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New species and new records of plant pathogenic fungi from northern Thailand

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Abstract

Plant pathogenic fungi are significant in agricultural ecosystems, causing substantial economic losses worldwide. Understanding their diversity and distribution by exploring the poorly studied niches is essential for agricultural and horticultural production and effective disease management strategies. In this study, we conducted a comprehensive survey of plant pathogenic fungi on the Mae Fah Luang University premises, Thailand. Through morphological and multi-gene phylogenetic analyses, eight new fungus-host and five new geographical records are reported. Additionally, a new species, *Neopestalotiopsis iridis*, is illustrated and described. The survey revealed a diverse assemblage of fungal pathogens infecting various plant hosts, including economically important crops and ornamental plants. Our findings contribute to the knowledge of fungal biodiversity in northern Thailand and provide valuable insights into new niches of the species. This study underscores the importance of continued research efforts to monitor plant pathogenic fungi in different niches to increase our understanding of species diversity, distributions and evolutionary relationships.

Keywords – *Bauhinia* sp. – *Iris pseudacorus* – Jack fruit – *Morinda citrifolia* – Niches – Phytopathogens

Introduction

Plant pathogenic fungi are a significant concern in agriculture, forestry, and natural ecosystems, causing diseases that lead to severe ecological problems and economic losses (Dayarathne et al. 2023). Determining the dynamic of interactions between plants and fungi in a particular ecological niche and creating efficient disease management plans requires an understanding of the diversity of plant pathogenic fungi (Burdon & Silk 1997). In the tropical setting of Thailand, with its unique climatic and ecological conditions, the prevalence and impact of plant pathogenic fungi necessitate detailed investigation (Ko et al. 2011, Withee et al. 2022).

Historically, the identification and characterization of plant pathogenic fungi have relied on classical morphological methods. However, recent advancements in molecular techniques have revolutionised fungal taxonomy, enabling more accurate identification and classification (Hariharan

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& Prasannath 2021). The significance of scientific exploration, encompassing the formal identification of novel taxa, resides in its pivotal role in rendering a species discernible within scientific inquiry (Chethana et al. 2021, Jayawardena et al. 2021). Without a scientifically assigned nomenclature, a species remains imperceptible to scholarly investigation, thereby severely limiting the avenues for probing its ecological dynamics, potential applications, inherent threats, and devising strategies for conservation (Cheek et al. 2020). Despite the progress, there remains a lack of comprehensive studies on plant pathogenic fungi in specific localities, including university premises.

Mae Fah Luang University (MFU) is situated in the northern region of Thailand and boasts a diverse array of plant species encompassing ornamental, agricultural, and indigenous varieties. This diversity provides an ideal setting to explore the interactions between plants and plant pathogenic fungi (Hyde et al. 2018). By conducting a systematic survey, this study aims to contribute valuable data on the presence of both known and potentially new species of fungi and their occurrence within the premises.

Materials & Methods

Sample collection and fungal isolation

Thirty fresh samples showing symptoms, including leaf and fruit anthracnose, leaf spots and leaf dieback were collected from different host plants in the Mae Fah Luang University (various places), Chiang Rai, Thailand, during 2021–2022. Samples collected from a specific host were segregated into distinct containers and transported to the laboratory for morphological examination and fungal isolation. Macro-morphological features were evaluated and documented through imaging facilitated by SZX16 (Olympus, Tokyo). Morphological characteristics were examined using an EZ4 stereomicroscope (Leica, Wetzlar, Germany), and photographed using a 600D Nikon camera (Tokyo, Japan). The dimensions of fungal characteristics were quantified employing Tarosoft (R) Image Frame Work v. 0.9.7. Visual representation was generated using Adobe Photoshop v. 21.1.2 software (Adobe Systems, San Jose, CA, USA). Fungi were isolated and purified through the single-spore isolation technique, as described by Senanayake et al. (2020), and the obtained strains were transferred to cryogenic tubes containing potato dextrose agar (PDA) and were kept at 2 °C for further examination. The culture characteristics were examined on PDA plates kept at 25±2 °C. The specimens and dried cultures were deposited in the Mae Fah Luang University Fungarium (MFLU), and living cultures were deposited in the Culture Collection (MFLUCC), Chiang Rai, Thailand.

DNA extraction, PCR amplification, and sequencing

DNA extraction was done using the Omega DNA Extraction Kit (Bio-Tek). The PCR reactions were prepared in 25 µL volumes, comprising 9.5 µL double-distilled water, 12.5 µL 2×PCR Master Mix (PROMEGA, Madison, USA), 1 µL DNA template (10–100 ng/µL), and 1 µL of each primer (20 µM). The specific markers, corresponding primers, and amplification conditions were employed for each fungal genus in this investigation (Supplementary material S1). A Master Cycler X50s (Eppendorf) was used to conduct PCR amplification. The sequencing service was provided by SolGent Company (Republic of Korea).

Alignments and phylogenetic analysis

The acquired genetic sequences were subjected to a BLASTn search in the NCBI. Subsequently, reference sequences were retrieved from the GenBank database. The sequences of each dataset were aligned using the MAFFT v. 7 (https://mafft.cbrc.jp/alignment/server/) (Katoh et al. 2019). The alignments were adjusted using BioEdit v. 7.0.9.0 (Hall 1999), and were automatically trimmed using TrimAl v 1.2 (Capella-Gutiérrez et al. 2009) under the -gapthreshold (0.8) option or -gappyout option. The optimal evolutionary model for specific gene region was determined using jModelTest 2 v.2.1.6 on XSEDE, as outlined by Darriba et al. (2012).

Maximum-likelihood (ML) was carried out using RAxML-HPC2 on XSEDE and IQ-TREE 2.2.2.7 on ACCESS (Minh et al. 2020), in the CIPRES Science Gateway (https://www.phylo.org/)

with the automatic substitution model selection (Kalyaanamoorthy et al. 2017) and 1000 ultrafast bootstraps. Bayesian inference (BI) was performed by MrBayes on XSEDE v.3.2.7a in the CIPRES Science Gateway portal with four independent Markov Chain Monte Carlo (MCMC) chains and four runs for 1,000,000–5,000,000 generations and sampled at every 100th generation. Maximum-parsimony (MP) analysis was performed by PAUP on XSEDE (Swofford 2002). Finally, the derived phylograms were rendered using FigTree v.1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/) and were annotated in Illustrator CC v. 22.0.0 (Adobe Systems).

Results

Morphological identification

Morphological characteristics, including the appearance of conidiomata, shape and size of the conidia, colour of conidia, conidial septa and appendages, shape and size of conidiophores and appearance of the colony were recorded for obtained strains. Based on morphological analysis, the obtained strains were divided into three genera: *Colletotrichum*, *Neopestalotiopsis*, and *Lasiodiplodia*. Since morphological characteristics are insufficient to identify the species accurately, multi-gene phylogeny was conducted after that.

Phylogenetic analysis

The combined sequence alignments encompassing each complex and genus were subjected to Maximum likelihood (ML), Maximum parsimony (MP), and Bayesian inference (BI) analyses. Phylogenetic analyses of *Colletotrichum* species complexes were performed using a concatenated dataset comprising ITS, *gapdh*, *chs-1*, *act*, and *tub2* gene regions. The phylogenetic relationships within *Lasiodiplodia* were resolved using a multi-locus dataset including ITS, *tef1*, *tub2*, and *rpb2*. For *Neopestalotiopsis*, evolutionary relationships were inferred based on a combined dataset of ITS, *tef1*, and *tub2* sequences. For *Neopestalotiopsis*, a combined dataset of ITS, *tef1*, and *tub2* sequence data was employed. Maximum likelihood analyses of the species complexes, namely *Colletotrichum dracaenophilum* and *C. orchidearum*, as well as for *Lasiodiplodia* were executed through RAxML-HPC2. Concurrently, ML analyses of the *Colletotrichum gloeosporioides* and *C. truncatum* species complex, alongside *Neopestalotiopsis*, were performed using IQ-TREE. Notably, the topology of the trees generated through ML, MP, and BI methods exhibited no significant differences. Consequently, the ML tree has been chosen and presented as the representative tree. The information related to the phylogenetic analysis and respective parameters are presented (Supplementary materials S2, S3). The details of all species and strains obtained in this study are shown (Table 1).

Taxonomy

Colletotrichum gloeosporioides species complex

In the phylogenetic assessments (Fig. 1), 12 isolates were classified within the *C. gloeosporioides* species complex. Among these, nine isolates formed a cluster within the *C. siamense* clade, and two isolates were grouped with the *C. fructicola* clade, and a single isolate was closely associated with *C. musae* (ex-type, ICMP 19119).

Table 1 Information of the isolates obtained in current research.

Species	Strain	Host	GenBank accession number					
			ITS	*tef1*	*gapdh*	*chs-1*	*act*	*tub2*
Colletotrichum fructicola	A9; MFLUCC 24-0210	*Ficus religiosa*	PP960250	—	PP982589	PP982575	PP975282	PP982559
C. fructicola	C42; MFLUCC 24-0214	*Bauhinia* sp.	PP960248	—	PP982587	PP982573	PP975280	PP982557
C. musae	C26; MFLUCC 24-0207	*Musa* sp.	PP960245	—	PP982584	PP982570	PP975277	PP982554
C. musicola	C30; MFLUCC 24-0206	*Lagerstroemia* sp.	PP960252	—	PP982591	PP982577	PP975284	PP982561
C. siamense	A3; MFLUCC 24-0203	*Asplenium nidus*	PP960243	—	PP982583	PP982568	PP975275	PP982552
C. siamense	A14; MFLUCC 24-0202	*Dendrobium* sp.	PP960242	—	PP982582	PP982567	PP975274	PP982551
C. siamense	C3; MFLUCC 24-0200	*Anthurium andraeanum*	PP960239	—	PP982579	PP982564	PP975271	PP982548
C. siamense	C4; MFLUCC 24-0201	*Artocarpus heterophyllus*	PP960241	—	PP982581	PP982566	PP975273	PP982550
C. siamense	C11; MFLUCC 24-0209	*Dracaena fragrans*	PP960244	—	—	PP982569	PP975276	PP982553
C. siamense	C29-2; MFLUCC 24-0208	*Bauhinia* sp.	PP960240	—	PP982580	PP982565	PP975272	PP982549
C. siamense	C36-1; MFLUCC 24-0204	*Morinda citrifolia*	PP960246	—	PP982585	PP982571	PP975278	PP982555
C. siamense	C36-2; MFLUCC 24-0205	*Morinda citrifolia*	PP960247	—	PP982586	PP982572	PP975279	PP982556
C. siamense	C45; MFLUCC 24-0215	*Ficus* sp.	PP960249	—	PP982588	PP982574	PP975281	PP982558
C. tropicicola	C41; MFLUCC 24-0213	*Citrus maxima*	PP960251	—	PP982590	PP982576	PP975283	PP982560
C. truncatum	A9-2; MFLUCC 24-0211	*Ficus religiosa*	PP960253	—	PP982592	PP982578	PP975285	—
Lasiodiplodia theobromae	C38; MFLUCC 24-0212	*Ficus* sp.	PP960254	PP982593	—	—	—	PP982562
Neopestalotiopsis iridis	C1; MFLUCC 24-0199^{T}	*Iris pseudacorus*	PP960255	PP982594	—	—	—	PP982563

Type strains are indicated with "^{T}".

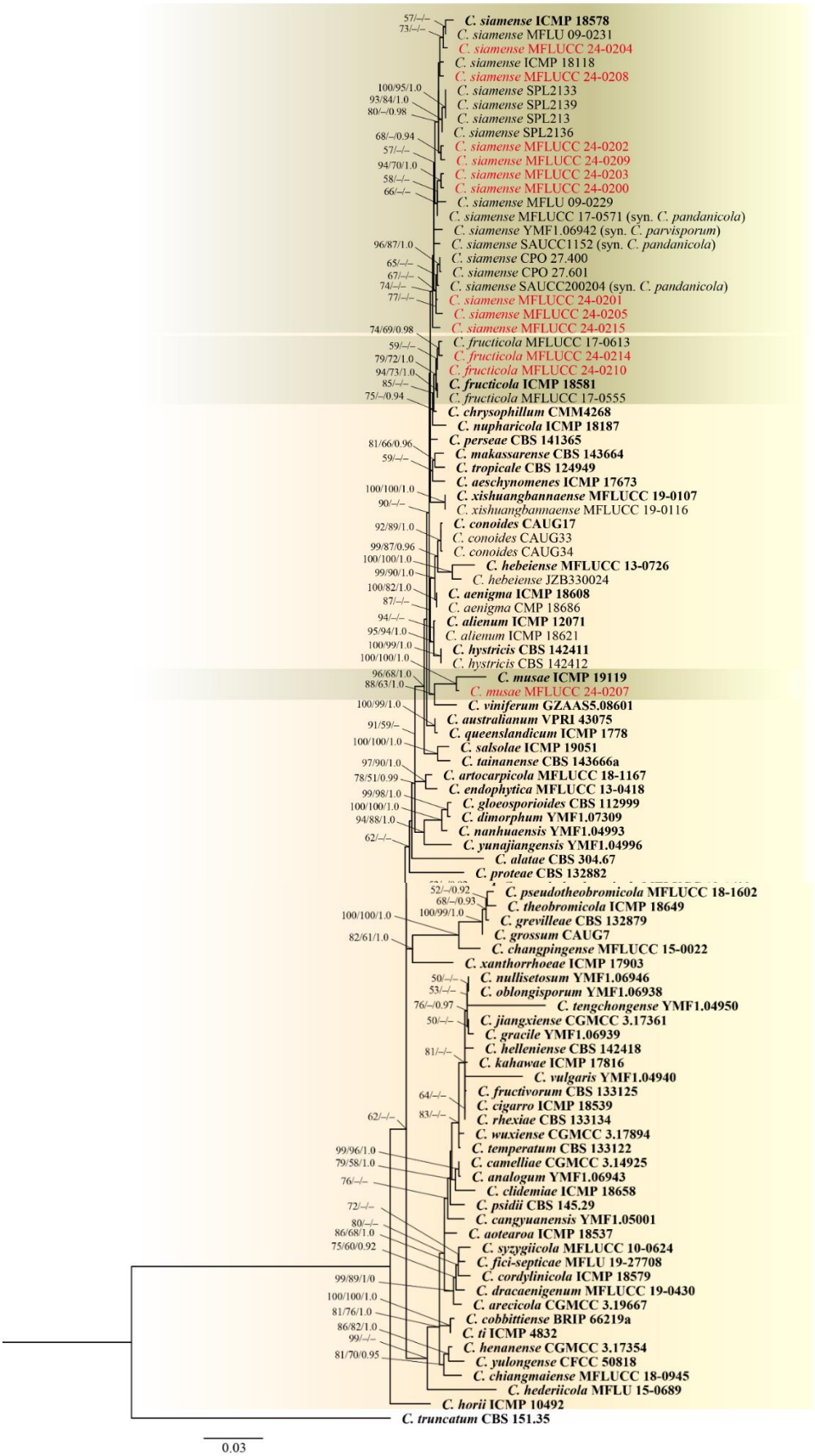

Fig. 1 – Phylogenetic tree constructed using maximum likelihood analysis of the *Colletotrichum gloeosporioides* species complex, based on the combined dataset of ITS, *gapdh*, *chs-1*, *act*, *tub*2. The tree was rooted with *Colletotrichum truncatum* (CBS 151.35). Maximum-likelihood and maximum-

parsimony bootstrap values above 50% and Bayesian posterior probabilities above 0.90 are shown near the nodes, respectively. Type strains are in bold, and the new isolates from this study are in red.

