless vascular plants, including ferns and club mosses. In our continuous investigations focused on identifying fungi associated with pteridophytes from northern Thailand, endophytic fungi were isolated from symptomless beach ferns. Within our cultures, a hyphomycetous fungus that exhibited nodulisporium-like branching patterns was identified. Mononematous conidiophores were observed in our isolate, with polyblastic conidiogenous cells. They were bearing hyaline to olivaceous, obovoid comparatively smaller conidia. We confirmed this fungus as *Daldinia eschscholtzii* using a combination of culture-induced asexual morphology and combined phylogenetic analysis of LSU, ITS, *rpb2* and *tub2* sequences. This discovery represents the first report of *Daldinia eschscholtzii* on *Phegopteris* species in Thailand.

Keywords – Basal vascular plants – Endophytes – Host record – *Hypoxylaceae* – *Sordariomycetes*

Introduction

Fungi play a crucial role in synthesizing a wide range of compounds while contributing significantly to the integrity and stability of ecosystems (Hyde et al. 2018). According to high throughput sequencing analysis, approximately 11.7 to 13.2 million fungal species are estimated (Hyde 2022). Despite this, only around 150,000 fungal species have been described (Bhunjun et al. 2022, Hyde et al. 2022, Phukhamsakda et al. 2022). The "missing fungi" will likely to be discovered in understudied hosts and geographical areas (Hyde 2001, Hyde et al. 2018). Hence, focusing on understudied basal vascular plants, including ferns, could result in a large diversity of novel taxa.

The pteridophytes such asferns, are a diverse and an ancient vascular plant lineage that differs from angiosperms in morphology, chemical composition, and growth habits (Olmo-Ruiz & Arnold 2014). They are the second-largest vascular plant group, with approximately 13,000 species (Kreft et al. 2010, Schneider et al. 2004). They are associated with diverse fungal groups including epiphytic lichens and microfungi (Godfrey 1974, Medel & Lorea-Hernández 2008), pathogens (Bauer et al. 1999, Berndt 2008, Ellett 1989, Helfer 2006), saprobes (Frankland 1966, Nagao & Doi 1996, Raitviir & Schneller 2007), and arbuscular mycorrhizae (Wang & Qiu 2006, Winther & Friedman 2007, Zhang

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et al. 2004). Endophytes from ferns have not been extensively studied due to their concealed presence within healthy plant tissues. The basal vascular plant *Phegopteris* is commonly known as the "beach fern" and contains 27 species (POWO 2023). *Phegopteris* is an apomictic tetraploid plant with a cosmopolitan distribution (Patel et al. 2019). Rust fungi such as *Herpobasidium filicinum* (Braun. 1982), *Hyalopsora* species, *Incrupila aspidii* (Kirschner et al. 2019, Ji et al. 2018) and *Uredinopsis* species (*Pucciniastraceae, Pucciniales*) (Bubner et al. 2019), *Leptopeltis gregaria, Nannfeldtia phegopteridis* (Hiratsuka et al. 1992) and Chytridiomycota like, *Synchytrium phegopteridis* (Ji et al. 2018) have been reported from *Phegopteris* species.

Xylariaceae is the largest family within *Xylariomycetideae* and the majority of species are wood-degrading fungi, and *Daldinia* was classified within *Xylariaceae* (Stadler 2011*).* Subsequently, Wendt et al*.* (2018) redefined genera like *Annulohypoxylon*, *Daldinia*, and *Hypoxylon*, along with others that have similar asexual and sexual characteristics, into the resurrected *Hypoxylaceae* family, based on phylogenetic analysis*.* Among them, *Daldinia* is one of the largest genera in the family *Hypoxylaceae* harbouring over 50 species (Wongkanoun et al. 2020). Some *Daldinia* species have prominent, stromata that develop on ligneous plants and can appear in masses at times (Stadler et al. 2014). Thus, they are unlikely to be overlooked. The two worldwide monographs of *Daldinia* were done by Child (1932) and Ju et al. (1997), respectively. The latest monograph was provided by Stadler et al. (2014) using molecular data, micromorphological data, and chemical profiles of cultured specimens, herbarium specimens, and fresh collections of *Daldinia* and other related species. Stadler et al. (2014) provided an extensive key and demonstrated that *Daldinia* species formed a distinct clade from *Annulohypoxylon* and *Hypoxylon*. Furthermore, Wendt et al. (2018) and Wibberg et al. (2021) reaffirmed the distinct position of *Daldinia* species within *Hypoxylaceae* through multi-gene analysis.

