Dissertation Title	Taxonomy and Phylogeny of Fungi Associated with Banana (*Musa* spp.)
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ABSTRACT

Bananas and plantains predominantly belong to *Musa* (*Musaceae, Zingiberales*). These fruit crops are cultivated and consumed in around 135 countries and are among the most traded fruits in the world. *Musa* harbors different lifestyles of fungi, including endophytes, pathogens, and saprobes. Due to the monetary value of the fruit, in-depth systematic studies have been conducted worldwide on fungal pathogens associated with *Musa*. However, only a few mycologists worldwide have studied *Musa's* saprobic and endophytic fungal niches. Several previously identified fungal taxa lack morphological illustrations, descriptions, and DNA sequence data in public databases. Hence, the taxonomy and phylogeny of these latter extant taxa are under-examined.

Wild and cultivated varieties of *Musa* plants, as well as commercial banana fruits available in local markets, were selected for the study. Samples were mainly collected throughout the year from Thailand and China. Fungi were primarily isolated from leaves, leaf sheaths, pseudo stems, and fruits. Endophytic fungi were recovered from fresh, asymptomatic leaf parts, while saprobic fungi were isolated from dead or decaying tissues. Symptomatic leaves were selected to isolate the fungal taxa associated with necrotic spots. Post-harvest pathogens were isolated from banana fruits available at commercial stalls.

Species identification was mainly conducted based on morphology and multi-gene phylogeny. The pathogenicity test was performed for pathogenic fungi in addition to morphological and phylogenetic analysis. Healthy banana fruits were artificially inoculated to evaluate the pathogenicity of fungi isolated from post-harvest fruit rots. Herbarium specimens of the fungi described in the thesis were deposited in the Mae Fah Luang University (MFLU) Fungarium, Chiang Rai, Thailand. Living cultures were deposited in the Culture Collection of Mae Fah Luang University (MFLUCC). The obtained DNA sequences were verified, submitted to GenBank, and made available for public reference.

A total of 368 fungal cultures representing endophytes, saprobes, pathogens, and fungi associated with leaf spots were isolated during the study, and 206 species were morphologically recorded. Among them, 150 fungal cultures were selected to generate molecular data and evaluate phylogenetic relationships. Based on morpho-molecular data, the present study identified 67 *ascomycetous* fungal species in 51 different genera, with a taxonomic hierarchy of 34 families and 13 orders in *Dothideomycetes*, *Eurotiomycetes*, and *Sordariomycetes*. Among these, 44 species were illustrated, taxonomic descriptions were provided, and their phylogenetic relationships were investigated. Additionally, 20 fungal taxa were identified to the generic level with the aid of DNA sequence data and morphology. Among the 44 species illustrated in the thesis, seven are new species, 26 are new hosts and geographical records, and six are new collections.

The newly described species are *Dematipyriforma teristiris* (*Pleurotheciaceae*), *Dictyoarthrinium musae* (*Didymosphaeriaceae*), *Neohelicosporium terrestris* (*Tubeufiaceae*), *Pyrenochaetopsis musae* (*Pyrenochaetopsidaceae*), *Smaragdiniseta musae* (*Stachybotryaceae*), *Spegazzinia musae* (*Didymosphaeriaceae*) and *Stachybotrys musae* (*Stachybotryaceae*).

In addition, 17 endophytic fungal genera, *viz. Albifimbria, Alternaria, Colletotrichum, Corynespora, Curvularia, Daldinia, Diaporthe, Epicoccum, Fusarium, Lasiodiplodia, Musicillium, Neopestalotiopsis, Pestalotiopsis, Phyllosticta, Pseudophaeocytostroma*, *Pseudopithomyces*, and *Spegazzinia*, were isolated from the fresh leaves of *Musa* and identified based on morphology and DNA. Among them, eight taxa, *viz. Albifimbria verrucaria* (*Stachybotryaceae*), *Corynespora torulosa* (*Corynesporascaceae*), *Daldinia eschscholtzii* (*Hypoxylaceae*), *Lasiodiplodia theobromae* (*Botryosphaeriaceae*), *Musicillium tropicale* (*Plectosphaerellaceae*), *Pseudophaeocytostroma sacchari* (*Diaporthaceae*), *Pseudopithomyces chartarum* (*Didymosphaeriaceae*), and *Setoarthopyrenia chromolaenae* (*Roussoellaceae*) are illustrated and described with confirmed morphology and molecular phylogeny.