Colletotrichum fructicola Prihast., L. Cai & K.D. Hyde (2009) Fig. 2

Index Fungorum Number: IF 515409; Facesoffungi Number: FoF 06767

Associated with leaf anthracnose of *Ficus religiosa*. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* acervular and pycnidial, dark brown. *Setae* not observed. *Conidiophores* branched or solitary, septate, hyaline, cylindrical to inflated. *Conidiogenous cells* smooth-walled, aseptate, cylindrical or clavate, hyaline, 13–22 × 3–4 µm ($\bar{x}$ = 15 × 3 µm, n = 30). *Conidia* unicellular, smooth-walled, hyaline, cylindrical, apex rounded, base sub-acute, guttulate, 9–20 × 4.5–5.5 µm ($\bar{x}$ = 15 × 5 µm, n = 30). *Appressoria*, irregular in shape, undulate, brown to dark brown, 3–7 × 3–5 µm ($\bar{x}$ = 4 × 3.7 µm, n = 30), produced directly on both hyphae and conidia on slide culture.

Culture characteristics – Colonies on PDA 60–73 mm diam. after seven days at 28 °C, velvety, circular, entire edge; surface smoke grey in the center and white at the margin; reverse the same colour.

Material examined – Mae Fah Luang University premises, Mueang Chiang Rai District, Chiang Rai Province, Thailand, on *Ficus religiosa* (Moraceae), 19 December 2021, Alireza Armand, A9 (MFLU 24-0212), living culture, MFLUCC 24-0210; *ibid*., on *Bauhinia* sp., living culture MFLUCC 24-0214.

Fig. 2 – *Colletotrichum fructicola* (MFLU 24-0212). a, b Symptomatic leaf of *Ficus religiosa*. c Pycnidia produced on the PDA. d, e Conidiophores and conidial attachment. f, g Conidia. h Appressoria. i Colony on the PDA. Scale bars: d, e = 20 µm, f–h = 10 µm.

Notes – Two isolates designated as *C. fructicola* (MFLUCC 24-0210 and MFLUCC 24-0214) exhibited morphological uniformity, and a comparative analysis of their morphology with the type strain of *C. fructicola* showed no morphological differences (Fig. 2). From a phylogenetic standpoint, the two newly isolated strains were grouped with the ex-type and other strains of *C. fructicola* within a distinct clade, supported by 79%, 72%, and 1.0 support values in the ML, MP, and BI analyses (Fig. 1). A pairwise comparison of our strains with the ex-type sequences revealed 1/229 (0.4%) bp difference in *chs-1* and 1/220 (0.4%) in *tub2* for the strain MFLUCC 24-0210. For the strain MFLUCC 24-0214, 2/217 (0.9%) bp differences were observed in *gapdh*, 2/229 (0.8%) in *chs-1*, and 2/420 (0.4%) in *tub2*. This is the first report of *C. fructicola* from *Bauhinia* sp. and *Ficus religiosa* globally.

Colletotrichum musae (Berk. & M.A. Curtis) Arx, (1957) Fig. 3

Index Fungorum Number: IF 295348; Facesoffungi Number: FoF 16992

Associated with the anthracnose of banana fruit. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* acervular, pale brown, with orange conidial mass on fruit. *Setae* not observed. *Conidiophores* branched or solitary, hyaline, septate, cylindrical. *Conidiogenous cells*, smooth-walled, aseptate, hyaline, cylindrical, 7–13 × 3–4 µm ($\bar{x}$ = 11 × 3.7 µm, n = 30). *Conidia* unicellular, smooth-walled, hyaline, oval, elliptical or cylindrical with a sub-acute base and obtuse apex, guttulate, 12–15.5× 5–7 µm ($\bar{x}$ = 14 × 6.3 µm, n = 30).

Culture characteristics – Colonies on PDA 75–85 mm diam. after seven days at 28 °C, velvety, circular, entire edge; aerial mycelium sparse, surface white at the beginning, becoming salmon after conidial masses developed directly from the hyphae, reverse white at the beginning, becoming pale fulvous after conidial masses developed.

Material examined – Mae Fah Luang University premises, Mueang Chiang Rai District, Chiang Rai Province, Thailand, on the fruit skin of *Musa* sp. (Musaceae), 12 December 2021, Alireza Armand, C26 (MFLU 24-0213), living culture, MFLUCC 24-0207.

Notes – Su et al. (2011) established an epitype for *Colletotrichum musae* utilizing the specimen *C. musae* (CBS 116870) as the reference. Morphological analysis comparing our strain of *C. musae* (MFLUCC 24-0207) with *C. musae* (CBS 116870) indicated an absence of discernible distinctions in the conidial shape and size. Nevertheless, a notable prevalence of oval-shaped conidia was observed in *C. musae* (MFLUCC 24-0207) compared to *C. musae* (CBS 116870), which produced mostly cylindrical conidia. Phylogenetic analysis revealed that *C. musae* (MFLUCC 24-0207) grouped alongside the ex-type within a distinct clade, showing 100% support values in both ML and MP, as well as BI analysis (Fig. 1). A pairwise comparison between MFLUCC 24-0207 and the ex-type sequence revealed 5/517 (0.9%) bp differences in ITS, 1/215 (0.4%) in *gapdh*, 1/210 (0.4%) in *act*, 6/405 (1.4%) in *tub2*.

Colletotrichum siamense Prihast., L. Cai & K.D. Hyde (2009) Fig. 4

Index Fungorum Number: IF 515410; Facesoffungi Number: FoF 03599

Associated with leaf anthracnose of *Morinda citrifolia*. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* acervular, dark brown. *Setae* not observed. *Conidiophores* branched or solitary, hyaline, septate, cylindrical to inflated. *Conidiogenous cells*, smooth-walled, aseptate, hyaline, cylindrical, 10–17 × 3–4 µm ($\bar{x}$ = 13.3 × 3.3 µm, n = 30). *Conidia* unicellular, smooth-walled, hyaline, ellipsoid or cylindrical, rounded at both ends, guttulate, 13–19× 4–5.5 µm ($\bar{x}$ = 17 × 5 µm, n = 30).

Culture characteristics – Colonies on PDA 58–70 mm diam. after seven days at 28 °C, velvety, circular, entire edge; surface white, becoming smoke grey with age; reverse the same colour.

Material examined – Mae Fah Luang University premises, Mueang Chiang Rai District, Chiang Rai Province, Thailand, on a symptomatic leaf of *Morinda citrifolia* (Rubiaceae), 03 February 2022, Alireza Armand, C36-2 (MFLU 24-0215), living culture, MFLUCC 24-0205; *ibid*., on a symptomatic leaf of *Asplenium nidus*, living culture MFLUCC 24-0203; *ibid*., on a symptomatic leaf of *Dendrobium* sp., living culture MFLUCC 24-0202; *ibid*., on a symptomatic leaf of *Anthurium*

andraeanum, living culture MFLUCC 24-0200; *ibid*., on a symptomatic leaf of *Artocarpus heterophyllus*, living culture MFLUCC 24-0201; *ibid*., on a symptomatic leaf of *Dracaena fragrans*, living culture MFLUCC 24-0209; *ibid*., on a symptomatic leaf of *Bauhinia* sp., living culture MFLUCC 24-0208; *ibid*., on a symptomatic leaf of *Morinda citrifolia*, living cultures MFLUCC 24-0204, MFLUCC 24-0205; *ibid*., on a symptomatic leaf of *Ficus* sp., living cultures MFLUCC 24-0204, MFLUCC 24-0215.

Notes – Nine isolates were unequivocally identified as belonging to *Colletotrichum siamense*, obtained from different hosts (Table 1). These isolates exhibited consistent morphological characteristics, including similarities in conidiogenous cells, conidial shape and size, as well as cultural characteristics. The morphological comparison confirmed that the isolates are similar to the ex-type. Phylogenetic analyses affirmed their consistent placement within the *C. siamense* clade with 74% ML support value along with the ex-type and other strains (Fig. 1). This study reported *C. siamense* from *Asplenium nidus*, *Dendrobium* sp., *Dracaena fragrans*, and *Morinda citrifolia* as new host records. Additionally, *C. siamense* was reported from *Anthurium andraeanum*, *Artocarpus heterophyllus*, *Bauhinia* sp., and *Ficus* sp. for the first time in Thailand.

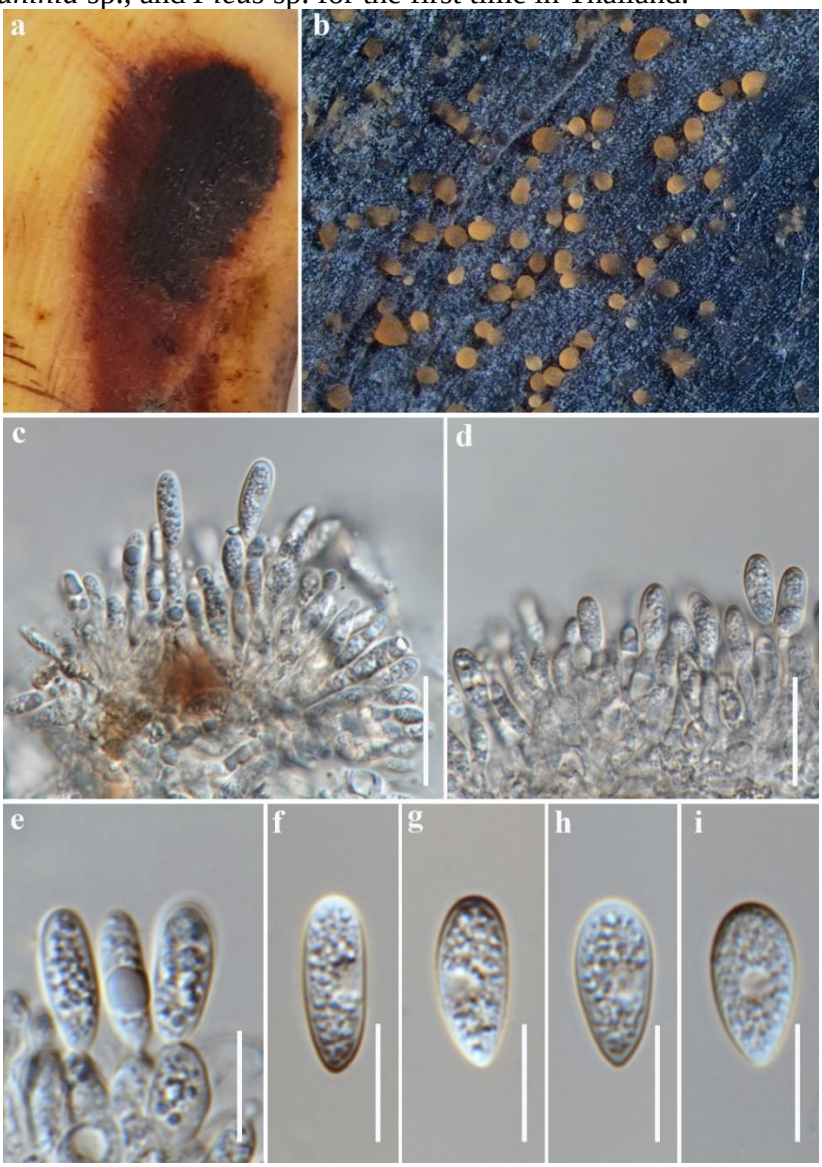

Fig. 3 – *Colletotrichum musae* (MFLU 24-0213). a Lesion on *Musa* sp. fruit peel. b Fruiting bodies and conidial masses on the fruit peel. c, d Conidiophores and conidia produced on PDA. e Conidiophores and conidial attachment. f–i Conidia. Scale bars: c, d = 20 µm, e–i = 10 µm.