Daldinia eschscholtzii is widely distributed in tropical regions and has previously been reported from marine algae, mangroves, nails, skin, human blood, as well as terrestrial plants (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 *D. eschscholtzii* sexual morph is defined by placentiform to turbinate stromata in exposed and warm environments, often on dead woody substrates of a few gymnosperms and various angiosperms (Stadler et al. 2014). In accordance with Stadler et al. (2014), the asexual form is identified by hyaline hyphomycetes, with a nodulisporium-like branching pattern. In this study, we describe *Daldinia eschscholtzii* as an endophyte for the first time from a basal fern.

Materials & Methods

Sampling and isolation

On 18th December 2022, healthy and fresh leaves of *Phegopteris* species were gathered at the Mushroom Research Centre (MRC) in Pha Deng Village, Mae Taeng, Chiang Mai Province (19.11758° N, 98.73099° E). They were kept in plastic bags and transported to the laboratory. Within 48 hours, the leaves were washed under running tap water after collecting. The washed leaves were dried and cut into approximately 1 × 1 cm segments. The leaves were surface sterilized by immersing in sodium hypochlorite (0.5%) for 2 minutes, and 3 subsequent rinses with sterile distilled water for 3 minutes. Subsequently, the leaves were dipped in ethanol (70%) for 2 minutes and thoroughly washed 3 more times with sterile distilled water for 3 minutes (Gao et al. 2019, Bhunjun et al. 2021). Afterward, they were dried on sterile filter paper. The sterilized leaf segment was divided into four pieces and transferred to a petri dish with potato dextrose agar (PDA) with 0.5 g/l streptomycin sulphate. The plate was incubated at 25 °C under dark conditions and hyphal tips growing out of the plant tissues were transferred to fresh PDA plates. The pure cultures were preserved on glycerol, PDA slants, and sterile distilled water and maintained at 4 °C for further studies.

Morphological studies

The isolate was initially identified based on morphological characteristics in a sporulated culture (Stadler et al. 2014). Pure culture was used to prepare microscopic slides of the isolated fungi for morphological identification. A small amount of mycelium was picked with sterilized needles and

mounted on clean slides with 1% KOH (Kumaresan et al. 2013, Senanayake et al. 2020). The micro-morphology was investigated using a Leica EZ4 Educational stereo microscope (Leica, Wetzlar, Germany) and images were captured with a Canon 750D digital camera attached to the Nikon ECLIPSE Ni compound microscope (Nikon, Japan). Tarosoft® Imageframework v.0.9.7 software (Tarosoft, Thailand) was used to measure fungal structures, and the plates were prepared using Adobe Photoshop CS6 version 13.1.2 (Adobe Systems, USA). Living and dried cultures were deposited in Mae Fah Luang University Herbarium (MFLU) and Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand. Plates and descriptions are submitted to the Greater Mekong Subregion website (Chaiwan et al. 2021).

DNA extraction and polymerase chain reaction

Fresh fungal mycelium on PDA was used to extract DNA with the E.Z.N.A.® Tissue DNA Kit. Polymerase chain reaction (PCR) was carried out to amplify four loci including internal transcribed spacer regions (ITS), partial large subunit rRNA (LSU), partial beta-tubulin gene (*tub2*), partial DNA-directed RNA polymerase II subunit gene (*rpb2*) with primers and conditions as shown in Table 1. Amplification was done in 25 µL reaction volume consisting of 12.5 µL 2x GoTaq® Green Master Mix (PROMEGA, USA), 1 µL of each primer (20 µM), 9.5 µL of double distilled, deionized water and 1 µL of genomic DNA. The acquired PCR products were sent to SolGent Co., Ltd., South Korea for obtaining the sequences.

