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Paradictyoarthrinium diffractum, a new host record on *Delonix regia* in northern Thailand

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Abstract

Woody substrates in tropical regions are important habitats for lignocellulolytic fungi. In this study, *Paradictyoarthrinium diffractum* was found on decaying wood of *Delonix regia* in Chiang Rai Province, Thailand. *Paradictyoarthrinium diffractum* was initially reported from a Rivulet in South Africa. Subsequently, it was also found to be associated with dead stumps and stems of *Tectona grandis.* Our isolate has similar characteristics as the type species having superficial, gregarious colonies and macronematous conidiophores. Our taxon differs from *P. diffractum* (MFLUCC 12–0557 and MFLUCC 13–0466) by having dark brown conidia with distinct septations, which turn black at maturity. The maximum likelihood and Bayesian inference analysis of combined LSU, ITS, and *rpb2* sequences revealed that our isolate clustered with isolates of *P. diffractum.* Based on the morphological and multigene phylogenetic evidence, we introduce *P. diffractum* strain MFLUCC 24–0257 as a new host record from *D. regia.* We also provide data on the distribution of *Paradictyoarthrinium* species from around the world.

Keywords – Asexual morph – New host record – Saprobes – *Pleosporales*

Introduction

Delonix regia is a member of *Fabaceae*, subfamily *Caesalpinioideae* (Babineau & Bruneau 2017). *Delonix regia* is native to Madagascar and is a semi-deciduous tree that grows up to 18 meters in height (Adje et al. 2008). The tree has antioxidant, anti-inflammatory, and anti-microbial properties (Rani et al. 2011). *Delonix regia* is mainly distributed throughout Africa, India, Madagascar, Northern Australia, Southern China and has spread over Europe, North America, and the Middle East (Chavan & Rasal 2012, Modi et al. 2016).

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Paradictyoarthrinium is the generic type of the family *Paradictyoarthriniaceae* (Liu et al. 2015, Hongsanan et al. 2020, Wijayawardene et al. 2022). *Paradictyoarthriniaceae* is similar to *Biatriosporaceae* and *Roussoellaceae* by having holoblastic conidiogenous cells and aseptate or septate conidia (Hongsanan et al. 2020). Five species *P. aquatica*, *P. diffractum* (type species), *P. hydei*, *P. salsipaludicola* and *P. tectonicola* are accepted in *Paradictyoarthrinium* (Index Fungorum 2024). Members of the genus have been reported as saprobes on decaying wood in freshwater habitats, spathe of *Cocos nucifera*, dead stems of *Tectona grandis* and decaying wood in mangroves from China, India, South Africa, and Thailand (Doilom et al. 2017, Liu et al. 2018, Prabhugaonkar & Bhat 2011). This study reports a new host record for *P. diffractum* on *D. regia* collected in Chiang Rai, Thailand. The strain was identified using morphology and multi-loci phylogenetic analysis based on LSU, ITS and *rpb2* sequences.

Materials and methods

Sample processing and morphological studies

The decaying woody litter of *D. regia* was collected from the Mae Fah Luang University, Chiang Rai Province, Thailand. The samples were placed into zip-lock plastic bags and examined following the methodology described in Senanayake et al. (2020). Detailed morphological characteristics were observed using a Leica EZ4 stereo microscope (Leica Microsystems (SEA) Pte Ltd, Singapore). The micro-characters were photographed using a stereomicroscope (Stereo Discovery v 8) attached to an Axio Cam ERc5s and a Nikon ECLIPSE Ni-U compound microscope (Nikon, Tokyo, Japan). The images were edited in Adobe Photoshop CS6 software (Adobe Systems Inc., USA). Measurements of the morphological structures were determined using the Tarosoft (R) Image Framework v 0.9.7. software (Informer Technologies Inc.). Single spore isolation was done using potato dextrose agar (PDA) following Senanayake et al. (2020) and the plates were incubated at 25 °C. Colony characters were observed and colony growth was measured. Herbarium specimens and living cultures were deposited at the Mae Fah Luang University Herbarium (MFLU) and Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand, respectively.