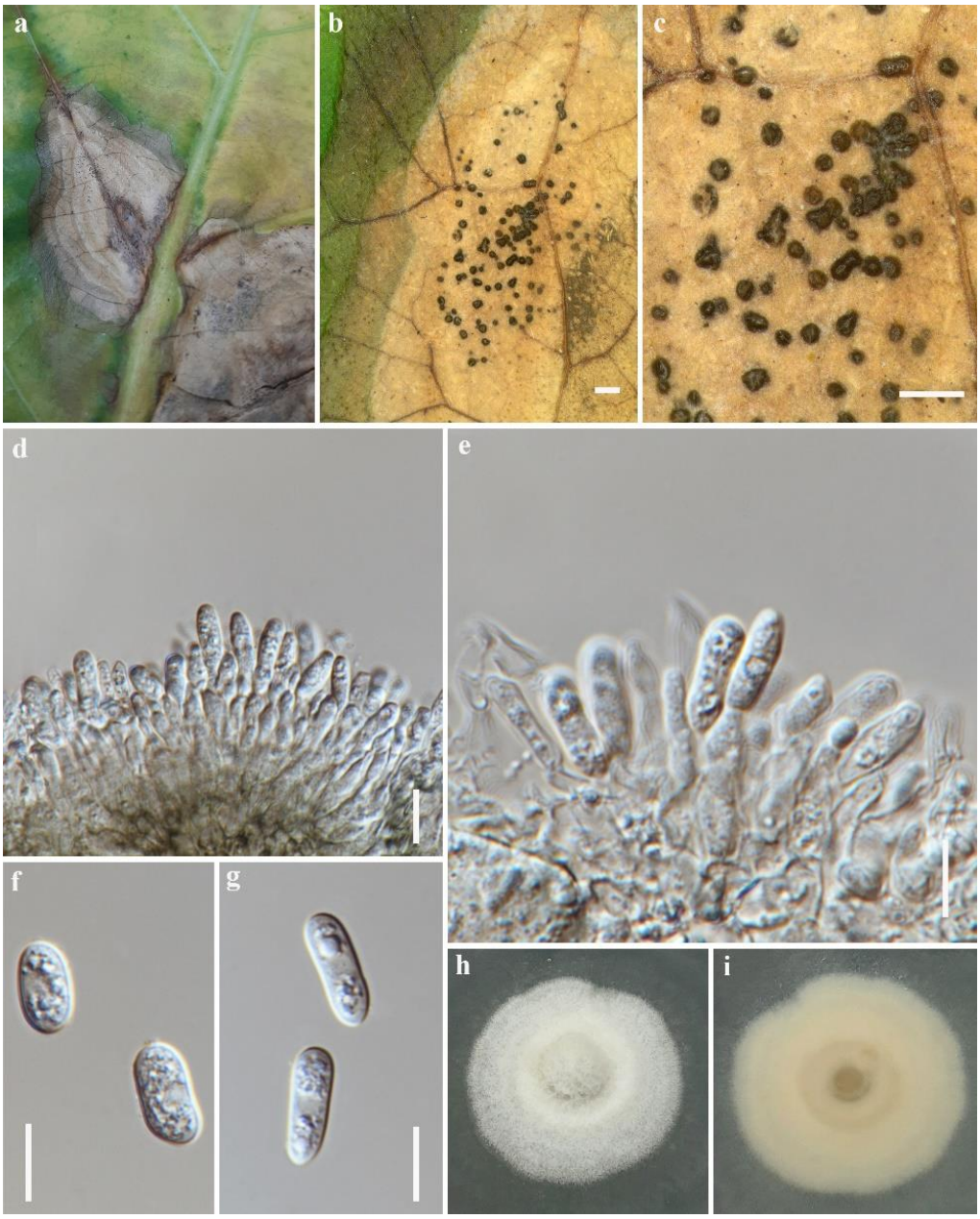

Fig. 4 – *Colletotrichum siamense* (MFLU 24-0215). a Symptoms on leaves of *Morinda citrifolia*. b, c. Fruiting bodies on the leaves. d, e Conidiophores and conidial attachment. f, g Conidia. h, i Upper and reverse view of colony on PDA. Scale bars: b–c = 500 µm, d–g = 10 µm.

***Colletotrichum orchidearum* species complex**

In the phylogenetic assessments (Fig. 5), a single isolate was identified in the *C. orchidearum* species complex, representing *C. musicola* (MFLUCC 24-0206).

Colletotrichum musicola Damm (2018) Fig. 6

Index Fungorum Number: IF 824225; Facesoffungi Number: FoF 16993

Associated with leaf spots of *Lagerstroemia* sp. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* acervular, dark brown. *Setae* pale to brown, verruculose, 60–100 µm long, 1–3-septate, base cylindrical to inflated. *Conidiophores* branched or solitary, hyaline, septate, cylindrical to inflated. *Conidiogenous cells*, smooth-walled, aseptate, hyaline, cylindrical, 8–18.5 × 3–4 µm ($\bar{x}$ = 14.5 × 3.5 µm, n = 30). *Conidia* unicellular, smooth-walled, hyaline or pale brown, cylindrical, rounded in the ends, straight, guttulate, (15–)17–20× 4–5.5 µm ($\bar{x}$ = 18 × 5 µm, n = 30).

Culture characteristics – Colonies on PDA 50–55 mm diam. after seven days at 28 °C, velvety, circular, entire edge; surface white, becoming smoke grey with age; reverse the same colour.

Material examined – Mae Fah Luang University premises, Mueang Chiang Rai District, Chiang Rai Province, Thailand, on a leaf of *Lagerstroemia* sp. (Lythraceae), 29 December 2021, Alireza Armand, C30 (MFLU 24-0214), living culture, MFLUCC 24-0206.

Notes – The isolate MFLUCC 24-0206 was morphologically similar to the *C. musicola* (ex-type, CBS 132885). It phylogenetically clustered with the ex-type and *C. musicola* (CBS 127557) with 100% ML, 100% MP, and 1.0 bootstrap value in BI analysis (Fig. 5). A pairwise comparison between our strain and the ex-type sequence showed 3/213 (1.4%) bp differences in *gapdh*, and 2/215 (0.9%) in *act*. This is a new host report of *C. musicola* associated with *Lagerstroemia* sp.

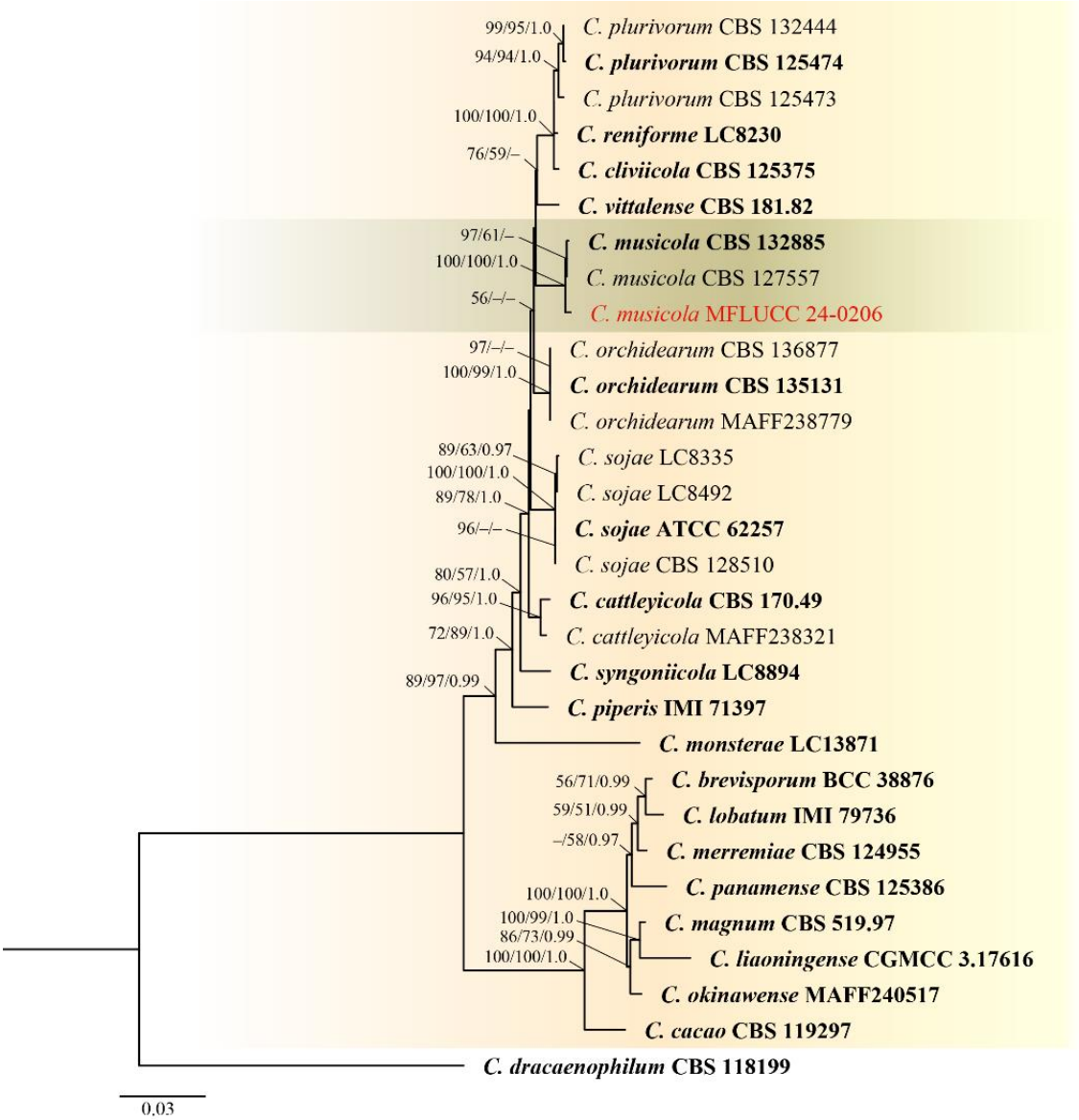

Fig. 5 – Phylogenetic tree generated from maximum likelihood analysis of the *Colletotrichum orchidearum* and *C. magnum* species complexes based on a concatenated ITS, *gapdh*, *chs-1*, *act*, *tub*2 sequence data. The tree was rooted with *Colletotrichum dracaenophilum* (CBS 118199). Maximum-likelihood and maximum-parsimony bootstrap values above 50% and Bayesian posterior probabilities above 0.90 are presented. Type strains are in bold, and the new isolates from this study are in red.

Fig. 6 – *Colletotrichum musicola* (MFLU 24-0214). a Symptoms on leaves of *Lagerstroemia* sp. b, c Fruiting bodies on the leaf. d Setae. e Conidiophores and conidial attachment. f Conidia. g. Upper and reverse view of the colony on PDA. Scale bars: d = 50 µm, e = 10 µm, f = 20 µm.

***Colletotrichum dracaenophilum* species complex**

In the phylogenetic assessments (Fig. 7), *Colletotrichum tropicicola* (MFLUCC 24-0213) was assigned to the *C. dracaenophilum* species complex, exhibiting a close clustering with its ex-type (MFLUCC 11-0114) with high support values of 91%, 96%, and 1.0 in the ML, MP, and BI analyses, respectively.

Colletotrichum tropicicola Phoulivong, Noireung, L. Cai & K.D. Hyde (2012) Fig. 8

Index Fungorum Number: IF 564159; Facesoffungi Number: FoF 16994

Associated with leaf anthracnose and die back of *Citrus maxima*. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* acervular, dark brown. *Setae* not observed. *Conidiophores* branched or solitary, hyaline, septate, cylindrical to inflated. *Conidiogenous cells* smooth-walled, aseptate, hyaline, cylindrical or clavate, 11–15(–18) × 3.5–5 µm ($\bar{x}$ = 12.5 × 4.5 µm, n = 30). *Conidia* unicellular, smooth-walled, hyaline, cylindrical, rounded ends, straight or slightly curved at base, guttulate, 16.5–19.5× 5–6 µm ($\bar{x}$ = 18 × 5.3 µm, n = 30).

Culture characteristics – Colonies on PDA 70–79 mm diam. after seven days at 28 °C, fluffy, circular, entire edge; mycelia sparse, surface olivaceous; reverse olivaceous. *Appressoria* produced on slide culture, pale brown to brown, circular or undulate, non-lobate, 4–10 µm in diam. ($\bar{x}$ = 5 µm, n = 30).

Material examined – Mae Fah Luang University premises, Mueang Chiang Rai District, Chiang Rai Province, Thailand, on a leaf of *Citrus maxima* (Rutaceae), 22 February 2022, Alireza Armand, C41 (MFLU 24-0216), living culture, MFLUCC 24-0213.

Notes – *Colletotrichum tropicicola* (MFLUCC 24-0213) exhibits morphological characteristics analogous to those of the ex-type strain, and was isolated from the same host plant from which the species was initially isolated. According to the phylogenetic analyses (Fig. 7), the strain MFLUCC 24-0213 is closely related to the ex-type strain, forming a clade supported by 91%, 96%, and 1.0 bootstrap values in the ML, MP, and BI. A pairwise comparison between our strain and the ex-type sequence revealed 2/203 (1.0%) bp differences in *gapdh*, 2/200 (1.0%) in *chs-1*, 1/210 (0.4%) in *act*, 2/400 (0.5%) in *tub2*.