Table 1: The genetic regions amplified in this study along with the corresponding PCR primers and procedures used.

Genetic loci	Primers (Forward/ Reverse)	Conditions	References
ITS	ITS5/ITS4	95 °C/45 s, 53 °C/45 s, 72 °C/120 s	White et al. (1990)
LSU	LR0R/LR7	94 °C/30 s, 48 °C/50 s, 72 °C/60 s	Vilgalys & Hester (1990)
rpb2	fRPB2-5f/fRPB2-7cR	95 °C/45 s, 57 °C/50 s, 72 °C/90 s	Liu et al. (1999)
tub2	T1/T22	95 °C/60 s, 54 °C/110 s, 72 °C/120 s	O'Donnell & Cigelnik (1997)

Initial denaturation at 95°C for 5 min and final extension at 72°C for 10 min with 35 cycles for all regions.

Phylogenetic analysis

BLAST searches were performed on the sequences in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The BLAST search results and preliminary morphological examinations indicated that our isolate is classified within the *Hypoxylaceae* family. Additional sequences for comparative analyses were sourced from GenBank, relying on recently published data (Gajanayake et al. 2021, Samarakoon et al. 2019, Stadler et al. 2014, Wendt et al. 2018, Wongkanoun et al. 2020) (Table 2). The single loci sequences were aligned using default settings in MAFFT v. 7.036 (http://mafft.cbrc.jp/alignment/server/index.html, Katoh et al. 2019) and edited where necessitated, utilizing BioEdit v. 7.0.5.2 (Hall. 1999). The alignments were trimmed using TrimAl (v.1.0) with GapThreshold set at 0.5 (Capella-Gutierrez et al. 2009). All the sequences of the new strain were deposited in GenBank.

The maximum likelihood (ML) tree was constructed in the CIPRES Science Gateway platform (Miller et al. 2010) using RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008). The parameters had been set up to 1000 pseudo-replicates. The best-fit model of evolution for Bayesian inference (BI) analyses was determined using jModelTest2 (Darriba et al. 2012). The BI was performed using MrBayes 3.2.2, with 4 simultaneous Markov chain Monte Carlo (MCMC) chains for 1,000,000 generations, with trees sampled every 100th generation. As part of the burn-in phase, the initial 25% trees were omitted, and the residual trees were employed in order to compute the posterior probabilities in the majority rule consensus tree.

Table 2 List of taxonomical epithets used in the phylogenetic study (Type strains are marked with a superscript 'T,' while the newly isolated strain is presented in bold).