DNA extraction, PCR amplification and sequencing

The mycelia on PDA were harvested into 1.5 ml tube after 40 days of incubation for genomic DNA extraction. The extraction was done using the E.Z.N.A.® Tissue DNA Kit (Omega Biotek Inc.) following the manufacturer's instructions. The primers utilized and the conditions for PCR amplification are mentioned in Table 1. PCR amplification and sequencing were conducted in the Laboratory of Photochemistry and Natural Medicines, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China. PCR amplifications were performed in a total volume of 25 µl, consisting with 12.5 µl of 2 × Power Taq PCR master mix, 9.5 µl of deionized water,1 µl from each primer (20 µM) and 1 µl of genomic DNA (178 ng/µl). Agarose gel electrophoresis (1.7%) was used to separate amplified PCR products and visualized by a compact Desktop UV Transmissometer (GL- 3120) gel documentation system (Haimen Kylin–Bell Lab Instruments Company Ltd).

Quality evaluation, sequence alignment and phylogenetic analyses

The quality of the sequences was checked using BioEdit v 7.0.9.0 (Hall 1999). Lasergene SeqMan Pro v 7 was used to generate the consensus sequences. Newly generated sequences were subjected to BLASTn searches in NCBI GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify highly similar sequences and related sequences were downloaded following related literature (Htet et al. 2023) (Table 2). The sequences were aligned using the MAFFT v 6.864b online tool (https://mafft.cbrc.jp/alignment/ server/index.html). The alignments were trimmed using TrimAl v 1.2 software with gappyout option (Capella-Gutiérrez et al. 2009). The ML analysis was completed using the IQ-Tree web server (http://iqtree.cibiv.univie.ac.at/). The bootstrap support for the process was obtained from 1,000 pseudo replicates (Nguyen et al. 2015). The BI analyses were

completed using MrBayes v 3.2.7a (Ronquist et al. 2012) on XSEDE in CIPRES Science Gateway (Miller et al. 2010). Fig Tree v 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) was used to visualize the phylogenetic trees and edited using Microsoft PowerPoint 2019 (USA).

Table 1 The amplified loci, primers, and PCR thermal cycle protocols used in this study.

Gene	PCR primers (forward/reverse)	PCR conditions	Reference
ITS	ITS4/ITS5	94 ºC for 3 min; 94 ºC for 30 s; 55 ºC for 50 s; 72 ºC for 90 s and 72 ºC for 10 min Amplification cycles: 40	White et al. (1990)
LSU	LROR/ LR5	94 ºC for 3 min; 94 ºC for 30 s; 55 ºC for 50 s; 72 ºC for 90 s and 72 ºC for 10 min Amplification cycles: 35	Vilgalys & Hester (1990)
rpb2	fRPB2-5F/ fRPB2-7cR	95 ºC for 5 min; 95 ºC for 15 s; 56 ºC for 50 s; 72 ºC for 120 s and 72 ºC for 10 min Amplification cycles: 40	Liu et al. (1999)

Table 2 Accession numbers of the taxa used in this study. The type-derived sequences are indicated in bold and denoted with T. The newly generated strain is in blue.