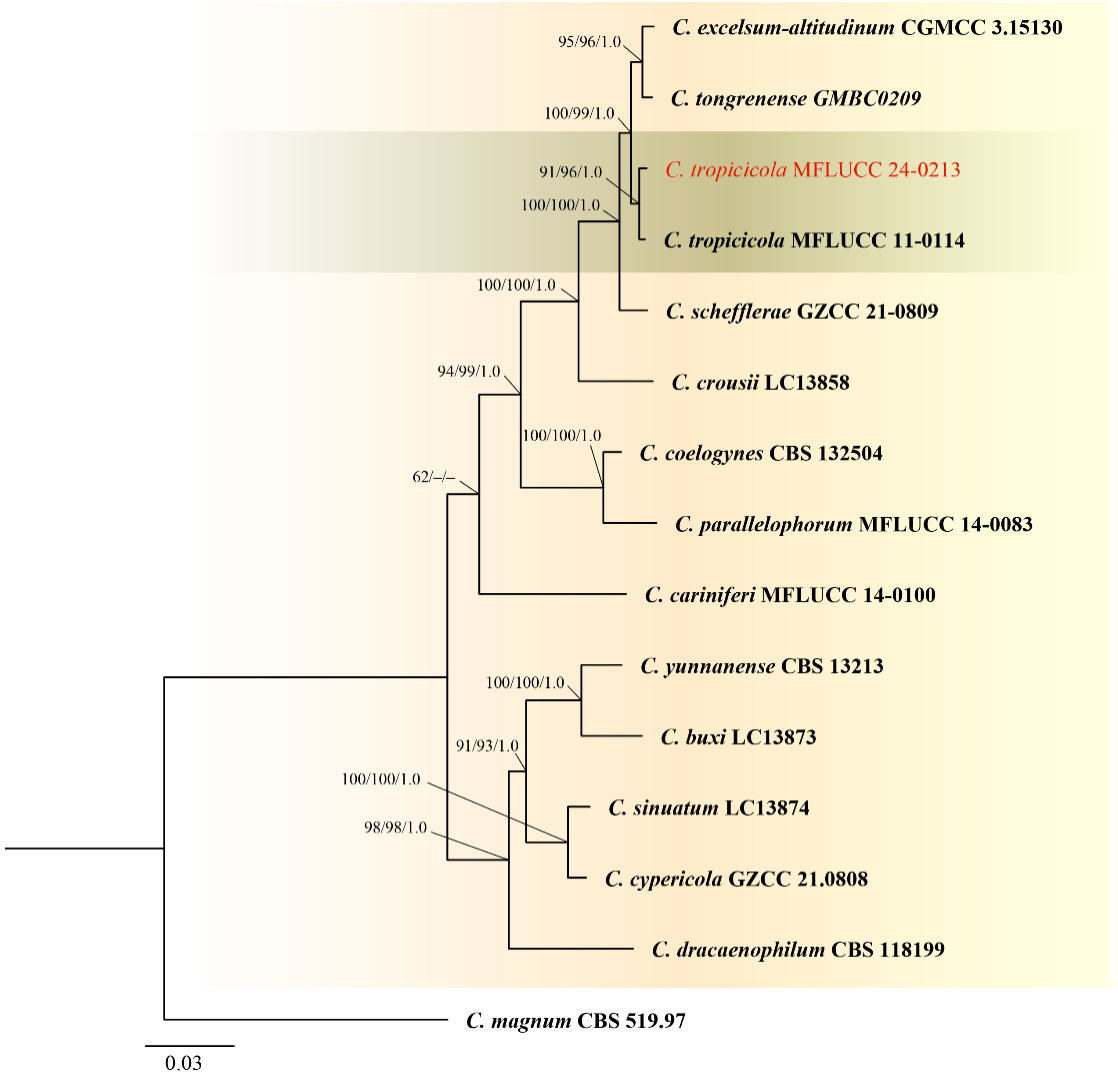

Fig. 7 – Phylogenetic tree produced through maximum likelihood analysis of the *Colletotrichum dracaenophilum* species complex based on the combined ITS, *gapdh*, *chs-1*, *act*, *tub*2 sequences. The tree was rooted with *Colletotrichum magnum* (CBS 519.97). Maximum-likelihood and maximum-parsimony bootstrap values above 50% and Bayesian posterior probabilities above 0.90 are shown, respectively. Type strains are in bold, and the new isolates from this study are in red.

***Colletotrichum truncatum* species complex**

In the phylogenetic assessments (Fig. 9), a single isolate was identified in the *C. truncatum* species complexes, representing *C. truncatum* (MFLUCC 24-0211).

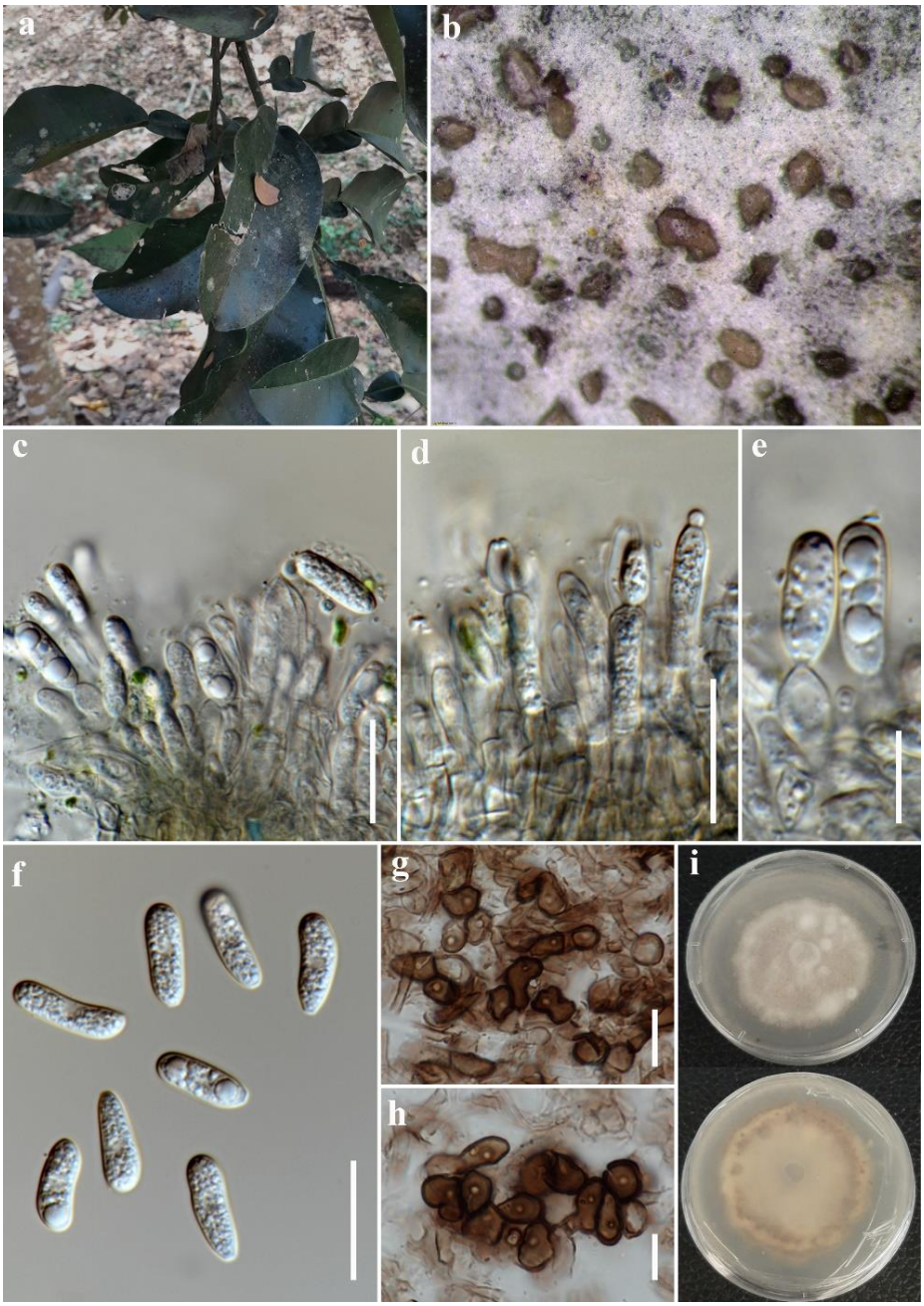

Fig. 8 – *Colletotrichum tropicicola* (MFLU 24-0216). a Symptoms on leaves of *Citrus maxima*. b Fruiting bodies on the leaf. c–e Conidiophores and conidial attachment. f Conidia. g, h Appressoria. i Upper and reverse view of the colony on PDA. Scale bars: c, d = 20 µm, e–h = 10 µm.

Colletotrichum truncatum (Schwein.) Andrus & W.D. Moore (1935) Fig. 10

Index Fungorum Number: IF 280780; Facesoffungi Number: FoF 03827

Associated with leaf anthracnose of *Ficus religiosa*. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* acervular, dark brown, bearing conidial mass and setae. *Setae* brown to dark brown, verruculose, 1–3 septate, 100–387 µm long ($\bar{x}$ = 250 µm, n = 10), base cylindrical, 6–8 µm diam. ($\bar{x}$ = 6.8 µm, n = 10), acute at the apex. *Conidiophores* branched or solitary, hyaline, septate. *Conidiogenous cells* hyaline, cylindrical, 10–15 × 2–4 µm ($\bar{x}$ = 12 × 3 µm, n = 30). *Conidia* unicellular, smooth-walled, hyaline, curved with parallel walls at the middle part, round and truncate at the base, tapering towards the acute and curved apex, guttulate, 21.5–27 × 3–4 µm ($\bar{x}$ = 24 × 3.5 µm, n = 30).

Culture characteristics – Colonies on PDA 32–35 mm in diam. after seven days at 28 °C, velvety, flat, entire edge, aerial mycelia medium dense, surface buff, reverse the same colour.

Material examined – Mae Fah Luang University premises, Mueang Chiang Rai District, Chiang Rai Province, Thailand, on a leaf of *Ficus religiosa* (Moraceae), 22 May 2022, Alireza Armand, A9-2 (MFLU 24-0217), living culture, MFLUCC 24-0211.

Notes – Morphologically, *C. truncatum* (MFLUCC 24-0211) was similar to the ex-epitype (CBS:151.35) (Damm et al. 2009). The strain (MFLUCC 24-0211) clustered with the ex-epitype and other strains in a clade with 100% ML, 100% MP, and 1.0 BI bootstrap values (Fig. 10). A pairwise comparison between our strain and the ex-type sequences revealed 2/205 (0.9%) bp differences in *gapdh*, 1/200 (0.5%) in *chs-1*, 1/210 (0.4%) in *act*, 3/410 (0.7%) in *tub2*. This study reported *C. truncatum* from *Ficus religiosa* as a new host record.

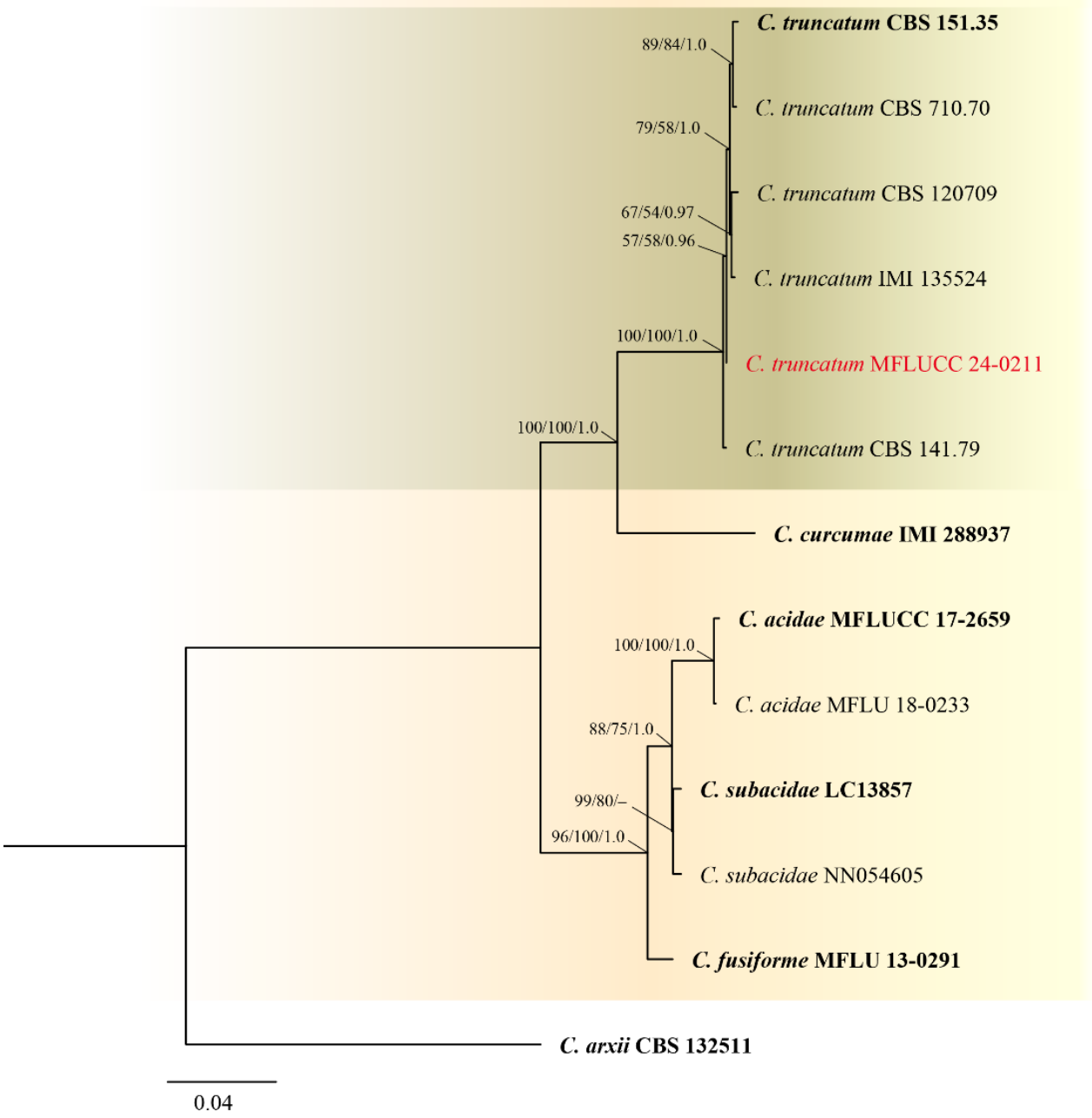

Fig. 9 – Phylogenetic tree generated by maximum likelihood analysis of the *Colletotrichum truncatum* species complex based on the concatenated sequence data of ITS, *gapdh*, *chs-1*, *act*, *tub*2. The tree was rooted with *Colletotrichum arxii* (CBS 132511). Maximum-likelihood and maximum-parsimony bootstrap values above 50% and Bayesian posterior probabilities above 0.90 are shown, respectively. Type strains are in bold, and the new isolates from this study are in red.