Species	**Strain number**	**GenBank accession numbers**			
		ITS	**LSU**	***rpb2***	***tub2***
Annulohypoxylon michelianum	CBS 119993	KX376320	KY610423	KY624234	KX271239
A. moriforme	CBS 123579	KX376321	KY610425	KY624289	KX271261
A. nitens	MFLUCC 12-0823	KJ934991	KJ934992	KJ934994	KJ934993
A. stygium	MUCL 54601	KY610409	KY610475	KY624292	KX271263
A. truncatum	CBS 140778	KY610419	KY610419	KY624277	KX376352
Daldinia andina^{T}	CBS 114736	–	KY610430	KY624239	KC977259
Daldinia Bambusicola^{T}	CBS 122872	KY610385	KY610431	KY624241	AY951688
Daldinia Brachysperma	BCC33676	MN153854	MN153871	–	MN172205
Daldinia caldariorum	MUCL 49211	AM749934	KY610433	KY624242	KC977282
Daldinia chiangdaoensis^{T}	BCC 88220	MN153850	MN153867	MN172208	MN172197
Daldinia concentrica	CBS 113277	AY616683	KY610434	KY624243	KC977274
Daldinia dennisii^{T}	CBS 114741	JX658477	KY610435	KY624244	KC977262
Daldinia eschscholtzii	**MFLUCC 23-0263**	**OR511809**	**OR511810**	**OR675446**	**OR675447**
Daldinia eschscholtzii	MUCL 45435	JX658484	KY610437	KY624246	KC977266
Daldinia eschscholtzii	CBS 113042	JX658497	–	–	–
Daldinia eschscholtzii	CBS 113047	AY616684	–	–	–
Daldinia eschscholtzii	CBS 116032	JX658500	–	–	–
Daldinia eschscholtzii	CBS 116035	JX658498	–	–	–
Daldinia eschscholtzii	CBS 116037	JX658492	–	–	–
Daldinia eschscholtzii	CBS 116037(2)	JX658499	–	–	–
Daldinia eschscholtzii	CBS 117735	JX658480	–	–	–
Daldinia eschscholtzii	CBS 117740	JX658481	–	–	–
Daldinia eschscholtzii	CBS 117741	JX658491	–	–	–
Daldinia eschscholtzii	CBS 122876	JX658438	–	–	–
Daldinia eschscholtzii	CBS 122877	JX658439	–	–	–
Daldinia eschscholtzii	CBS 122878	JX658440	–	–	–
Daldinia eschscholtzii	KC 1616	JX658496	–	–	–
Daldinia eschscholtzii	KC1699	JX658490	–	–	–
Daldinia eschscholtzii	MUCL 38740	JX658493	–	–	–
Daldinia eschscholtzii	MUCL 41777	JX658486	–	–	–
Daldinia eschscholtzii	MUCL 41778	JX658494	–	–	–
Daldinia eschscholtzii	MUCL 43508	JX658495	–	–	–
Daldinia eschscholtzii	MUCL 47965	JX658482	–	–	–
Daldinia eschscholtzii	MFLUCC19-0153	MK587661	MK587748	MK625012	MK636691
Daldinia eschscholtzii	MFLUCC 20-0232	MW672318	–	–	MW682336
Daldinia eschscholtzii	MFLUCC 20-0233	MW672319	MW672325	–	MW682337
Daldinia flavogranulata^{T}	BCC 89363	MN153856	MN153873	MN172211	MN172200
Daldinia korfii^{T}	STMA14089	KY204020	–	–	KY204016
Daldinia kretzschmarioides	TBRC 8875	MH938531	MH938540	MK165425	MK165416
Daldinia loculatoides^{T}	CBS 113279	MH862918	KY610438	KY624247	KX271246
Daldinia macaronesica^{T}	CBS 113040	KY610398	KY610477	KY624294	KX271266
Daldinia petriniae^{T}	MUCL 49214	–	KY610439	KY624248	KC977261
Daldinia phadaengensis^{T}	BCC 89349	MN153852	MN153869	MN172206	MN172195
Daldinia placentiformis	MUCL 47603	AM749921	KY610440	KY624249	KC977278
Daldinia pyrenaica^{T}	MUCL 53969	KY610413	–	KY624274	KY624312
Daldinia steglichii^{T}	MUCL 43512	KY610399	KY610479	KY624250	KX271269
Daldinia subvernicosa	TBRC 8877	MH938533	MH938542	MK165430	MK165421
Daldinia theissenii^{T}	CBS 113044	KY610388	KY610441	KY624251	KX271247
Daldinia vernicosa^{T}	CBS 119316	KY610395	KY610442	KY624252	KC977260
Entonaema liquescens	ATCC 46302	KY610389	KY610443	KY624253	KX271248

Table 2 Continued.