Species	Strain Number	LSU	ITS	*rpb2*
Melanomma pulvis-pyrius	CBS 124080	GU456323	N/A	GU456350
Melanomma pulvis-pyrius	CBS 125577	MH875177	MH863714	N/A
***Neoroussoella bambusae*^T**	**MFLUCC 11-0124**	**KJ474839**	**KJ474827**	**KJ474856**
***Nigrograna mackinnonii*^T**	**CBS 674.75**	**KF015612**	**NR_132037**	**KF015703**
***Nigrograna norvegica*^T**	**CBS 141485**	**KX650556**	**KX650556**	**KX650578**
***Nigrograna obliqua*^T**	**CBS 141477**	**KX650560**	**KX650560**	**KX650580**
***Occultibambusa bambusae*^T**	**MFLUCC 13-0855**	**KU863112**	**KU940123**	**KU940170**
***Occultibambusa pustula*^T**	**MFLUCC 11-0502**	**KU863115**	**KU940126**	**N/A**
***Paradictyoarthrinium aquatica*^T**	**MFLUCC 16–1116**	**MG747495**	**MG747496**	**MG780231**
Paradictyoarthrinium diffractum	MFLUCC 13-0466	KP744498	KP744455	KX437764
Paradictyoarthrinium diffractum	MFLUCC 12-0557	KP744497	KP744454	KX437765
Paradictyoarthrinium diffractum	MFLUCC 24-0257	PQ199634	PQ198077	PQ272735
***Paradictyoarthrinium hydei*^T**	**MFLUCC 17-2512**	**MG747497**	**MG747498**	**MG780232**
Paradictyoarthrinium hydei	KUNCC 10440	OQ146990	OQ135178	N/A
***Paradictyoarthrinium salsipludicola*^T**	**MFLUCC 22-0054**	**OR589801**	**OR589800**	**N/A**
***Paradictyoarthrinium tectonicola*^T**	**MFLUCC 13-0465**	**KP744500**	**KP744456**	**KX437763**
Paradictyoarthrinium tectonicola	MFLUCC 14-0630	KP744499	KP744456	N/A
***Roussoella nitidula*^T**	**MFLUCC 11-0182**	**KJ474843**	**KJ474835**	**KJ474859**
Roussoellopsis macrospora	MFLUCC 12-0005	KJ474847	N/A	KJ474862
***Seriascoma didymospora*^T**	**MFLUCC 11-0179**	**KU863116**	**KU940127**	**KU940173**
***Thyridaria acacia*^T**	**CBS 138873**	**KP004497**	**KP004469**	**N/A**
Thyridaria broussonetiae	CBS 141481	KX650568	KX650568	KX650586
Thyridaria broussonetiae	CBS 141482	KX650570	KX650570	KX650587
Torula herbarum	CBS 111855	KF443386	KF443409	KF443396
***Torula hollandica*^T**	**CBS 220.69**	**KF443384**	**KF443406**	**KF443393**
***Versicolorisporium triseptatum*^T**	**JCM 14775**	**AB330081**	**AB365596**	**N/A**
***Xenomassariosphaeria clematidis*^T**	**MFLUCC 14-0923**	**MT214571**	**MT310616**	**N/A**
***Xenomassariosphaeria rosae*^T**	**MFLUCC 15-0179**	**NG059883**	**N/A**	**N/A**

Abbreviation: CBS: Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands**;** JCM: Japan Collection of Microorganisms, Japan**;** KUNCC: Kunming Institute of Botany Culture Collection, Kunming, China**;** MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand. N/A: sequence data are not available.

Results

Phylogenetic analysis

Sequences of LSU, ITS, and *rpb2* were utilized in the phylogenetic analyses. The topology of the maximum likelihood tree was similar to the BI analysis. Based on base pair comparisons, the ITS region was 99% identical without gaps to *P. diffractum* (MFLUCC 12-0557) and 99% similar with three gaps to *P. diffractum* (MFLUCC 13-0466). The LSU sequence was 99% identical with one gap to the strain MFLUCC 12-0557, while MFLUCC 13-0466 was 99% identical without gaps. The *rpb2* region showed 99% identity without gaps to *P. diffractum* MFLUCC 12-0557 and MFLUCC 13-0466. This confirms that our isolate is *P. diffractum*.

Furthermore, our tree topology is also in accordance with the phylogenetic tree of Liu et al. (2018) and Htet et al. (2023). The best-scoring ML tree had a final optimization likelihood value of -14320.09 (Fig. 1). The matrix had 917 distinct alignment patterns. Estimated base frequencies were as follows: A = 0.250, C = 0.250, G = 0.250, T = 0.250; substitution rates AC = 1.4427, AG = 4.35996, AT = 1.4427, CG = 1.00000, CT = 8.8636 and GT = 1.00000; invariable site = 0.482 and gamma distribution shape parameter alpha = 0.676. The average standard deviation of split frequencies in the BI analysis was 0.0099 after 2,000,000 generations.