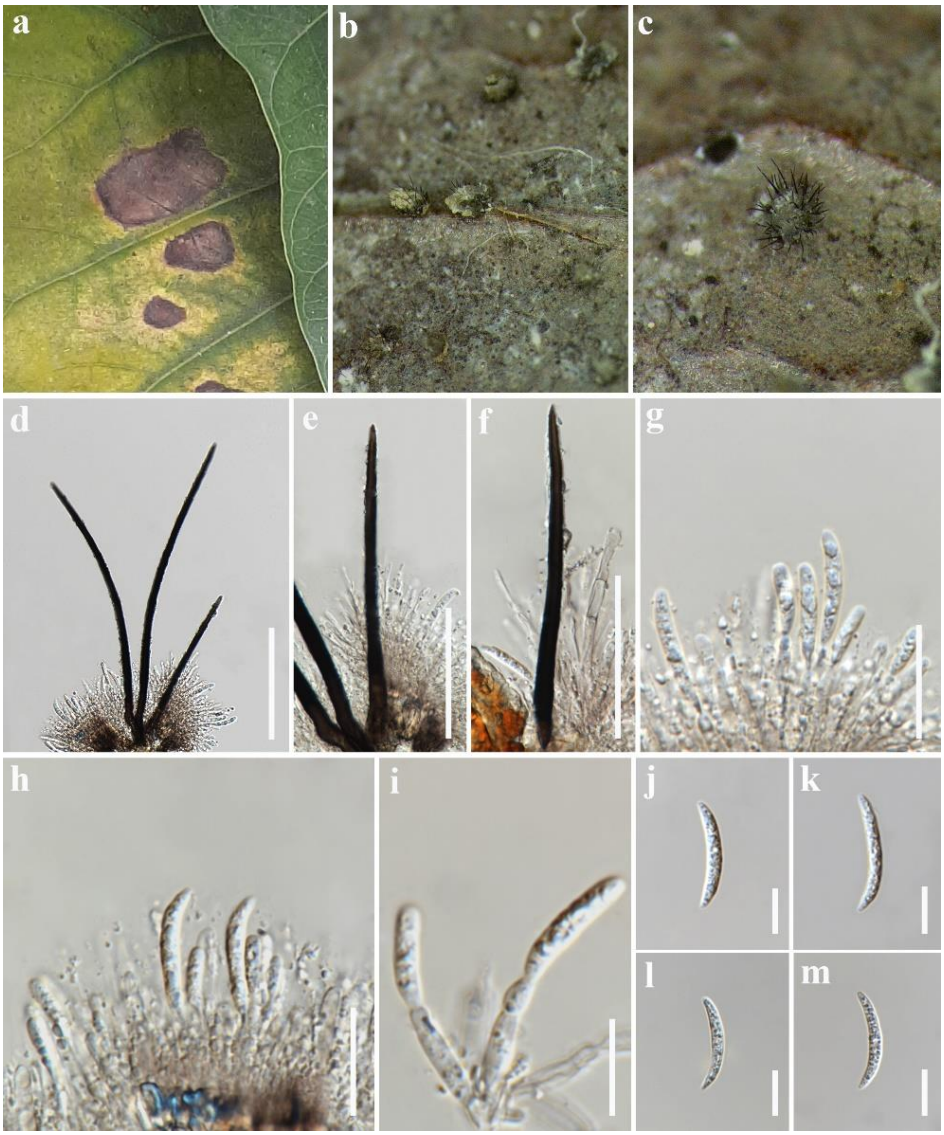

Fig. 10 – *Colletotrichum truncatum* (MFLU 24-0217). a Symptoms on leaves of *Ficus religiosa*. b, c Fruiting bodies on the leaf. d–f Setae. g–i Conidiophores and conidial attachment. j–m Conidia. Scale bars: d = 100 µm, e, f = 50 µm, g, h = 20 µm, i–m = 10 µm.

Lasiodiplodia theobromae (Pat.) Griffon & Maubl. (1909) Fig. 11

Index Fungorum Number: IF 188476; Facesoffungi Number: FoF 00167

Associated with the leaf die back of *Ficus*. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* pycnidial, 200–350 µm high × 150–290 µm diam. ($\bar{x}$ = 283 × 220 µm, n = 10), solitary or aggregated, scattered, immersed, becoming erumpent at maturity, globose to subglobose, black. *Peridium* 17–66 µm wide, consisting of 3–6 layers, outer layer thick-walled, dark brown cells of *textura angularis* to *textura globulosa*, inner layer thin-walled, light brown cells of *textura angularis* to *textura globulosa*. *Paraphyses* 20–60 × 2–3 µm ($\bar{x}$ = 32 × 2.2 µm, n = 30), hyaline, cylindrical, aseptate, not branched. *Conidiogenous cells* 5–11 × 3–6 µm ($\bar{x}$ = 9 × 4 µm, n = 20), hyaline, cylindrical, discrete or occasionally integrated. *Conidia* 20–26 × 12.5– 14.5 µm ($\bar{x}$ = 23 × 13 µm, n = 30), unicellular, oblong to ovoid, straight, rounded at both ends, hyaline, thick-walled, guttulate, becoming brown and 1-septate at maturity.

Culture characteristics – Colonies on PDA fast growing, reaching 80–90 mm diam. after seven days at 28 °C, circular, medium dense, flat, cottony to fluffy, white in the initial stage and become black with age, reverse the same colour.

Material examined – Mae Fah Luang University premises, Mueang Chiang Rai District, Chiang Rai Province, Thailand, on the leaf of *Ficus* sp. (Moraceae), 23 April 2022, Alireza Armand, C38 (MFLU 24-0218), living culture, MFLUCC 24-0212.

Notes – Morphological comparison of *L. theobromae* (MFLUCC 24-0212) with the neotype (MBT176098) showed similarities between these two strains (Phillips et al. 2013). The phylogenetic analysis indicated that *L. theobromae* isolate (MFLUCC 24-0212) clustered to the ex-type of *L. theobromae* with 63% ML and 0.94 BI values (Fig. 12). A pairwise comparison between our strain and the ex-type sequence revealed 1/310 (0.3%) bp difference in *tef1* and 1/370 (0.2%) in *tub2*. This is a new geographical report of *Lasiodiplodia theobromae* associated with *Ficus* sp. in Thailand.

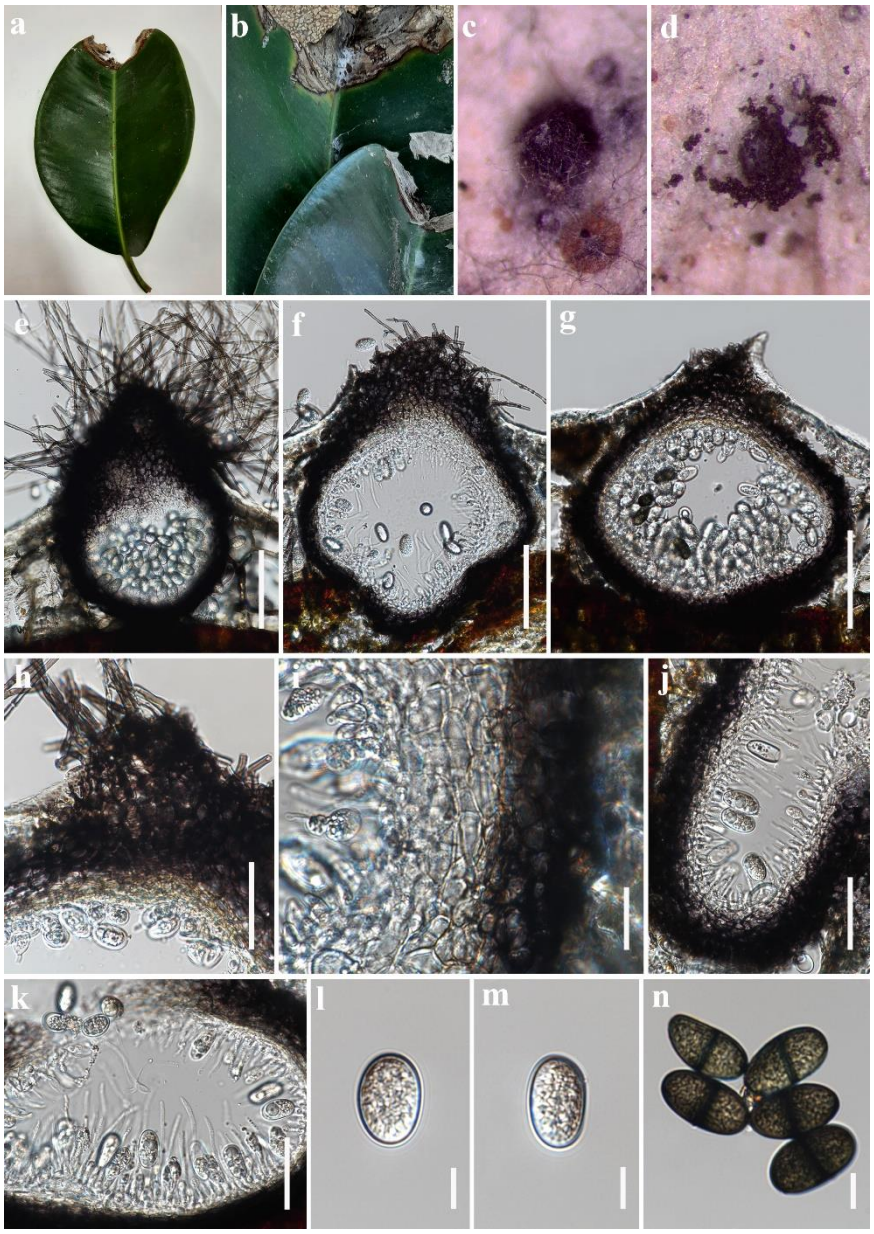

Fig. 11 – *Lasiodiplodia theobromae* (MFLU 24-0218). a, b Symptoms on leaves of *Ficus* sp. c, d Fruiting bodies on the leaf. e–g Cross section of pycnidium. h Ostiole. i Peridium. j, k Conidiogenous cells and conidial arrangement inside the fruiting body. l, m Immature conidia. n Mature conidia. Scale bars: c–d = 200 µm, e–g = 100 µm, h = 50 µm, i = 20 µm, j–k = 50 µm, l–n = 10 µm.

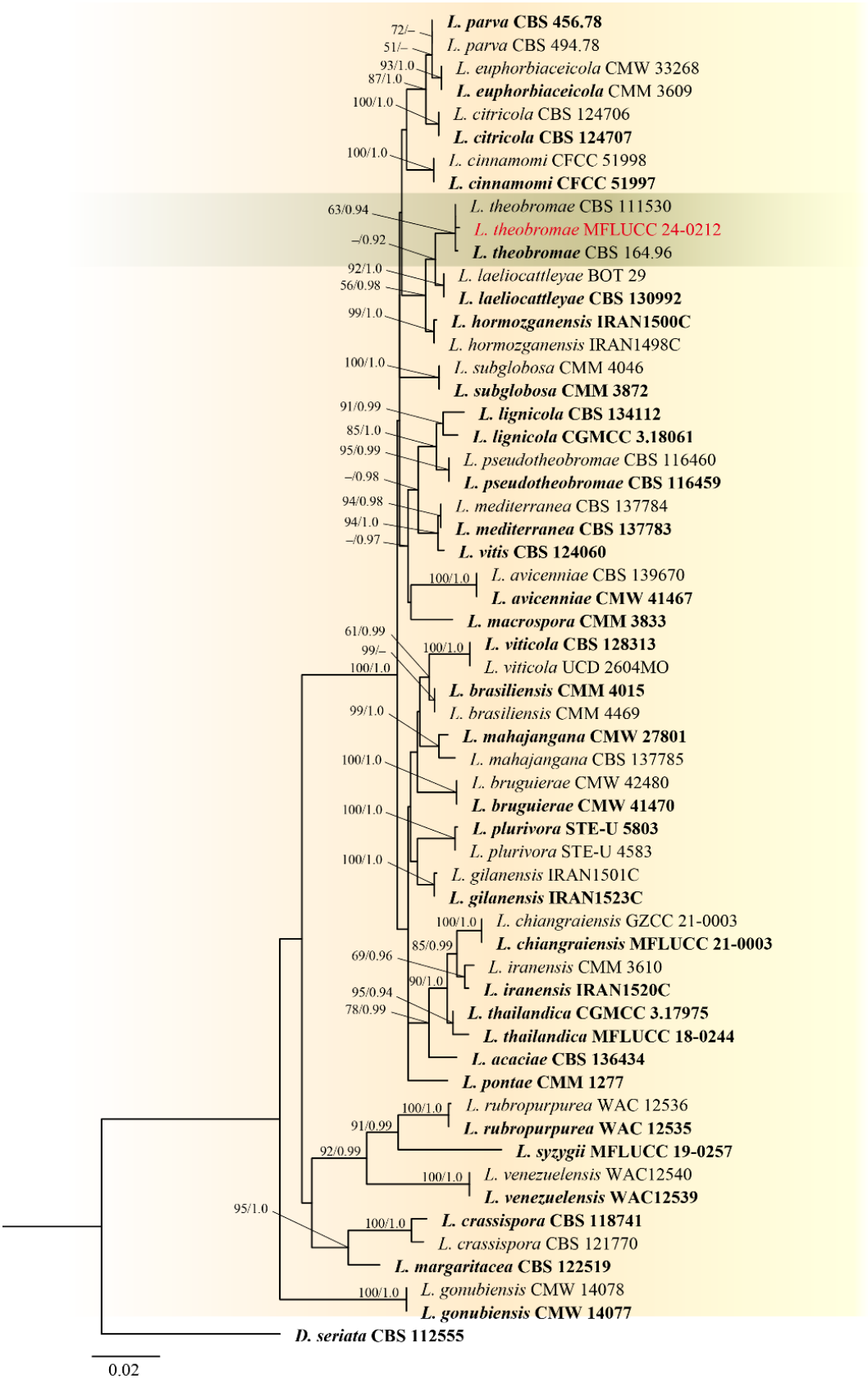

Fig. 12 – Phylogenetic tree created by maximum likelihood analysis of *Lasiodiplodia* based on a concatenated ITS, *tef1*, *tub2*, *rpb2* sequence data. The tree was rooted with *Diplodia seriata* (CBS 112555). Maximum-likelihood bootstrap values above 50% and Bayesian posterior probabilities above 0.90 are shown near the nodes, respectively. Type strains are in bold, and the new isolates from this study are in red.

