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Morphology and muti-gene phylogenetic analyses reveal *Dothiorella chiangmaiensis* sp. nov. (*Botryosphaeriaceae*, *Botryosphaeriales*) from Thailand

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Abstract

Dothiorella species occur on a wide range of plants as endophytes, saprobes and pathogens. This genus is characterized by pigmented, 1-septate ascospores, and conidia that become brown and 1-septate while still attached to the conidiogenous cells. In the current study, we introduce a novel saprobic species, *Dothiorella chiangmaiensis*, from dead branches of *Tamarindus indica* (*Fabaceae*) in Thailand. This novel taxon was recognized based on morphological examinations coupled with phylogenetic analyses. Multigene phylogenetic analyses were performed by maximum likelihood and Bayesian inference analyses of an ITS, *tef*1 and *tub2* sequence alignment. Conidia of *D. chiangmaiensis* are dark brown, 1-septate and guttulate. The novel taxon is described and illustrated. This study contributes to expanding the taxonomic framework for *Dothiorella* by introducing a new species.

Keywords – 1-new species – Dothideomycetes – morphology – phylogeny

Introduction

Dothiorella was introduced by Saccardo (1880) with *D. pyrenophora* as the type species. Crous & Palm (1999) studied the holotype of *D. pyrenophora* and synonymized *Dothiorella* with *Diplodia*. However, based on morphology and molecular data, Phillips et al. (2005) reinstated *Dothiorella* to accommodate species with conidia that become brown and 1-septate while still attached to the conidiogenous cells and thus differ from *Diplodia* species, which have hyaline conidia that become pigmented and septate only sometime after release from the pycnidia (Abdollahzadeh et al. 2014, Dissanayake et al. 2016). *Dothiorella* and *Spencermartinsia* were earlier considered to be two separate genera in *Botryosphaeriaceae* (Phillips et al. 2008, 2013). Yang et al. (2017) synonymized *Spencermartinsia* under *Dothiorella*, and Hongsanan et al. (2020) and Wijayawardene et al. (2020) accepted this.

The sexual morph of *Dothiorella* species is characterized by erumpent or superficial ascomata, bitunicate, fissitunicate asci with pigmented, 1-septate ascospores (Phillips et al. 2013). The asexual morph of *Dothiorella* has immersed, erumpent conidiomata. Paraphyses have not been

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reported. Conidiogenous cells are holoblastic and hyaline (Phillips et al. 2013). Conidia become brown and 1-septate while still attached to the conidiogenous cells (Phillips et al. 2005, Dissanayake et al. 2016, Hongsanan et al. 2020).

Dothiorella species have worldwide distribution and occur on a wide range of hosts (Jayawardena et al. 2019). The genus includes endophytic, saprobic, and plant pathogenic species associated with canker, die-back and fruit rots (Liu et al. 2012, Phillips et al. 2013, Dissanayake et al. 2016, Jayawardena et al. 2019). *Dothiorella* species are known to have weak pathogenicity on ecologically and economically important plants (Úrbez-Torres & Gubler 2009, Úrbez-Torres et al. 2012). For example, *Dothiorella americana*, *D. iberica* and *D. viticola* are weakly virulence on grapevines (Úrbez-Torres & Gubler 2009, Úrbez -Torres et al. 2012). *Dothiorella* species were introduced mainly on their host association (Abdollahzadeh et al. 2014), which resulted in more than 350 species names listed in MycoBank and 395 species names in Index Fungorum. However, molecular data and cultures are available for only 36 species (Wu et al. 2021, Wijayawardene et al. 2022). Due to the cosmopolitan distribution of *Dothiorella* species in different hosts, additional taxonomic and ecological studies are needed.

This study introduces a new species of *Dothiorella* from Thailand. Morphological illustration of the novel taxon is provided, together with the phylogenetic placement based on maximum likelihood (ML) and Bayesian inference (BI) analyses of a combined ITS, *tef*1 and *tub2* sequence alignment.