The morpho-molecular data revealed the occurrence of 48 genera of saprobic fungi *viz. Acrostalagmus*, *Albifimbria*, *Alternaria*, *Apiospora*, *Bahusandhika*, *Cladosporium*, *Clonostachys*, *Colletotrichum*, *Corynespora*, *Curvularia*, *Dematipyriforma*, *Dendryphiella*, *Diaporthe*, *Dictyoarthrinium*, *Durispora*, *Epicoccum*, *Fusarium*, *Gibellulopsis*, *Gliomastix*, *Hermatomyces*, *Kalmusia*, *Koorchaloma*, *Lasiodiplodia*, *Lasionectria*, *Lophiotrema*, *Memnoniella*, *Musicillium*, *Nectria*, *Neocordana*, *Neohelicosporium*, *Neopestalotiopsis*, *Nigrospora*, *Ophioceras*, *Periconia*, *Pestalotiopsis*, *Phaeosphaeria*, *Phyllosticta*, *Plectosphaerella*, *Pseudocercospora*, *Pseudophaeocytostroma*, *Pseudopithomyces*, *Pyrenochaetopsis*, *Setophoma*, *Smaragdiniseta*, *Spegazzinia*, *Stachybotrys* and *Torula* on *Musa* substrates. Among them, 33 species *viz. Albifimbria verrucaria* (*Stachybotryaceae*), *Apiospora locuta-pollinis* (*Apiosporaceae*), *Bahusandhika indica* (*Lentimurisporaceae*), *Colletotrichum musae* (*Glomerellaceae*), *Corynespora torulosa* (*Corynesporascaceae*), *Dematipyriforma teristiris* (*Pleurotheciaceae*), *Dendryphiella vinosa* (*Dictyosporiaceae*), *Dictyoarthrinium musae*, *D. thailandica*, *D. sacchari* (*Didymosphaeriaceae*), *Gibellulopsis serrae* (*Plectosphaerellaceae*), *Lasiodiplodia theobromae* (*Botryosphaeriaceae*), *Lophiotrema hydei*

(*Lophiotremataceae*), *Memnoniella levispora* (*Stachybotryaceae*), *Musicillium elettariae* (*Plectosphaerellaceae*), *Neocordana musae* (*Pyriculariaceae*), *Neohelicosporium terrestris* (*Tubeufiaceae*), *Nigrospora sphaerica* (*Apiosporaceae*), *Periconia cortaderiae, P. delonicis, P. echinochloae* (*Periconiaceae*), *Pseudopithomyces chartarum* (*Didymosphaeriaceae*), *Smaragdiniseta musae* (*Stachybotryaceae*), *Spegazzinia deightonii, S. musae, S. radermacherae* (*Didymosphaeriaceae*), *Stachybotrys microsporus, S. musae, S. subcylindrosporus* (*Stachybotryaceae*), *Torula chromolaenae, T. fici, T. mackenziei,* and *T. masonii* (*Torulaceae*) are included with full descriptions, illustrations and molecular phylogeny.