Neopestalotiopsis iridis A. Armand, Jayawar. & K.D. Hyde, sp. nov. Fig. 13

Index Fungorum Number: IF 902440; Facesoffungi Number: FoF 16400

Etymology – The epithet refers to the plant host genus, *Iris*, from which the fungus was isolated.

Associated with the leaf spots of *Iris pseudacorus*. **Sexual morph:** Not observed. **Asexual morph:** *Conidiomata* acervular, on PDA culture, sporodochial, solitary, scattered, black, exuding black glistening conidial masses. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 5–10 × 2.6–4.5 µm ($\bar{x}$ = 8 × 3.5 µm, n = 30), discrete, cylindrical, ampulliform to flask shape, hyaline, smooth, thin-walled, annelidic, collarette visible. *Conidia* (17–)21–25 × 6–7 µm ($\bar{x}$ = 22 × 6.3 µm, n = 50), fusoid or clavate, straight or slightly curved, four-septate, smooth, septa constricted; basal cell obconic, truncate in the base, thin-walled, hyaline, (3–)4.2–5.1 µm long ($\bar{x}$ = 4 µm, n = 50); three median cells (10.2–)13–14.7(–15.5) µm long ($\bar{x}$ = 13.5 µm, n = 50), smooth-walled, brown, septa darker than the cells; second cell from the base (3.3–)4–4.7(–5.7) µm long, pale brown; third cell brown, (2.8–)3.7–4.5 µm long; fourth cell pale brown, (3.3–)4.2–5.1 µm long; with a septum between the third and fourth cell more darker than the other septa; apical cell 3–4 µm long, conic, subacute in the apex, thin-walled, smooth, hyaline, with 2–4 tubular apical appendages (mostly three), unbranched, centric, and straight or slightly bent, inserted at different loci in the apical crest, (18–)20–22.5(–26.7) × 1–1.5 µm ($\bar{x}$ = 23 × 1.2 µm, n = 50); basal appendage single, filiform, unbranched, centric, (1.5–)3.5–4(–5.3) µm long, and (0.5–)0.7–0.9(–1.1) µm wide (n = 50).

Culture characteristics – Colony on PDA 80–90 mm diam. after seven days at 28 °C, dirty white, with fluffy white aerial mycelia. Reverse pale buff.

Material examined – Mae Fah Luang University campus, Mueang Chiang Rai District, Chiang Rai Province, Thailand, on the leaf of *Iris pseudacorus* (Iridaceae), 21 September 2021, Alireza Armand, C1 (MFLU 24-0219, holotype), ex-type living culture, MFLUCC 24-0199.

Notes – *Neopestalotiopsis coffeae-arabicae* (= *Pestalotiopsis ceffeae-arabicae*) was initially isolated from *Coffea arabica* in China, where it was associated with red-brown leaf spots (Song et al. 2013). *Neopestalotiopsis thailandica* and *N. sonneratae* were first described in association with leaf spots found on mangrove species, *Rhizophora mucronata* (Rhizophoraceae), and *Sonneronata alba* (Lythraceae) from Thailand, respectively (Norphanphoun et al. 2019). In this study, *N. iridis* was isolated from *Iris pseudacorus* (Iridaceae), which is a monocot species. *Neopestalotiopsis iridis* was identified as belonging to a distinct lineage (97% ML, 0.90 PP), positioned basally within a clade comprising three species: *N. sonneratae*, *N. thailandica*, and *N. coffeae-arabicae* (Fig. 14). *Neopestalotiopsis iridis* produced bigger conidiogenous cells and basal cells than those of *N. sonneratae*, *N. thailandica*, and *N. coffeae-arabicae*. Moreover, apical appendages produced by *N. iridis* were longer than those of *N. coffeae-arabicae* and *N. sonneratae*, while it was considerably shorter than those of *N. thailandica*. Base pair differences (Table 2) and morphological comparisons (Table 3) between *N. iridis* and the aforementioned closest species are provided.

Table 3 Base pair differences comparison between three loci of *Neopestalotiopsis iridis* and its closely related species.

Species	Loci		
	ITS	***tef1***	***tub2***
N. coffeae-arabicae (HGUP4019)	0/465 bp	2/205 bp	12/400 bp
N. thailandica (MFLUCC17-1730)	1/465 bp	3/205 bp	9/412 bp
N. sonneratae (MFLUCC17-1745)	0/465 bp	3/205 bp	9/412 bp

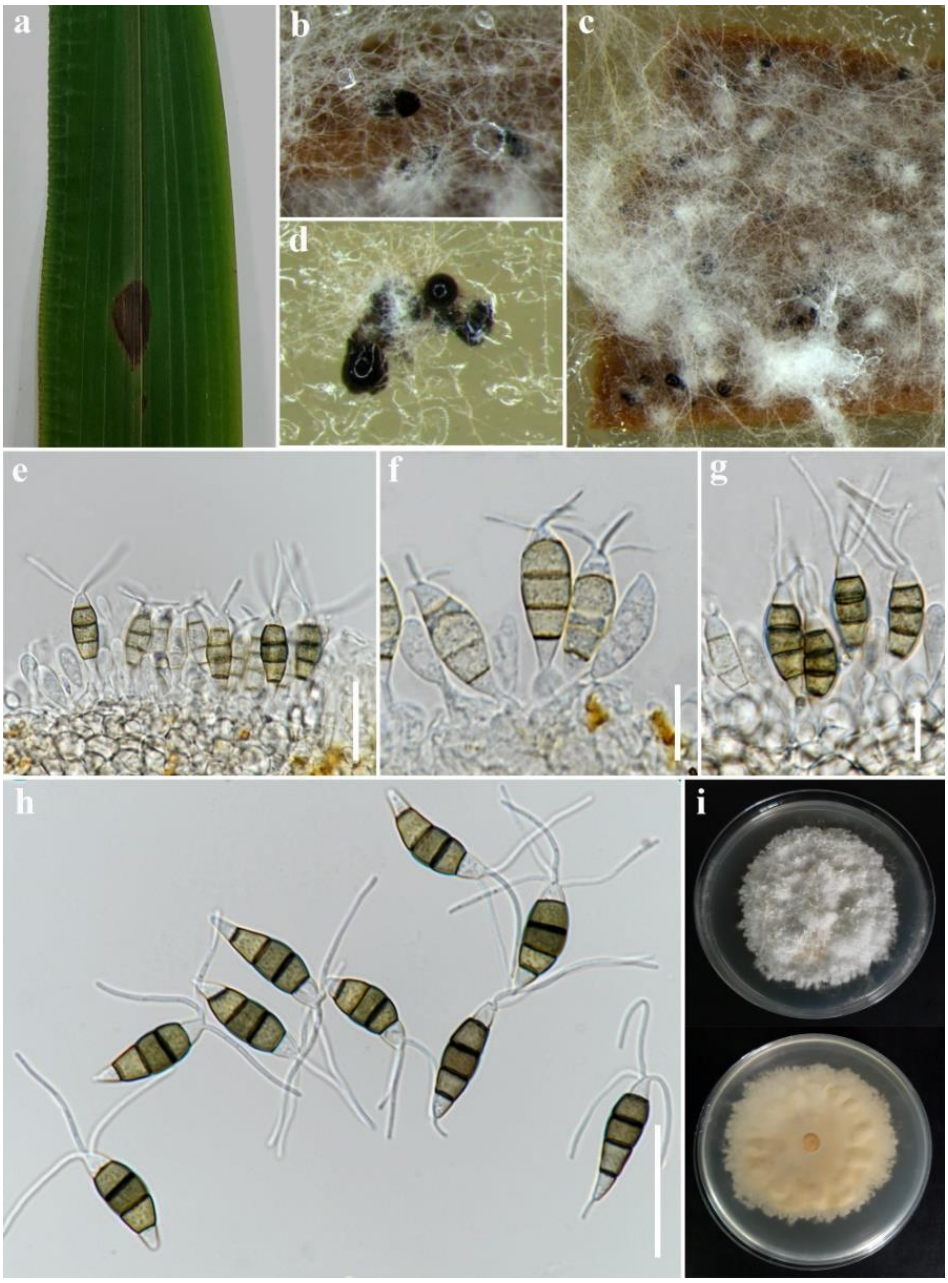

Fig. 13 – *Neopestalotiopsis iridis* (MFLU 24-0219, **holotype**). a Symptoms on the leaf of *Iris pseudacorus*. b–d Fruiting bodies produced on the host leaf scattered on PDA. e–g Conidiogenous cells and conidial attachment. h Conidia. i Upper and reverse view of the colony on the PDA. Scale bars: e = 20 µm, f, g = 10 µm, h = 20 µm.

Discussion

Colletotrichum siamense stands out as a prominent member within the *C. gloeosporioides* species complex, having been consistently identified across a diverse range of plant hosts globally (Talhinhas & Baroncelli 2021). This species has been documented in various orchards and fruits in Thailand, including *Mangifera indica* (Rattanakreetakul et al. 2023), *Persea americana* (Armand & Jayawardena 2024), and *Syzygium samarangense* (Khuna et al. 2023a). Moreover, it has been reported to be associated with both native plants and ornamentals in Thailand (Aliya et al. 2022, Khuna et al. 2023b). In this investigation, nine isolates were unequivocally identified as *Colletotrichum siamense* through meticulous morphological characterization and phylogenetic analyses of multi-gene sequence data sourced from various plant genera (Table 1).

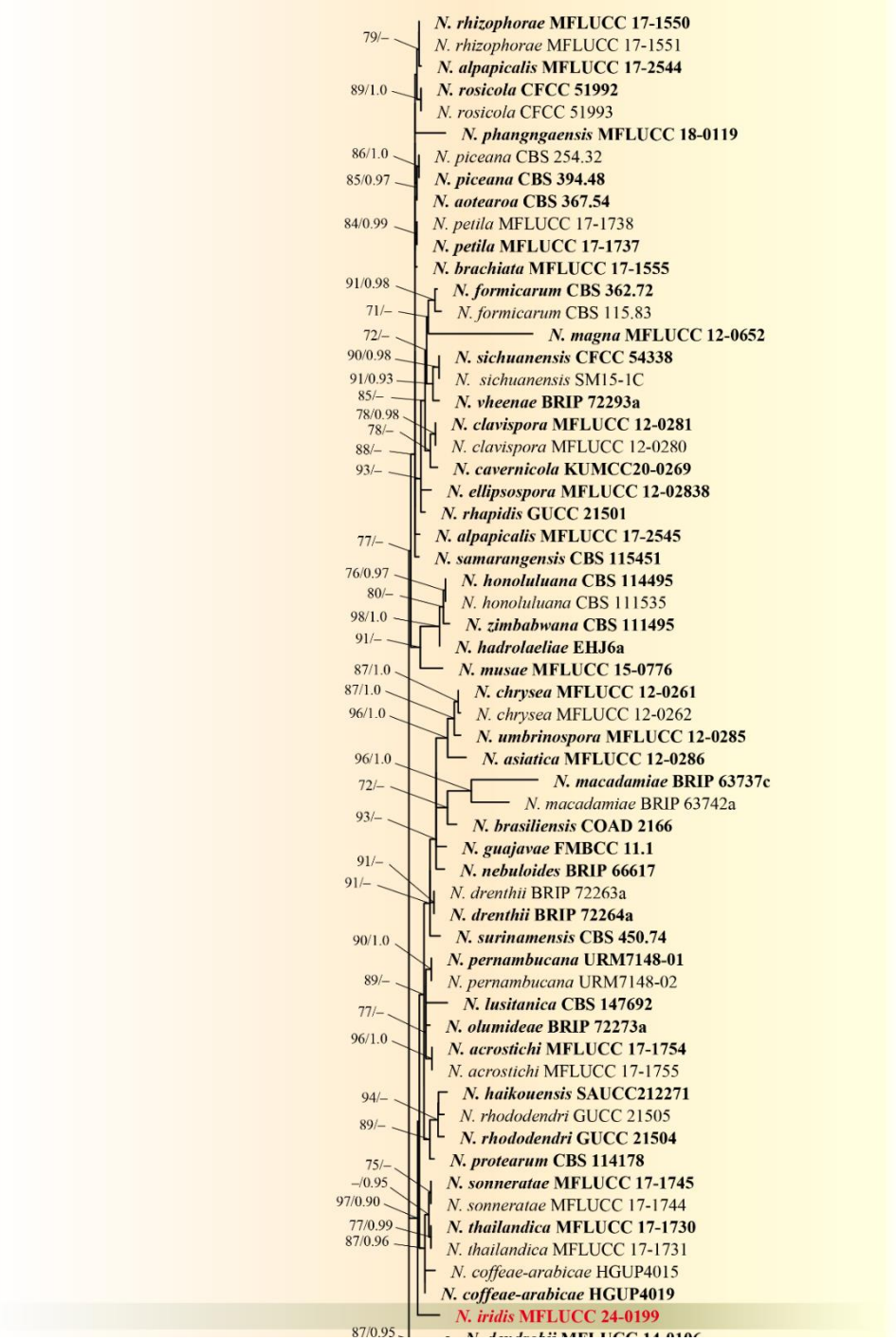

Fig. 14 – A phylogenetic tree was obtained from the maximum likelihood analysis of *Neopestalotiopsis*, based on a combined ITS, *tef1*, and *tub2* sequences. The tree was rooted with *Pestalotiopsis trachicarpicola* (OP068) and *P. diversiseta* (MFLUCC 12-0287). Maximum-likelihood bootstrap values above 50% and Bayesian posterior probabilities above 0.90 are presented, respectively. Type strains are in bold, and the new isolates from this study are in red.