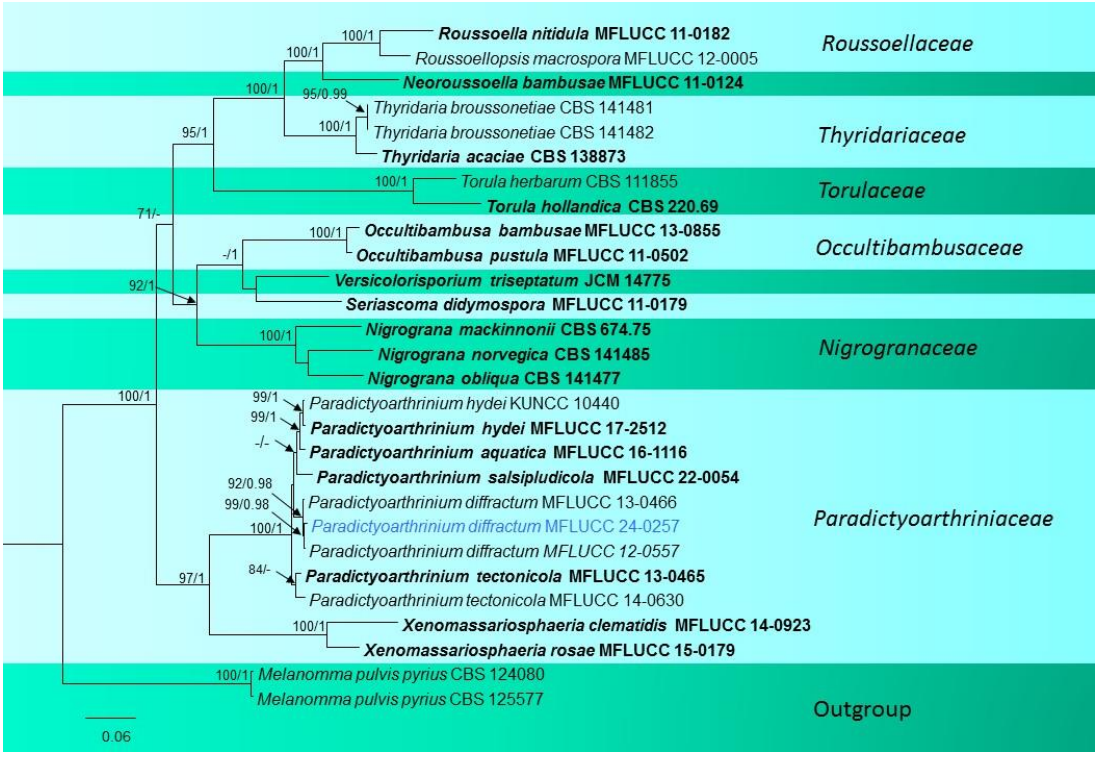

Fig. 1 – Tree of the combined alignment of LSU, ITS and *rpb2* sequence data. The tree was rooted with *Melanomma pulvis-pyrius* (CBS 124080 and CBS 125577). Bootstrap support values equal to or greater than 70% for ML and BI values equal to or greater than 0.95 are shown as ML/BI at respective nodes. The isolate presented in this study is in blue. Ex-type strains are indicated in bold. The scale bar represents the number of nucleotide substitutions per site.

Taxonomy

Paradictyoarthrinium diffractum Matsush, *Matsush. Mycol. Mem*. 9: 18 (1996)

Index Fungorum number: IF 415849

Facesoffungi number: FoF 00315

Saprobic on decaying wood of *D. regia* (Boj. ex Hook.) Sexual morph: Undetermined. Asexual morph: Hyphomycetous. *Colonies* grow on the surface of natural substrate, superficial,

powdery, scattered, gregarious, light brown to black. *Conidiophores* 3.3–4.5 × 3–4.5 µm ($\bar{x}$ = 3.8 × 3.5 µm, n = 12), macronematous, mononematous, erect, cylindrical, slightly curved, brown. *Conidiogenous cells* 2.5–4.5 × 3.3–4 µm ($\bar{x}$ = 2.5 × 3.5 µm, n = 12), holoblastic, integrated, determinate, terminal, cylindrical, brown to dark brown. *Conidia* 8.5–17.7 × 7–14 µm ($\bar{x}$ = 13 × 11 µm, n = 15), solitary or in branched chains, truncated at base, multi-dictyoseptate, 1–4-transeptate, verrucose, thick-walled, hard to separate, circular to irregular, dark brown to black at maturity.