Materials & Methods

Specimen collections, morphological studies and isolations

Dead branches of *Tamarindus indica* were collected from a forested area at the Mushroom Research Centre (MRC), Chiang Mai, Thailand on 10 September 2020. Specimens were brought to the laboratory in zip-lock bags, and samples were examined following the methods described in Senanayake et al. (2020). Morphological observations were made using a LEICA EZ4 stereomicroscope (Leica Microsystems Company, Germany), AXIOSKOP 2 PLUS compound microscope (Carl Zeiss Microscopy Company, Germany) and photographed with a Canon 550D digital camera fitted to the microscope. All measurements were made with ZEN2 (blue edition) software. The photoplate was prepared with Adobe Photoshop CS3 Extended version 10.0. Measurements were made with the Tarosoft (R) Image Frame Work program, and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA).

Single spore isolations were carried out following the method described in Senanayake et al. (2020). Germinated conidia were transferred to potato dextrose agar (PDA) plates and incubated at 25°C. Pure cultures were obtained by subculturing, and culture characters were recorded after one week. The holotype material was deposited in the Mae Fah Luang University Herbarium (MFLU), and the living culture was deposited at the Culture Collection of Mae Fah Luang University (MFLUCC). Faces of fungi number and Index Fungorum number were obtained as in Jayasiri et al. (2015) and Index Fungorum (2022), respectively. The details were added to the Greater Mekong Subregion webpage (Chaiwan et al. 2021).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh mycelium (50–100 mg) using a DNA rapid Extraction Kit (Aidlab Biotechnologies Co., Ltd., China) by following the manufacturer's instructions. Extracted DNA was stored at 4°C and -20°C for short and long-term storage respectively. The internal transcribed spacers region (ITS) and partial translation elongation factor 1-α gene (*tef*1) were amplified by polymerase chain reactions (PCR) as described in Rathnayaka et al. (2021). PCR reactions were carried out in a final volume of 25 µl, which contained 12.5 µl of 2× Easy Taq PCR SuperMix (TransGen Biotech, Beijing, China), 1 µl of each forward and reverse primers, 2 µl of genomic DNA and 8.5 µl of sterilized, deionized water. PCR products were

visualized on 1% agarose electrophoresis gel and sequenced at Guangzhou Tianyi Science and Technology Co., Ltd. (Guangzhou, China). Newly generated nucleotide sequences were deposited in GenBank (Table 1).

Phylogenetic analyses

The quality of the sequences was assessed by checking their chromatograms with BioEdit v 7.0.9.0 (Hall 1999). Newly generated sequences were initially subjected to BLASTn searches at NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and relevant literature was consulted to select sequences for inclusion in the phylogenetic analyses (Rathnayaka et al. 2022). Sequences generated from this study and isolates retrieved from GenBank are shown in Table 1. Each locus (ITS, *tef*1 and *tub2*) was aligned individually with MAFFT 6.864b (Katoh et al. 2019) and trimmed using trimAl v1.2 software (Capella-Gutiérrez et al. 2009). Single-gene and multi-gene aligned datasets were analysed separately by maximum likelihood (ML) and Bayesian inference (BI). MrModeltest v. 2.2 (Nylander 2004) under the AIC (Akaike Information Criterion) implemented in PAUP v. 4.0b10 was used to find the best fit models for BI and ML analyses. The GTR+G model was selected as the best model for both ML and BI analyses for all gene regions.

The ML analyses were carried out with RAxML-HPC2 on XSEDE (v. 8.2.10) (Stamatakis 2014) in the CIPRES Science Gateway (Miller et al. 2010) using the GTR+G substitution model. The nonparametric bootstrap iterations were run for 1,000 replications. The BI analyses were conducted with MrBayes v. 3.2.6 (Ronquist et al. 2012). The Markov Chain Monte Carlo (MCMC) algorithm of six chains was initiated for 1,000,000 generations. The trees were sampled at every 100th generation resulting in 10,000 trees. The first 10% of trees were discarded as the burn-in phase, while the remaining 9,000 trees were used to calculate the posterior probabilities (PP) in the majority rule consensus tree. FigTree v1.4.0 program (Rambaut 2012) was used to visualise trees, which were then edited with Microsoft PowerPoint (2010).