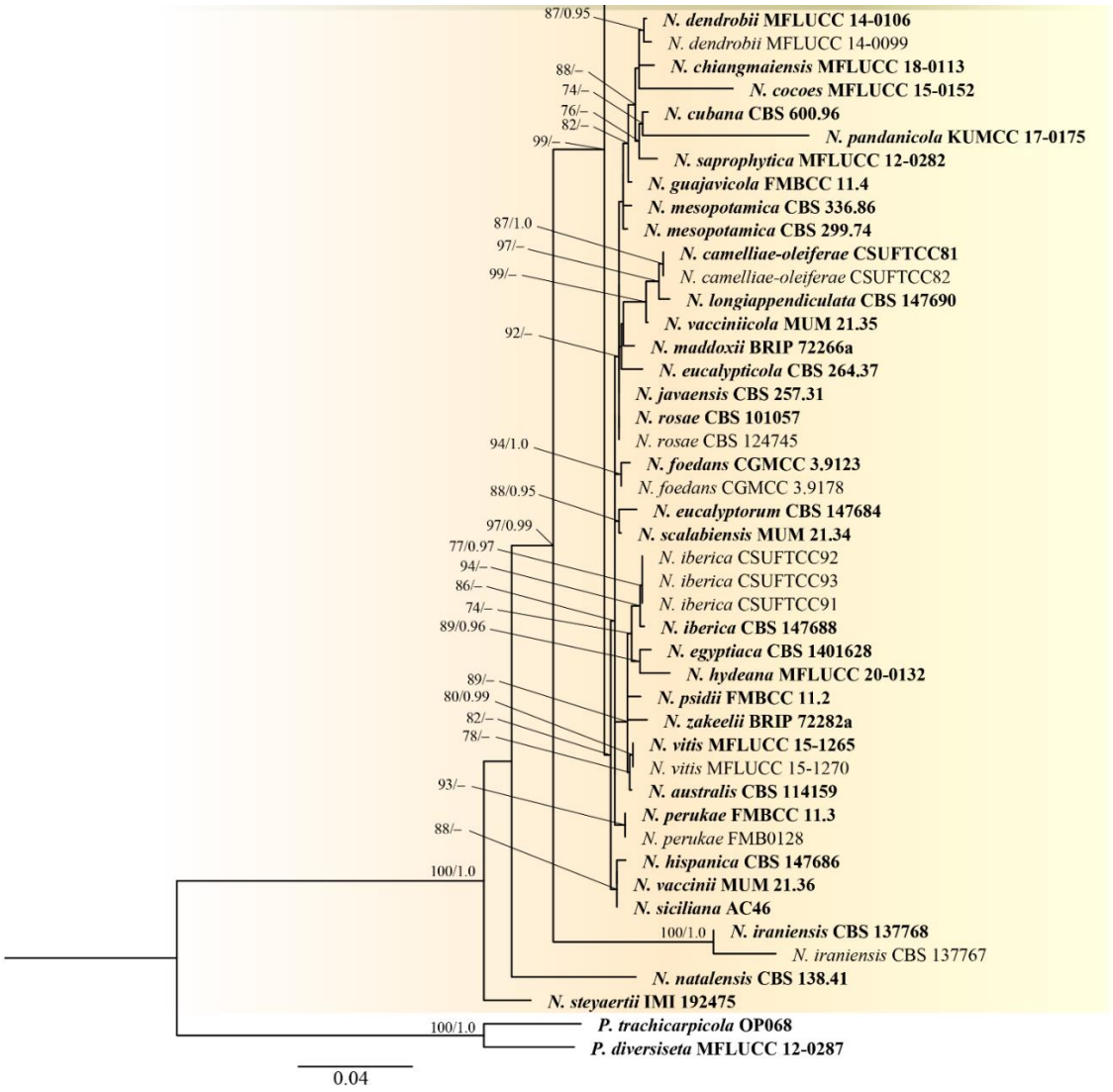

Fig. 14 – Continued.

Based on a comprehensive literature review encompassing notable contributions such as those by Talhinhas & Baroncelli (2023), and augmented by data sourced from the USDA host-fungus database (https://fungi.ars.usda.gov/, accessed on 22 January 2024), it is evident that occurrence of *C. siamense* infection targeting *Anthurium andraeanum* has been restricted to a report from Sri Lanka (Vithanage et al. 2021). Notably, before the current study, there had been a conspicuous absence of documented instances of *C. siamense* infection in *Anthurium andraeanum* within the geographical confines of Thailand. *Colletotrichum artocarpocola* documented by Bhunjun et al. (2019) from Thailand, and *C. siamense* reported in Australia (Shivas et al. 2016) and Brazil (Borges et al. 2023), have been recognized on *Artocarpus heterophyllus* (jackfruit) as saprobe, endophyte, and pathogen, respectively. Consequently, this study represents the inaugural documentation of *C. siamense* associated with leaf spots on jackfruit as a new fungus-host record. *Morinda citrifolia* (morinda), a fruit-bearing tree belonging to the Rubiaceae family and native to Southeast Asia and Australasia (Almeida et al. 2019), has been the focus of limited investigation regarding *Colletotrichum* species. Previous studies have identified *C. gloeosporioides* in India (Kumar et al. 2012) and *C. tropicale* in Mexico from *Morinda citrifolia* (Ayyar-Serna et al. 2018). It is noteworthy, however, that both studies substantiated species identification primarily through morphological characteristics and ITS sequence data, methodologies prone to species misidentification, as highlighted by Hyde et al. (2013), Jeewon & Hyde (2016), and Jayawardena et al. (2021).

Table 2 Morphological comparison of *Neopestalotiopsis iridis* with the closely related species.

Species	Conidia	Basal cell	3 median cells	Apical cell	Basal appendages	Apical appendages	Ref.
Neopestalotiopsis iridis	(17–)21–25 × 6–7 µm, fusoid or clavate, straight or slightly curved, four-septate, smooth, septa constricted	obconic, truncate in the base, thin-walled, hyaline, (3–)4.2–5.1 µm long	(10.2–)13–14.7(–15.5) µm long, smooth-walled, brown, septa darker than the cells; second cell from the base (3.3–)4–4.7(–5.7) µm long, pale brown; third cell brown, (2.8–)3.7–4.5 µm long; fourth cell pale brown, (3.3–)4.2–5.1 µm long; with septum between the third and fourth cell darker than the other septa	3–4 µm long, conic, subacute in the apex, thin-walled, smooth, hyaline, with 2–4 tubular apical appendages	single, filiform, unbranched, centric, (1.5–)3.5–4(–5.3) µm long, and (0.5–)0.7–0.9(–1.1) µm wide	unbranched, centric, and straight or slightly bent, inserted at different loci in the apical crest, (18–)20–22.5(–26.7) × 1–1.5 µm	This study
***N. coffeae-arabicae* (HGUP4019)**	Fusoid to ellipsoid, straight to slightly curved, 4-septate, 16–20 × 5–7 µm	Short, conic to obconic, hyaline, verruculose	11.8–13.5 µm long, dark brown, septa and periclinal walls darker than the rest of the cell, versicolorous; second cell from base pale brown, 3.5–4.5 µm; third cell darker brown, 3.5–5µm; fourth cell darker, 3.7–4.5 µm	2.4–3.1 µm, hyaline, obconic to subcylindrical, with 2–4 appendages (mostly 3)	3–5 µm long, filiform	11–16 µm long, tubular, arising from the apex of the apical cell	Song et al. (2013)
***N. thailandica* (MFLU 19-0783)**	(20–)21–25(–25.5) × 6–7(–7.5) µm, fusiform to clavate, straight to slightly curved, 4(–7)-septate	Obconic with a truncate base, hyaline or sometimes pale brown, (2.5–)3–4(–4.5) µm long	(12–)12.5–15(–16) µm long, brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4–)4.5–5(–5.5) µm long; third cell brown, (3.5–)11–26(–27.5) µm long; fourth cell brown, (4–)5–5.5(–6) µm long	(3.5–)4–5.5(–6) µm long, hyaline, conic to acute, with 1–2 appendages	Single, tubular, unbranched, centric, (3–)6–9(–10) µm long	Inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (30–)32.5–38(–40) µm long,	Norphanphoun et al. (2019)
***N. sonneratae* (MFLU 19-0781)**	(21.5–)24–26(–28) × 7–7.5(–8), fusiform to clavate, straight to slightly curved, 4-septate	Obconic with a truncate base, hyaline or sometimes pale brown, (2–)3–3.5(–4) µm long	(14.5–)15–16.5(–17.5) µm long, brown, septa and periclinal walls darker than rest of the cell, versicolored, wall rugose; second cell from base pale brown, (4.5–)5–6(–7) µm long; third cell brown, (4–)5–5.5(–6) µm long; fourth cell brown, (4–)5–6(–7) µm long	(3.5–)4–4.5(–5) µm long, hyaline, conic to acute, with 1–3 tubular appendages	Single, tubular, unbranched, centric, (2.5–)3–4(–5) µm long	Inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (5.5–)7–8(–14) µm long	Norphanphoun et al. (2019)

In contrast, the current study introduces a groundbreaking revelation, identifying two strains of *C. siamense* (MFLUCC 24-0204, MFLUCC 24-0205) for the first time on a global scale, associated with anthracnose on *Morinda citrifolia*. This discovery is based on a robust analytical framework involving a five-loci phylogeny coupled with a morphological approach, thereby enhancing the accuracy and reliability of species identification in the *Colletotrichum* genus. Additionally, this study marks the first geographical record of *C. siamense* on *Bauhinia* sp. and *Ficus* sp. from Thailand. Furthermore, *C. siamense* was reported on *Asplenium nidus* (bird's-nest fern), *Dracaena fragrans*, and *Dendrobium* sp. (Orchidaceae) for the first time worldwide (Table 1).

Colletotrichum fructicola is a pivotal pathogenic species predominantly distributed in tropical regions, as highlighted by Talhinhas & Baroncelli (2021). Its presence in Thailand has been documented across diverse plant species, including reports by Prihastuti et al. (2009), Phoulivong et al. (2010), and the recent contribution by Armand et al. (2023). *Colletotrichum siamense* from Argentina and India (Larran et al. 2015, Sharma et al. 2015), along with *C. endophytica* and *C. gloeosporioides* from China (Li et al. 2016, Liang et al. 2023), have been isolated from *Bauhinia* spp., a genus known for its substantial biochemical properties and biotechnological applications (Cagliari et al. 2018). Intriguingly, no documented instances of *C. fructicola* have been reported on *Bauhinia* spp., neither in Thailand nor elsewhere, until the present study. Therefore, *C. fructicola* (MFLUCC 24-0214) and *C. siamense* (MFLUCC 24-0208) are illustrated as a new host and geographical records from Thailand, respectively. Furthermore, *C. fructicola* (MFLUCC 24-0210) is reported as a new fungus-host record in the present study.

Colletotrichum truncatum has been documented on various plant species in Thailand, including *Capsicum* sp., *Gossypium* sp., *Manihot esculenta, Solanum melongena* (Hyde et al. 2018), and *Durio zibethinus* (Armand et al. 2023). Notably, its isolation from *Ficus religiosa* had not been reported globally until this investigation. Therefore, this study presents the inaugural documentation of the association of *C. truncatum* (MFLUCC 24-0211) with anthracnose in *Ficus religiosa*. This finding adds a novel dimension to the host range of *C. truncatum* and contributes to a broader understanding of its ecological interactions and potential impact on diverse plant species.

Colletotrichum musicola, initially described by Damm et al. (2019), isolated from a restricted range of plant species found in Brazil and Mexico (Cavalcante et al. 2019, Vásquez-López et al. 2019, Boufleur et al. 2020). However, the present investigation has expanded its known host spectrum, revealing *C. musicola* association with *Lagerstroemia* sp. (Lythraceae) leaf spots, reported for the first time worldwide. Furthermore, the occurrence of *Lasiodiplodia theobromae* on *Ficus* spp. had not been documented in Thailand until the current inquiry. Herein, *L. theobromae* (MFLUCC 24-0212) was identified as a fungal pathogen associated with leaf dieback through an analysis encompassing both morphological and molecular methodologies. *Colletotrichum tropicicola* and *C. musae* were isolated from *Citrus maxima* and *Musa* sp., respectively. While *C. musae* has been extensively studied as the causal agent of banana anthracnose, this marks the second documented instance of *C. tropicicola* associated with anthracnose of *Citrus maxima* in Thailand. In the current study, *Colletotrichum* species were isolated from fruit and leaf anthracnose, leaf spots, and leaf dieback across various plant families, including Moraceae, Musaceae, Lythraceae, Rubiaceae, and Rutaceae.