Culture characteristic – Conidia germinating on PDA within 24 hours. Colonies on PDA reaching a diam. of 12–20 mm after one week at 25 °C. The colony is initially white to pale grey, turning dark grey to dark brown within one week, circular, smooth, velvety; reverse: dark brown.

Material examined – THAILAND. Chiang Rai Province, decaying wood of *D. regia* (*Fabaceae*), 24 January 2024, Balagamage D. Thilanga, (MFLU 24–0262); living culture, MFLUCC 24–0257.

Notes – Our isolate (MFLU 24-0262) nested with *P. diffractum* (MFLUCC 12-0557 and MFLUCC 13-0466) based on phylogenetic analyses of ITS, LSU, and *rpb2* sequences with 0.98 PP and 99% ML statistical support. The mycelium and conidiogenous cells exhibited macronematous, curved brown, cylindrical and holoblastic structure that are brown to dark brown (Fig. 2). These characteristics are similar to other members of *Paradictyoarthrinium* (Liu et al. 2015, Matsushima 1996, Prabhugaonkar & Bhat 2011). The dark brown to black, thick-walled conidia form solitary or branched chains with a rounded, truncated base which resemble the morphology of *P. diffractum* (MFLUCC 12-0557) (Matsushima 1996, Prabhugaonkar & Bhat 2011, Tian et al. 2024). Based on morphological and phylogenetic evidence, we identify the isolate MFLU 24–0262 as *P. diffractum* and as the first report on *D. regia* in Thailand.

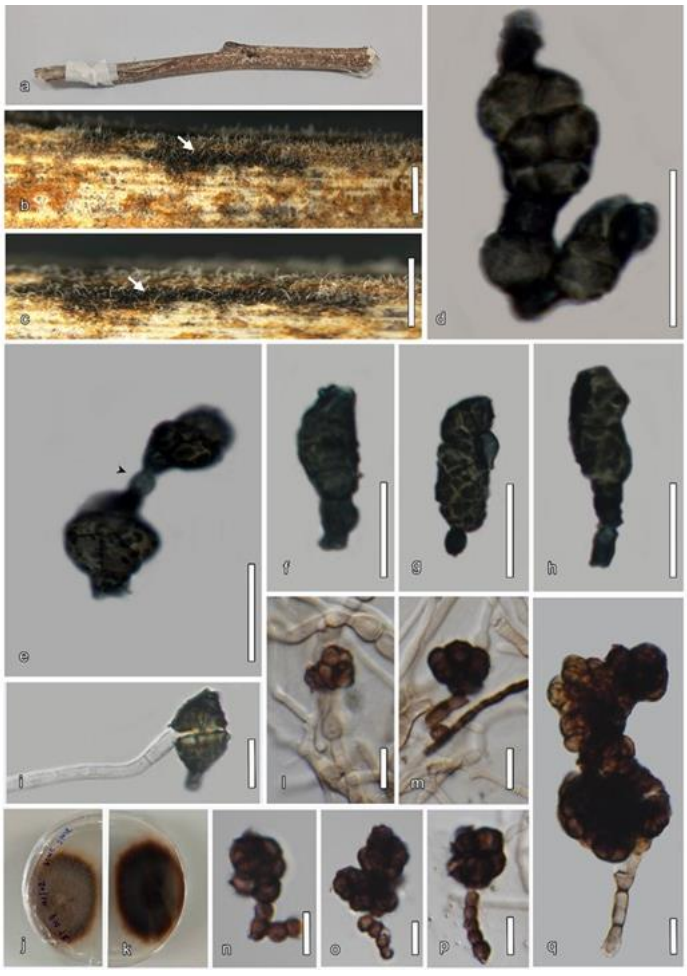

Fig. 2 – ***Paradictyoarthrinium diffractum*** (MFLU 24–0262) a Host. b-c Colonies on the host surface (indicated with arrow). d Conidia aggregation. e Conidia and conidiogenous cells (indicated