Table 1 Taxa used in the phylogenetic analysis and their GenBank accession numbers. Newly generated sequences are in blue and ex-type strains are in bold.

Species	Strain no.	GenBank accession no.		
		ITS	***tef*1**	***tub2***
Dothiorella acacicola	**CPC 26349**	NR_145255	KX228376	N/A
D. acericola	KUMCC 18-0137	MK359449	MK361182	N/A
D. albiziae	**MFLUCC 22-0057**	ON751762	ON799588	ON799590
D. alpina	CGMCC 3.18001	KX499645	KX499651	N/A
D. brevicollis	**CMW 36463**	NR_111703	JQ239390	JQ239371
D. capri-amiss	CBS:121763	EU101323	EU101368	KX464850
D. casuarini	CBS 120688	DQ846773	DQ875331	N/A
D. chiangmaiensis	**MFLUCC 22-0106**	OP598812	OP614928	N/A
D. chiangmaiensis	MFLU 22-0161	OP598811	OP614929	N/A
D. citricola	ICMP16828	EU673323	EU673290	EU673145
D. diospyricola	**CBS 145972**	MT587398	MT592110	MT592581
D. dulcispinae	CMW:36460	JQ239400	JQ239387	JQ239373
D. dulcispinae	CMW 25407	EU101300	MT592120	KX464862
D. iranica	IRAN1587C	KC898231	KC898214	N/A
D. lampangensis	MFLUCC 18-0232	MK347758	MK340869	MK412874
D. longicollis	CBS 122068	EU144054	EU144069	N/A
D. magnoliae	CFCC 51563	KY111247	KY213686	N/A
D. mangifericola	**CBS 121760**	EU101290	EU101335	KX464877
D. mangifericola	IRAN1584C	KC898221	KC898204	N/A
D. moneti	**MUCC505**	EF591920	EF591971	EF591954
D. obovata	**MFLUCC 22-0058**	ON751763	ON799589	ON799591
D. ostryae	**JZB3150026**	MN533805	MN537429	N/A
D. plurivora	IRAN1557C	KC898225	KC898208	N/A
D. pretoriensis	**CMW 36480**	JQ239405	JQ239392	JQ239376

Table 1 Continued.

Species	Strain no.	GenBank accession no.		
		ITS	***tef*1**	***tub2***
D. prunicola	CAP187	EU673313	EU673280	EU673100
D. rhamni	**CBS 140852**	KT240287	MT592111	MT592582
D. santali	**MUCC 509**	EF591924	EF591975	EF591958
D. sarmentorum	**CBS 128309**	HQ288218	MT592106	MT592577
D. sarmentorum	MFLUCC 17-0242	KY797637	N/A	MT592585
D. sarmentorum	CBS 115041	AY573202	AY573222	EU673096
D. sarmentorum	MFLUCC 17-0951	MG828897	MG829267	MT592592
D. sarmentorum	**CBS 392.80**	KX464133	KX464626	KX464897
D. sarmentorum	IRAN1579C	KC898234	KC898217	N/A
D. sarmentorum	IRAN1583C	KC898236	KC898219	N/A
D. sarmentorum	MFLUCC 13-0498	KJ742379	KJ742382	N/A
D. sarmentorum	CBS 725.79	KX464130	KX464622	KX464888
D. sarmentorum	**IMI 63581b**	AY573212	AY573235	MT592612
D. striata	ICMP 16819	EU673320	EU673287	EU673142
D. striata	**DAR80992**	KJ573643	KJ573640	N/A
D. tectonae	MFLUCC12-0382	KM396899	KM409637	KM510357
D. thailandica	**MFLUCC 11-0438**	NR_111794	JX646861	JX646844
D. thripsita	BRIP 51876	KJ573642	KJ573639	KJ577550
D. uruguayensis	**CBS 124908**	NR_156208	N/A	KX464886
D. vinea-gemmae	**B116-3**	KJ573644	KJ573641	KJ577552
D. viticola	**WA10NO01**	HM009376	HM800511	HM800519
D. viticola	WA10NO02	HM009377	HM800512	HM800520
D. yunnana	CGMCC 3.18000	KX499644	KX499650	N/A
Neofusicoccum luteum	**CBS 562.92**	KX464170	KX464690	KX464968
N. luteum	**CMW 41365**	NR_147360	KP860702	KP860779