Species	Strain number	GenBank accession numbers			
		ITS	LSU	RPB2	TUB2
Graphostroma platystomum	CBS 270.87	JX658535	DQ836906	KY624296	HG934108
Hypomontagnella monticulosa	BCC58592	MN153864	MN153881	MN172219	MN172204
Hypomontagnella monticulosa^{T}	MUCL 54604	KY610404	KY610487	KY624305	KX271273
Hypoxylon crocopeplum	CBS 119004	KC968907	KY610445	KY624255	KC977268
Hypoxylon fragiforme^{T}	MUCL 51264	KC477229	KM186295	KM186296	KX271282
Hypoxylon fuscum^{T}	CBS 113049	KY610401	KY610482	KY624299	KX271271
Hypoxylon griseobrunneum^{T}	CBS 331.73	KY610402	KY610483	KY624300	KC977303
Hypoxylon haematostroma^{T}	MUCL 53301	KC968911	KY610484	KY624301	KC977291
Hypoxylon hypomiltum	MUCL 51845	KY610403	KY610449	KY624302	KX271249
Hypoxylon investiens	CBS 118183	KC968925	KY610450	KY624259	KC977270
Hypoxylon lateripigmentum^{T}	MUCL 53304	KC968933	KY610486	KY624304	KC977290
Hypoxylon papillatum^{T}	ATCC 58729	KC968919	KY610454	KY624223	KC977258
Hypoxylon petriniae^{T}	CBS 114746	KY610405	KY610491	KY624279	KX271274
Hypoxylon porphyreum	CBS 119022	KC968921	KY610456	KY624225	KC977264
Hypoxylon pulicicidum^{T}	CBS 122622	JX183075	KY610492	KY624280	JX183072
Hypoxylon rubiginosum^{T}	MUCL 52887	KC477232	KY610469	KY624266	KY624311
Hypoxylon samuelsii^{T}	MUCL 51843	KC968916	KY610466	KY624269	KC977286
Hypoxylon trugodes^{T}	MUCL 54794	KF234422	KY610493	KY624282	KF300548
Jackrogersella minutella	CBS 119015	KY610381	KY610424	KY624235	KX271240
Jackrogersella multiformis^{T}	CBS 119016	KC477234	KY610473	KY624290	KX271262
Pyrenopolyporus hunteri	MUCL 52673	KY610421	KY610472	KY624309	KU159530
Pyrenopolyporus laminosus	TBRC 8871	MH938527	MH938536	MK165424	MK165415
Pyrenopolyporus nicaraguensis	CBS 117739	AM749922	KY610489	KY624307	KC977272
Pyrenopolyporus symphyon	TBRC 8873	MH938529	MH938538	MK165428	MK165419
Rhopalostroma angolense	CBS 126414	KY610420	KY610459	KY624228	KX271277
Rostrohypoxylon terebratum^{T}	CBS 119137	DQ631943	DQ840069	DQ631954	DQ840097
Ruwenzoria pseudoannulata^{T}	MUCL 51394	KY610406	KY610494	KY624286	KX271278
Thamnomyces dendroidea	CBS 123578	FN428831	KY610467	KY624232	KY624313
Xylaria hypoxylon^{T}	CBS 122620	KY610407	KY610495	KY624231	KX271279
Xylaria polymorpha	MUCL 49884	KY610408	KY610464	KY624288	KX271280

Results

Phylogenetic analysis

The concatenated dataset had 3,538 characters with gaps (LSU: 776 bp, ITS: 585 bp, *rpb2*: 802 bp, *tub2*: 1,375 bp). Figure 1 displays the BI tree, which was constructed using the combined dataset of 81 epithets. This dataset includes species from the *Hypoxylaceae* family and three outgroup species. For the BI analysis, the best-fit models were TIM2ef+I+G for ITS and GTR+I+G for LSU, *rpb2,* and TPM3uf+I+G for the *tub2* gene. The final tree received a standard deviation of split frequencies of 0.006472 of Bayesian posterior probabilities (BPP).

The combined dataset resulted in the best-scoring ML tree with a final ML optimization likelihood value of -50072.834903 with the RAxML analysis. The matrix had 1871 distinct alignment

patterns, with 28.10% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.237039, C = 0.261992, G = 0.257985, T = 0.242984; substitution rates AC = 1.332226, AG = 4.423858, AT = 1.448245, CG = 1.014681, CT = 5.944248, GT = 1.000000; gamma distribution shape parameter α = 0.274756.

The phylograms from the BI and ML analyses had similar topologies and did not vary significantly at the generic level which is consistent with the former study by Gajanayake et al. (2021) based on multi-gene phylogeny.