Eight fungal taxa, *viz. Colletotrichum siamense* (*Glomerellaceae*), *Curvularia umbiliciformis* (*Pleosporaceae*), *Metulocladosporiella musae* (*Herpotrichiellaceae*), *Neocordana musae* (*Pyriculariaceae*), *Neopestalotiopsis clavispora*, *N. musae* (*Sporocadaceae*), *Phaeosphaeria oryzae* (*Phaeosphaeriaceae*) and *Pyrenochaetopsis musae* (*Pyrenochaetopsidaceae*), associated with *Musa* leaf spots, are reported. Three fungal taxa, *viz. Albifimbria verrucaria* (*Stachybotryaceae*), *Lasiodiplodia theobromae* (*Botryosphaeriaceae*) and *Pseudopithomyces chartarum* (*Didymosphaeriaceae*), are identified as having both endophytic and saprobic lifestyles on the leaves of Nam Wa banana (*Musa acuminata* × *M. balbisiana*; ABB genomic group; Pisang Awak). Interestingly, the endophytic, pathogenic, and saprobic lifestyles of *Colletotrichum musae* (*Glomerellaceae*) and *Corynespora torulosa* (*Corynesporascaceae*) were recorded in Nam Wa banana leaves and fruits. These results were confirmed with pathogenicity tests combined with morpho-molecular evidence. These findings further support the hypotheses of lifestyle transitions of fungi on the same host.

Two banana fruit diseases, anthracnose and speckle, were carefully investigated during the study. Both symptomatic banana fruits were collected from the local markets in Northern Thailand's Chiang Mai, Chiang Rai, and Nan Provinces. To isolate fungi, surface-sterilized segments of infected banana peels were placed on potato dextrose agar (PDA). *Colletotrichum musae* and *C. siamense* were identified from the post-harvest anthracnose lesions in Namwa (*Musa acuminata* × *M. balbisiana*; ABB genomic group; Pisang Awak) and Khai bananas (*M. acuminata*; AA genomic group; Sucrier). In addition, *Corynespora torulosa* was found in the speckles of Nam Wa (*Musa acuminata* × *M. balbisiana*; ABB genomic group; Pisang Awak) fruits. To establish Koch's postulates, fresh and unripe banana fruits were artificially inoculated with the identified strains, which confirmed their pathogenicity. This is the first confirmed occurrence of *Cory. torulosa* causing banana fruit speckles in Thailand. In addition, the presence of *C. siamense* causing post-harvest anthracnose in Khai bananas (*M. acuminata*; AA genomic group; Sucrier) was reported. With the updated molecular phylogeny for the *Pyriculariaceae*, a thorough review of the plant pathogenic genus *Neocordana* that causes leaf spots on *Musa* was also conducted. The findings of this study contribute to a better understanding of post-harvest disease control in the commercial banana industry in Thailand.

Furthermore, this study documents the fungi associated with *Musa* hosts globally, and the findings are presented here as a checklist. Seven hundred sixty (760) fungal species associated with *Musa* are listed, along with their respective geographical localities, updated classifications, and literature. These taxa belong to four major phyla: *Ascomycota* is the most species-rich (93%), followed by *Basidiomycota* (6%) and *Mucoromycota* (1%). This compilation serves as a valuable resource for further research in fungi associated with *Musa*.

The overall study provides some insights into the systematics of microfungi associated with *Musa* in the tropics. Molecular data were generated for several extant species, and the taxonomic placements were resolved. Saprobic lifestyles of previously reported endophytes and pathogens were discovered, which further justified lifestyle changes in fungi. DNA sequence data were made available in public databases for correct identification and future studies. Some selected fungal strains

that are known to produce bioactive compounds were reported, and those strains were made available for chemical analysis and screening in future works. The documentation of novel taxa contributes to the correct estimation of fungal diversity and the number of fungi in Asia and the world. The results of this study will provide improved awareness of pre- and post-harvest fungal disease management in Thailand for commercial banana producers, traders, and stakeholders in the banana post-harvest handling chain.

Keywords: *Ascomycota*, Coelomycetes, *Dothideomycetes*, Endophytes, Hyphomycetes, Pathogens, Post-harvest diseases, Saprobes, *Sordariomycetes*.