N/A - Sequences not available

Results

Phylogenetic analyses

The combined ITS, *tef*1 and *tub2* dataset included 47 ingroup taxa with two isolates of *Neofusicoccum luteum* (CBS 562.92 and CMW 41365) as the outgroup. The final alignment consisted of 1201 characters, including gaps (ITS = 477, *tef*1 = 294, *tub2* = 430). Both ML and BI analyses resulted in trees with similar topology. The best-scoring RAxML tree with a final likelihood value of -6138.3611 is presented in Fig. 1. The matrix of the combined dataset included 460 distinct alignment patterns with 18.98% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.20636, C = 0.30773, G = 0.249379, T = 0.236531; substitution rates AC = 1.508092, AG = 3.080246, AT = 1.452568, CG = 1.161038, CT = 5.884853, GT = 1.0; gamma distribution shape parameter α = 0.201084. The average standard deviation of split frequencies was 0.009 after 1,000,000 generations of runs. According to the phylogenetic analyses, our new strains, MFLUCC 22-0106 and MFLU 22-0161 formed a separate clade within *Dothiorella* with 70% ML, 0.93 pp.

Taxonomy

Dothiorella chiangmaiensis Rathnayaka & K.D. Hyde, sp. nov. Fig. 2

Index Fungorum number: IF558398; Facesoffungi number: FoF 12894

Etymology – The epithet *chiangmaiensis* refers to Chiang Mai Province, where the fungus was collected.