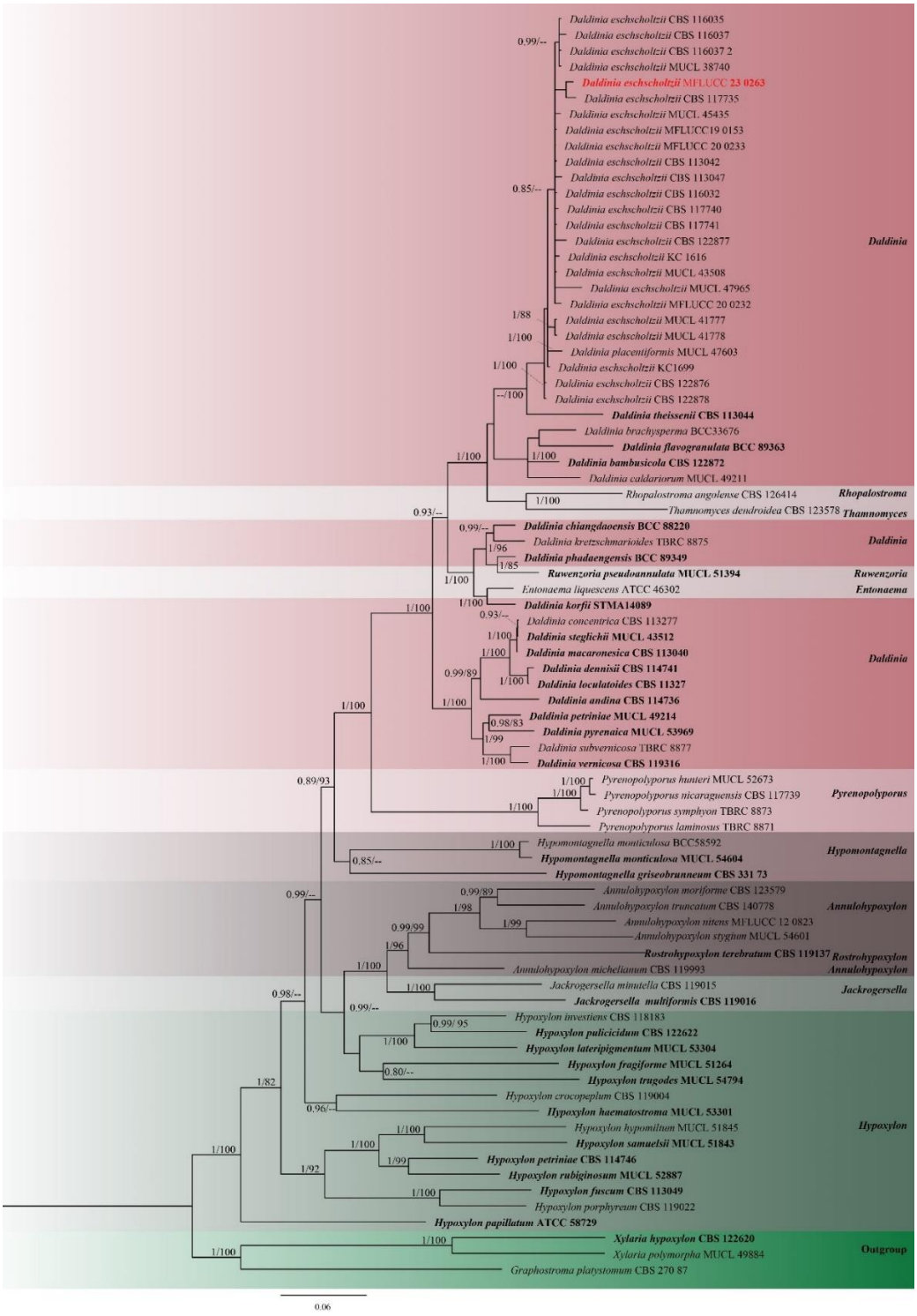

Fig 1 – Bayesian analyses inferred from a combined dataset of LSU, ITS, *rpb2*, and *tub2* sequence data of 78 genera of *Hypoxylaceae* species and three species as the outgroup. ML bootstrap (≥80 %) and BPP (≥ 0.80 PP) are displayed at the branches. The type strains are in bold while the strain

generated in this study is in red. The tree is rooted with *Xylaria hypoxylon* (CBS 122620), *X. polymorpha* (MUCL 49884), and *Graphostroma platystomum* (CBS 270.87). The scale bar indicates 0.06 nucleotide changes per site.