with arrow). f-h Conidia. i Germinating conidia. j Culture on PDA above. k reverse. l-q Developing conidia in culture condition. Scale bar: b-c = 2.5 mm. d-h = 10 µm. i-m = 10 µm. n-q = 10 µm.

Discussion

This study reports *P. diffractum* on *D. regia*, Northern Thailand for the first time. Around seventy fungal species have been recorded on *D. regia* (Farr et al. 2021, Perera et al. 2020, Somrithipol et al. 2002). *Delonix regia* exhibits a wide range of pharmaceutical properties, including cytotoxic, gastroprotective, hepatoprotective, antibacterial, anti-inflammatory, and wound-healing effects (Modi et al. 2016). Therefore, the exploration of fungal fauna associated with *D. regia* bare critical ecological and economical importance.

Paradictyoarthriniaceae are saprobes associated with aquatic, terrestrial, and marine environments on decaying wood from China, India, Thailand and South Africa (Doilom et al. 2017, Htet et al. 2023, Liu et al. 2015). *Paradictyoarthrinium* is associated with *Arecaceae* and *Lamiaceae* (Doilom et al. 2017, Liu et al. 2015, 2018, Matsushima 1996, Prabhugaonkar & Bhat 2011). Members of *Paradictyoarthriniaceae* share similar morphological features and are difficult to differentiate based on morphology alone. Thus, *Paradictyoarthrinium* species delineation is usually based on multilocus phylogeny (Hongsanan et al. 2020).

Paradictyoarthrinium diffractum is common in low altitudes around 2000 meters above sea level (Boonmee et al. 2021). Vitasse et al. (2021) reported the influence of global climate change on the distribution patterns of fungi with an increase in decaying wood materials and soil. The geographical and host distribution of *Paradictyoarthrinium* species is shown in Table 3. *Paradictyoarthrinium* has been reported on monocotyledon and dicotyledon plant species and associated with mangrove environment habitat. This suggests the potential development of salinity tolerance in response to the environmental conditions of the habitat. Exploring and reporting new host records are valuable to understanding the fungal biodiversity and ecological significance. It helps reveal novel relationships between fungi and hosts as the roles of saprobic, symbiont or pathogens.

Table 3 Geographical and host distribution of *Paradictyoarthrinium* species.

Species	Habitat/Host	Countries	References
Paradictyoarthrinium aquatica	Saprobic on decaying wood in freshwater habitats	China	Liu et al. (2018)
Paradictyoarthrinium diffractum	Saprobic on dead twigs in freshwater habitats / ondead spathe of *Cocos nucifera* / dead stumps and stems of *Tectona grandis*	India, South Africa, Thailand	Matsushima (1996), Prabhugaonkar & Bhat (2011), Doilom et al. (2017), Capital & Lao (2020), Tian et al. (2024)
	Saprobic on *Mangifera indica* and *Pinus taeda*	China	
	Saprobic on decaying wood of *Delonix regia*	**Thailand**	**This study**
Paradictyoarthrinium hydei	Saprobic on decaying wood of unidentified plant	Thailand	Liu et al. (2018)
	Saprobic dead inner branch of *Quercus variabilis*	China	Capital & Lao (2020)
	Saprobic on decaying wood in a freshwater stream	China	Xu et al. (2023)
	Saprobic on *Pinus* sp	China	Tian et al. (2024)
Paradictyoarthrinium salsipaludicola	Saprobic on decaying wood in mangrove habitats	Thailand	Htet et al. (2023)
Paradictyoarthrinium. tectonica	Saprobic on dead stumps of *Tectona grandis*	Thailand	Liu et al. (2015)

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