Holotype – MFLU 22-0161

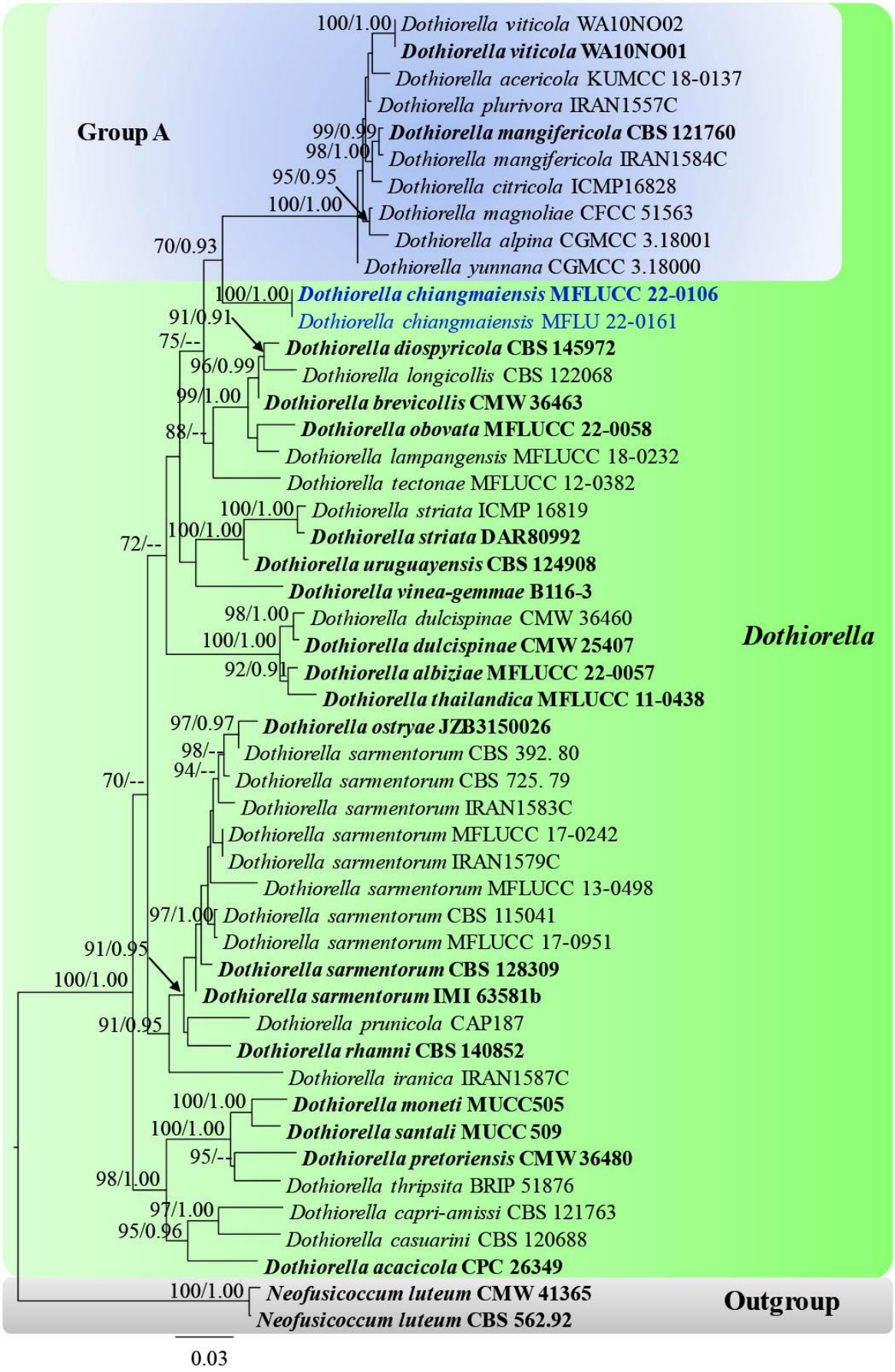

Fig. 1 – Phylogenetic tree generated from ML analysis based of the combined ITS, *tef*1 and *tub2* sequence dataset. The tree was rooted to *Neofusicoccum luteum* (CMW 4165 and CBS 562.26). Tree topology is similar to that in the previous study done by Rathnayaka et al. (2022). Bootstrap

support values for ML ≥ 70 % and Bayesian posterior probabilities (PP) ≥ 0.9 are noted at the nodes. Strain numbers are noted after the species names. Strains isolated in this study are represented as blue and type strains are in bold.

Fig. 2 – *Dothiorella chiangmaiensis* on dead branches of *Tamarindus indica* (MFLU 22-0161). a, b Conidiomata on host surface. c Vertical section through a conidioma. d Ostiole. e Peridium of conidioma. f–h Conidia attached to conidiogenous cells. i–k Conidia. l Germinated conidium. m, n Colony on PDA (m upper, n lower). Scale bars: a =1 mm, b =100 µm, c =200 µm, d =50 µm, e, i–l = 20 µm, f–h = 10 µm.

Saprobic on dead branches of *Tamarindus indica*. Sexual morph: Undetermined. Asexual morph: Coelomycetous. *Conidiomata* 445–500 μm high × 460–500 μm diam. ($\bar{x}$ = 485 × 475 μm, n = 10), pycnidial, solitary, formed in uniloculate stromata, immersed, becoming erumpent at maturity, globose to sub globose, ostiolate. *Ostiole* 50–85 μm diam., central, papillate. *Conidiomata wall* 33–83 μm diam., composed of two layers, outer layer composed of thick-walled, dark brown to brown cells of *textura angularis*, inner layer composed of thin-walled, pale brown to hyaline cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 6–12 μm × 5–9 μm ($\bar{x}$ = 9.5 × 7 μm, n = 20), holoblastic, lining the pycnidial cavity, hyaline, cylindrical, discrete, determinate, smooth-walled. *Conidia* 30–40 μm ×12–17 μm ($\bar{x}$ = 36 × 15 μm, n = 20, l/w = 2.4), ellipsoid, straight or slightly curved, rounded at both ends, initially hyaline and aseptate becoming dark brown and 1-septate often while attached to conidiogenous cell, slightly constricted at the septum, guttulate.