Taxonomy

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

Index Fungorum Number: IF544992

Facesoffungi Number: FoF02990

Endophytic on *Phegopteris* species **Sexual morph**: See Stadler et al. (2014). **Asexual morph**: White to greyish-black effused colonies on PDA. *Mycelium* 2.4–5.2 µm ($\bar{x}$ = 3.4 µm, n = 10) wide, coenocytic to septate, internal to superficial, vacuolic hypha, light brown to olivaceous, delicately verruculose mostly at the end of the conidiophores, tubular and slightly thickened cell walls with nodulisporium-like branching patterns. *Conidiophores* 36–186 × 2.4–4.7 µm ($\bar{x}$ = 101 × 4 µm, n = 7), mononematous, erect to flexuous, arising terminally or laterally from hypha, cylindrical-oblong, branched, aseptate, hyaline to olivaceous, verruculose, warted, *primary branches* 36–129 × 3–7 µm ($\bar{x}$ = 88.8 × 4.5 µm, n = 10), *secondary branches* 16.8–161 × 1.7–4.5 µm ($\bar{x}$ = 61.3 × 3.2 µm, n = 10) bearing 1–3 phialides. *Conidiogenous cells* 11.4–19 × 1.9–5.2 µm ($\bar{x}$ = 14.6 × 3.6 µm, n = 5), polyblastic, terminal or intercalary, cylindrical to occasionally ampulliform, pale brown to hyaline, smooth-walled to verruculose. *Conidia* 1.3–5.5 × 0.7–2.8 µm ($\bar{x}$ = 2.7 × 1.7 µm, n = 30), ovoid, aseptate, hilum basally displayed, guttulate, hyaline to olivaceous, thick, smooth-walled.

Culture characteristics: Colonies on PDA reached a diameter 90 mm after 14 days at 25 °C, slightly rough surface, edge undulate to entire, medium dense. Colony from above: pale grey to olivaceous at the marginal end, light green to white in the centre; reverse: white to grey at the marginal end, blackish brown in the centre; mycelium hyaline to light brown. Colonies sporulated after 19 days. Colonies on CMA reached a diameter 55mm after 8 days at 25 °C, soft edges, medium to dense. Colony from above: pale olivaceous to white at the marginal end, mycelium hyaline to olivaceous.

Material examined: THAILAND, Chiang Mai Province, Mae Taeng, Pha Deng Village, Mushroom Research Centre, on symptomless fresh leaves of *Phegopteris* sp. (*Thelypteridaceae*), 18 December 2022, AG. Gunarathne, TAU 0007 (MFLU 23-0414); living culture MFLUCC 23–0263.

GenBank accession numbers: MFLUCC 23–0263: ITS: OR511809; LSU: OR511810; *rpb2*: OR675446; *tub2*: OR675447.