To justify the introduction of our strain as a new species, we conducted comprehensive morphological and phylogenetic analyses using two strains for each of the three species: *Neopestalotiopsis coffeae-arabicae*, *N. thailandica*, and *N. sonneratae*, ensuring accurate phylogenetic positioning. The study demonstrated that our species formed a distinct lineage separate from the type and additional strains of the closely related species (Fig. 14). While the ITS region, which is more conserved in the *Neopestalotiopsis* genus, showed minimal variation, significant nucleotide differences were observed in the protein-coding regions *tef1-alpha* (tef) and *beta-tubulin* (tub2) between our species and closely related species (Table 3). Based on the result of both morphological and phylogenetic analysis (Table 2, 3), the isolate was introduced as a new taxon. To date, no studies have addressed fungal species diversity associated with *Iris* spp. in Thailand. Remarkably, before this investigation, the presence of *Neopestalotiopsis* species on *Iris* spp. had not

been recorded (https://fungi.ars.usda.gov/; accessed on 23 March 2024). This study isolated and characterized *Neopestalotiopsis iridis* as a novel species from leaf spots of *Iris pseudacorus* (Iridaceae).

In summary, the present study has unveiled one new species, documented eight previously unreported host records, including *C. siamense* from *Asplenium nidus*, *Dendrobium* sp., *Dracaena fragrans*, and *Morinda citrifolia*; *C. fructicola* from *Bauhinia* sp. and *Ficus religiosa*; *C. truncatum* from *Ficus religiosa*; *C. musicola* from *Lagerstroemia* sp., and identified five new geographical occurrences of plant pathogenic species in Thailand, including *C. siamense* from *Anthurium andraeanum*, *Artocarpus heterophyllus*, *Bauhinia* sp., and *Ficus* sp.; *Lasiodiplodia theobromae* from *Ficus* sp.. These observations underscore the novel contribution of the present study, shedding light on a hitherto unreported host and ecological niches for fungal species, especially *C. siamense*, thus enriching our understanding of its distribution and pathogenic potential. As such, these findings contribute to the specific understanding of pathogenic fungi and provide a broader framework for comprehending the ecological dynamics of fungal species, paving the way for further investigations.

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Data availability

The datasets used for the phylogenetic analyses in the present study are available from the corresponding author by request and as supplementary files.

References

Aliya SS, Nusaibah SA, Mahyudin MM, Yun WM et al. 2022 – *Colletotrichum siamense* and *Pestalotiopsis jesteri* as potential pathogens of new rubber leaf spot disease via detached leaf assay. Journal of Rubber Research 25(3), 195–212. Doi 10.1007/s42464-022-00157-4

Almeida ÉS, de Oliveira D, Hotza D. 2019 – Properties and applications of *Morinda citrifolia* (noni): A review. Comprehensive Reviews in Food Science and Food Safety 18(4), 883–909. Doi 10.1111/1541-4337.12456

Armand A, Jayawardena RS. 2024 – Morphomolecular identification and pathogenicity of *Colletotrichum* species associated with avocado anthracnose in northern Thailand. Plant Pathology 73(1), 186–197. Doi 10.1111/ppa.13792

Armand A, Hyde KD, Jayawardena RS. 2023 – First report of *Colletotrichum fructicola* causing fruit rot and leaf-tip dieback on pineapple in northern Thailand. Plants 12(4), 971. Doi 10.3390/plants12040971

Ayvar-Serna S, Díaz-Nájera JF, Vargas-Hernández M, Plancarte-Galán PJ et al. 2018 – *Colletotrichum tropicale* causal agent of anthracnose on noni plants (*Morinda citrifolia*) in Guerrero, Mexico. Plant Pathology & Quarantine 8(2), 165–169. Doi 10.5943/ppq/8/2/8

Bhunjun CS, Jayawardena RS, Wei D-P, Huanraluek N et al. 2019 – Multigene phylogenetic characterisation of *Colletotrichum artocarpicola* sp. nov. from *Artocarpus heterophyllus* in northern Thailand. Phytotaxa 418, 273–286. Doi 10.11646/phytotaxa.418.3.3

Borges RCF, Rossato M, Santos MDM, Vieira WAS et al. 2023 – Characterization of *Colletotrichum* isolates causing anthracnose on *Artocarpus heterophyllus* in Brazil. Journal of Plant Pathology 105, 299–305. Doi 10.1007/s42161-022-01252-6

Boufleur TR, Castro RR, Rogério F, Ciampi-Guillardi M et al. 2020 – First report of *Colletotrichum musicola* causing soybean anthracnose in Brazil. Plant Disease 104(6), 1858. Doi 10.1094/PDIS-12-19-2627-PDN

Burdon JJ, Silk J. 1997 – Sources and patterns of diversity in plant-pathogenic fungi. Phytopathology 87(7), 664–669. Doi 10.1094/PHYTO.1997.87.7.664

Cagliari R, Kremer FS, da Silva Pinto L. 2018 – *Bauhinia lectins*: Biochemical properties and biotechnological applications. International Journal of Bbiological Macromolecules 119, 811–820. Doi 10.1016/j.ijbiomac.2018.07.156

Capella-Gutiérrez S, Silla-Martinez JM, Gabaldón T. 2009 – TrimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25, 1972–1973. Doi 10.1093/bioinformatics/btp348G

Cavalcante GR, Barguil BM, Vieira WA, Lima WG et al. 2019 – Diversity, prevalence, and virulence of *Colletotrichum* species associated with lima bean in Brazil. Plant disease 103(8), 1961–1966. Doi 10.1094/PDIS-11-18-2002-RE

Cheek M, Lughadha EN, Kirk P, Lindon H et al. 2020 – New scientific discoveries: plants and fungi. Plants People Planet 2(5), 371–388. Doi 10.1002/ppp3.10148

Chethana KT, Manawasinghe IS, Hurdeal VG, Bhunjun CS et al. 2021 – What are fungal species and how to delineate them?. Fungal Diversity 109(1), 1–25.

Damm U, Sato T, Alizadeh A, Groenewald JZ, Crous PW. 2019 – The *Colletotrichum dracaenophilum*, *C. magnum* and *C. orchidearum* species complexes. Studies in Mycology 92(1), 1–46. Doi 10.1016/j.simyco.2018.04.001

Damm U, Woudenberg JH, Cannon PF, Crous PW. 2009 – *Colletotrichum* species with curved conidia from herbaceous hosts. Fungal Diversity 39, 45.

Darriba D, Taboada GL, Doallo R, Posada D. 2012 – JModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9(8), 772. Doi 10.1038/nmeth.2109

Dayarathne MC, Thambugala KM, Daranagama DA, Mridha AU et al. 2023 – Global challenges in identifying plant pathogenic fungi: An overview with suggestions for the way forward. Chiang Mai Journal of Science 50(4), 1–17. Doi 10.12982/cmjs.2023.039

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.

Hariharan G, Prasannath K. 2021 – Recent advances in molecular diagnostics of fungal plant pathogens: A mini review. Frontiers in Cellular and Infection Microbiology 10, 600234. Doi 10.3389/fcimb.2020.600234

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. Doi 10.1007/s13225-018-0415-7

Hyde KD, Udayanga D, Manamgoda DS, Tedersoo L et al. 2013 – Incorporating molecular data in fungal systematics: a guide for aspiring researchers. Current Research in Environmental and Applied Mycology. Doi 10.48550/arXiv.1302.3244

Jayawardena RS, Hyde KD, de Farias AR, Bhunjun CS et al. 2021 – What is a species in fungal plant pathogens?. Fungal Diversity 109(1), 239–266. Doi 10.1007/s13225-021-00484-8

Jeewon R, Hyde KD. 2016 – Establishing species boundaries and new taxa among fungi: recommendations to resolve taxonomic ambiguities. Mycosphere 7(11), 1669–1677. Doi 10.5943/mycosphere/7/11/4

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017 – ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14, 587–589. Doi 10.1038/nmeth.4285

Katoh K, Rozewicki J, Yamada KD. 2019 – MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4), 1160–1166. Doi 10.1093/bib/bbx108

Ko TW, McKenzie EH, Bahkali AH, To-Anun C et al. 2011 – The need for re-inventory of Thai phytopathogens. Chiang Mai Journal of Science 38(4), 625–637.

Kumar K, Singh DR, Amaresan N, Madhuri K. 2012 – Isolation and pathogenicity of *Colletotrichum* spp. causing anthracnose of Indian mulberry (*Morinda citrifolia*) in tropical islands of Andaman and Nicobar, India. Phytoparasitica 40(5), 485–491. Doi 10.1007/s12600-012-0249-x

Khuna S, Kumla J, Thitla T, Lumyong S, Suwannarach N. 2023a – First report of rose apple leaf spot caused by *Colletotrichum siamense* in Thailand. Plant Disease 107(5), 1625. Doi 10.1094/PDIS-08-22-1869-PDN

Khuna S, Kumla J, Thitla T, Suwannarach N. 2023b – First report of anthracnose on giant philodendron caused by *Colletotrichum siamense* in Thailand. Plant Disease (10), 3301. Doi 10.1094/PDIS-05-23-0878-PDN

Larran S, Bahima JV, Dal Bello G, Franco E, Balatti P. 2015 – *Colletotrichum siamense* causing anthracnose in *Bauhinia forficata* subsp. *pruinosa* in Argentina. Australasian Plant Disease Notes 10, 7. Doi 10.1007/s13314-015-0160-7

Li PL, Liu D, Zheng XJ, Chen HB, Gong GS et al. 2016 – First report of *Colletotrichum gloeosporioides* causing anthracnose on *Bauhinia blakeana*. Plant Disease 100(10), 2165. Doi 10.1094/PDIS-03-16-0367-PDN

Liang L, Li S, Han S, Qiao T et al. 2023 – First report of *Colletotrichum endophyticum*, a causal agent of leaf spot of *Bauhinia blakeana*, in Southwest China. Plant Disease (3), 956. Doi 10.1094/PDIS-05-22-1230-PDN

Minh BQ, Schmidt HA, Chernomor O, Schrempf D et al. 2020 – IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution 37(5), 1530-1534. Doi 10.1093/molbev/msaa015

Norphanphoun C, Jayawardena RS, Chen Y, Wen TC et al. 2019 – Morphological and phylogenetic characterization of novel pestalotioid species associated with mangroves in Thailand. Mycosphere 10, 531–578. Doi 10.5943/mycosphere/10/1/9

Phillips AJ, Alves A, Abdollahzadeh J, Slippers B et al. 2013 – The Botryosphaeriaceae: genera and species known from culture. Studies in Mycology 76(1), 51–167. Doi 10.3114/sim0021

Phoulivong S, Cai L, Chen H, McKenzie EH et al. 2010 – *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. Fungal Diversity 44, 33–43. Doi 10.1007/s13225-010-0046-0

Prihastuti H, Cai L, Chen H, McKenzie EH, Hyde KD. 2009 – Characterization of *Colletotrichum* species associated with coffee berries in northern Thailand. Fungal Diversity 39(1), 89–109.

Rattanakreetakul C, Keawmanee P, Bincader S, Mongkolporn O et al. 2023 – Two newly identified *Colletotrichum* species associated with mango anthracnose in central Thailand. Plants 12(5), 1130. Doi 10.3390/plants12051130

Senanayake IC, Rathnayaka AR, Marasinghe DS, Calabon MS et al. 2020 – Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. Mycosphere 11(1), 2678–754. Doi 10.5943/mycosphere/11/1/20

Song YU, Geng KU, Hyde KD, Zhao WS et al. 2013 – Two new species of *Pestalotiopsis* from Southern China. Phytotaxa 126(1), 22–32. Doi 10.11646/PHYTOTAXA.126.1.2

576 Su YY, Noireung P, Liu F, Hyde KD et al. 2011 – Epitypification of *Colletotrichum musae*, the
577 causative agent of banana anthracnose. Mycoscience 52(6), 376–382. Doi 10.1007/S10267-
578 011-0120-9
579 Swofford DL. 2002 – PAUP*. Phylogenetic analysis using parsimony (* and other methods). Ver.
580 4.0b10. Sunderland, MA: Sinauer Associates.
581 Sharma G, Pinnaka AK, Shenoy BD. 2015 – Resolving the *Colletotrichum siamense* species complex
582 using ApMat marker. Fungal Diversity 71, 247. Doi 10.1007/s13225-014-0312-7
583 Shivas RG, Tan YP, Edwards J, Dinh Q et al. 2016 – *Colletotrichum* species in Australia.
584 Australasian Plant Pathology 45, 447–464. Doi 10.1007/s13313-016-0443-2
585 Talhinhas P, Baroncelli R. 2023 – Hosts of *Colletotrichum*. Mycosphere 14(2), 158–261. Doi
586 10.5943/mycosphere/14/si2/4
587 Talhinhas P, Baroncelli R. 2021 – *Colletotrichum* species and complexes: geographic distribution,
588 host range and conservation status. Fungal Diversity 110(1), 109–198. Doi 10.1007/s13225-
589 021-00491-9
590 Vásquez-López A, Palacios-Torres RE, Camacho-Tapia M, Granados-Echegoyen C et al. 2019 –
591 *Colletotrichum brevisporum* and *C. musicola* causing leaf anthracnose of taro (*Colocasia*
592 *esculenta*) in Mexico. Plant Disease 103(11), 2963. Doi 10.1094/PDIS-05-19-0967-PDN
593 Vithanage IK, Yakandawala DM, Maharachchikumbura SS, Jayasinghe L, Adikaram NK. 2021 –
594 *Colletotrichum* species causing anthracnose disease in *A. andraeanum*, manifested as spathe rot
595 also in addition to spadix rot and leaf spot. Running title: *Colletotrichum* spp. causing
596 *anthurium* anthracnose. European Journal of Plant Pathology 161, 837–846. Doi
597 10.1007/s10658-021-02366-w
598 Withee P, Haituk S, Senwanna C, Karunarathna et al. 2022 – Identification and pathogenicity of
599 *Paramyrothecium* species associated with leaf spot disease in northern Thailand. Plants 11(11),
600 1445. Doi 10.3390/plants11111445