Culture characteristics – Conidia germinating on PDA within 24 h. Germ tubes produced at one side of the conidia. Colonies on PDA reaching 1.5–2.5 cm diam. after 6 days at 25°C, colonies circular in shape, medium dense, flat or effuse, slightly raised, fluffy to fairly fluffy, aerial, black to grey colour in the upper side and black colour in the lower side.

Material examined – Thailand, Chiang Mai, Mushroom Research Centre (MRC), on dead branches of *Tamarindus indica* (*Fabaceae*), 10 September 2020, Pahoua Pasouvang (MFLU 22-0161, holotype), ex-type living culture MFLUCC 22-0106.

Note – *Dothiorella chiangmaiensis* fits within the generic concept of *Dothiorella* in having 1-septate conidia that become brown while attached to the conidiogenous cells (Phillips et al. 2005, Dissanayake et al. 2016). In the multi-gene phylogeny (ITS, *tef*1 and *tub2*), the novel taxon formed a distant lineage basal to *Dothiorella* species in group A, i.e., *Dothiorella acericola, D. alpina*, *D. citricola, D. magnoliae, D. mangifericola, D. plurivora, D. viticola and D. yunnana* with 70% ML and 0.93 BYPP support (Fig. 1). Detailed morphological comparison and base pair differences between species in group A and *D. chiangmaiensis* are provided in Tables 2, 3, respectively.

Among the *D. chiangmaiensis* and group A species, only *D. chiangmaiensis* has guttulate conidia. Compared to other species in group A, our novel taxon has the highest L/W ratio (2.4) (Table 2). Therefore, our novel taxon is distinguished from species in group A by having the largest conidia with guttules. Based on distinct morphology and phylogenetic evidence, we introduce *D. chiangmaiensis* as a new species in *Dothiorella*.

Table 2 Synopsis of morphological characters of asexual morphs among the *Dothiorella chiangmaiensis* and species in group A.

Species	**Conidia**				**References**
	Size (μm)	**Average (μm)**	**Ratio (L/W)**		
Dothiorella acericola	17–22(–23) × 7–10(–13)	20.8 × 9.2	2.2	dark brown, slightly constricted at the septum, smooth-walled	Phookamsak et al. (2019)
D. alpina	22–25(–28) × 10–12 (–13)	24.4 × 11.1	2.19	brown to dark brown, not constricted at the septum, smooth-walled	Hyde et al. (2020)
D. chiangmaiensis	30–40 ×12–17	36 × 15	2.4	dark brown, slightly constricted at the septum, guttulate	This study
D. citricola	(23.7–)24 – 27(– 28) × (9.5 –)10 –12(–14.1)	25.8 ± 1.1 × 12.2 ± 1.3	2.1 ± 0.2	brown, occasionally slightly constricted at the septum, externally smooth, internally finely verruculose	Abdollahzadeh et al. (2014)

Table 2 Continued.