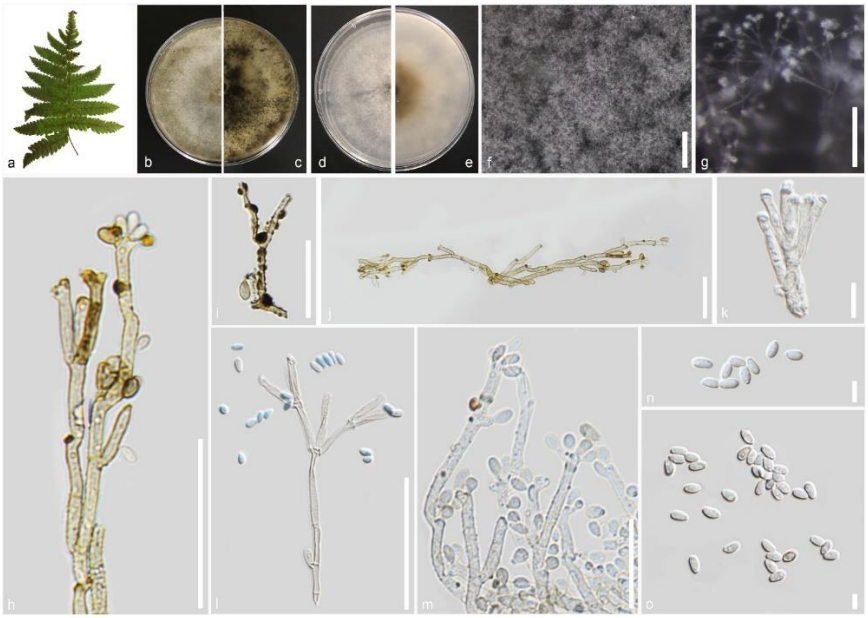

Fig. 2 – *Daldinia eschscholtzii* (MFLUCC 23–0263). **a** *Phegopteris* host. **b, c** Sporulated colonies on PDA after 19 days. **c, d** Sporulated colony on CMA after 17 days. **f–m** Conidiophores with

nodulisporium-like branching patterns, and intercalary conidiogenous cells bearing conidia (**k** Conidiophores sporulated on CMA, **f–h, j** in 5 % KOH, **l** in Lactophenol cotton blue). **n** Conidia on PDA. **o** Conidia on CMA. Scale bars: d = 200 µm, e = 100 µm, f = 50 µm, g–j = 20 µm, k = g–j = 10 µm, n, o = 5 µm.

Discussion

This study presents a novel finding regarding endophytic *Daldinia eschscholtzii* associated with *Phegopteris* from Northern Thailand. Notably, this region exhibits a remarkable fungal species novelty ranging from 55% to 96% (Hyde et al. 2018).

The strain MFLU 23–0414 clustered with 24 strains of *D. eschscholtzii* based on the phylogenetic approach with LSU, ITS, *rpb2* and *tub2* data. The scope of our phylogenetic analysis, it was observed that the sequences associated with the *D. eschscholtzii* MUCL 45435 and Our isolate MFLU 23-0414 closely resemble *D. eschscholtzii* based on Stadler et al. (2014) (Table 3.0). Hence, in the capacity of a foliaceous endophyte, we confer the first documented occurrence of *D. eschscholtzii* from *Phegopteris* species. Furthermore, this is the first report of an ascomycete from *Phegopteris* species in Thailand.

Daldinia eschscholtzii has been identified as an endophyte from various other host organisms, including *Aloe vera* from Bangladesh (Ahmmed et al. 2022), *Psidium guajava* from India (Chutulo & Chalannavar 2020), and *Musa* species from Thailand (Samarakoon et al. 2019). It has also been observed as a saprobe on both angiosperms and gymnosperms (Stadler et al. 2014), and as a contaminant on the growing substrate of oyster mushrooms (Gajanayake et al. 2021). This indicates the potential for *D. eschscholtzii* to be a non-clavicipitaceous endophyte which can opportunistically transition its life mode from endophytic to saprobic (Bhunjun et al. 2023).

Daldinia eschscholtzii is known for its capability to produce dalesconole, which is an immunosuppressant (Zhang et al. 2011), cytochalasins, spirodalesol, concentricols and binaphthalene derivatives (Stadler et al. 2014), tetralones, chromones (Becker & Stadler 2021). In most instances, the investigation of secondary metabolites in *Daldinia* necessitates the presence of stromata, which we were unable to observe in our study. Forthcoming research endeavours will be required for bioprospecting of our *D. eschscholtzii* isolate and to probe the purpose of these chemical substances in relation to the host organism or potential industrial applications.

Table 3 Asexual morphological comparison of selected *Daldinia eschscholtzii.*

Character	***Daldinia eschscholtzii*** (This study)	***D. eschscholtzii*** (Stadler et al. 2014)
Mycelium	Hyaline to light brown	Initially whitish and later turns to dull-green
Conidiogenous structure	Nodulisporium-like branching patterns	Nodulisporium-like branching patterns
Conidiophore	Hyaline to olivaceous	Hyaline
	Comprising primary and secondary branches	Di-or tri-chotomously branched

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Accessibility of data

Dry culture **-** MFLU 23-0414

Living culture - MFLUCC 23-0263

GenBank accession numbers - MFLUCC 23-0263*:* ITS - OR511809, LSU - OR511810, *rpb2* - OR675446, *tub2* - OR675447

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