Species	Conidia				References
	Size (µm)	Average (µm)	Ratio (L/W)		
D. magnoliae	(16.00–) 20.63–22.50 (–31.35) × (8.14–) 10.87–12.03(–15.55)	21.56 × 11.45	1.88	brown, always deeply constricted at the septum, externally smooth, internally finely verruculose	You et al. (2017)
D. mangifericola	(14.4–)17–22(–22.5) × (6.3–)8–10(–11)	19 ± 1.6 × 9 ± 0.9	2.1 ± 0.2	brown, occasionally slightly constricted at the septum, externally smooth, internally finely verruculose	Abdollahzadeh et al. (2014)
D. plurivora	(18–)20–25(–27) × (8.9–)10–13(–14.4)	22.5 ± 1.7 × 11 ± 1.1	2.1 ± 0.2	brown, occasionally slightly constricted at the septum, externally smooth, internally finely verruculose	Abdollahzadeh et al. (2014)
D. viticola	(16–)20.2–20.6(–26) × (7–)9.2– 9.4(–12)	20.4 ± 0.1 × 9.3 ± 0.1	2.2 ± 0.02	brown, occasionally slightly constricted at the septum, externally smooth, internally finely verruculose	Luque et al. (2005)
D. yunnana	(18.4–)19.6–21(–22.2) × (8.1–)8.6–9.2(–9.6)	20.3 ± 1.5 × 8.9 ± 0.9	2.3 ± 0.2	brown, occasionally slightly constricted at the septum, externally smooth	Zhang et al. (2016)

Table 3 Base pair comparison of *Dothiorella chiangmaiensis* (MFLUCC 22-0106) and species in group A (without gaps).

Species	Strain no.	ITS	*tef*1
Dothiorella acericola	KUMCC 18-0137	2.5% (12/472 bp)	20.1% (34/169 bp)
D. alpina	CGMCC 3.18001	3.4% (15/447 bp)	18.7% (40/213 bp)
D. citricola	ICMP16828	2.3% (11/472 bp)	17.7% (37/208 bp)
D. magnoliae	CFCC 51563	2.8% (12/436 bp)	17.6% (37/210 bp)
D. mangifericola	CBS 121760	2.5% (12/472 bp)	22.4% (40/178 bp)
D. plurivora	IRAN1557C	2.5% (12/472 bp)	18.4% (39/211 bp)
D. viticola	WA10NO01	2.5% (12/472 bp)	20.2% (39/193 bp)
D. yunnana	CGMCC 3.1800	2.6% (12/460 bp)	17.6% (37/210 bp)

Discussion

In this study, we introduced a new species in *Dothiorella*, *Dothiorella chiangmaiensis* with most of the criteria for establishing new species in *Dothideomycetes* fulfilled (Chethana et al. 2021, Jayawardena et al. 2021, Pem et al. 2021). The novel taxon described here occurred as a saprobe and was collected from a terrestrial habitat in Thailand in September 2020.

Thailand is a tropical country with a rich fungal diversity (Rathnayaka & Jayawardena 2019). As shown in Table 4, very few *Dothiorella* species have been recorded and except for *D. dulcispinae* (synonym: *D. oblonga*), another five *Dothiorella* species were introduced as new from Thailand. Based on previous studies, all these *Dothiorella* species were recorded as saprobes (Table 4).

This study has expanded the taxonomic framework of *Dothiorella* in Thailand by revealing another new species. Therefore, this finding contributes to the basic knowledge of the fungal diversity in Thailand. However, further investigations are needed to discover the hidden diversity of *Dothiorella* species with different life modes and hosts in Thailand.

Table 4 *Dothiorella* species recorded from Thailand.

Species	Host	Life mode	References
**Dothiorella albiziae*	dry pod of *Albizia lebbeck*	Saprobic	Rathnayaka et al. (2022)
D. dulcispinae (synonym: *D. oblonga*)	*Chromolaena odorata*	Saprobic	Mapook et al. (2020)
**D. obovata*	*Pavonia odorata*	Saprobic	Rathnayaka et al. (2022)
**D. tectonae*	*Tectona grandis*	Saprobic	Doilom et al. (2015)
**D. thailandica*	*Bamboo culm*	Saprobic	Liu et al. (2012)
**D. lampangensis*	fallen fruit pericarp of *Rutaceae*	Saprobic	Jayasiri et al. (2019)

*Holotype

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