Dissertation Title	Taxonomy and Phylogeny of Soil Ascomycetes in Thailand
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ABSTRACT

Soil covers a large area of the earth's crust and supports a high biodiversity. Fungi are a highly abundant group of organisms found in soils worldwide. Soil fungi use extensive hyphal networks to break down complex organic materials, transport nutrients, and improve the soil structure. In soil ecosystems, fungi maintain interactions with plant roots and other micro and macro soil organisms while providing ecosystem services. They have saprobic, pathogenic or mutualistic nutritional modes. Understanding fungi is essential as they are key drivers of below-ground ecosystem functions. Taxonomy of soil-associated fungal species is important as it paves the way to biodiversity, ecology, and biotechnological studies. Ascomycota is one of the largest and most abundant fungal phyla in edaphic habitats. Currently, there are two methods available for soil fungal detection; High-throughput sequencing (HTS) and culture-dependent methods, but most of the modern studies focus on HTS methods. Culture-dependent methods are less focused yet essential in identifying the species and genera in the soil. Although HTS provides an intangible estimate of fungal number, only culturing methods can yield living specimens necessary to understand the true number of Ascomycota species recovered from soil. However, soil-associated Ascomycota species and genera identified through culturing methods have not been compiled in recent studies, which poses a challenge to determine the exact number of Ascomycota species and genera worldwide. Ascomycota species have a wide distribution in different types of soil ecosystems in the world. Some of the Ascomycota species are commonly found in soil and can easily be detected, however, there are some uncommon species that can rarely be detected from the isolation methods. Thus, isolating the uncommon soil fungal groups is a challenge. As reported by the Global Fungi

website, fungal diversity in Asia is well studied, but most of the soil habitats in Thailand are poorly explored; thus, there is a need to focus the taxonomic studies on soil fungi in Thailand.

This study provides a comprehensive review on the value of culturing soil fungi in the modern molecular era, providing a compilation of soil-inhabiting Ascomycota genera worldwide, with a web-based platform to record soil Ascomycota, and offers a taxonomic account of uncommon soil ascomycetes found in both disturbed and undisturbed habitats in Thailand.

For the taxonomy and detection of soil fungi, several approaches have been used over the years, including both culture-dependent and culture-independent methods. The culture-dependent methods are based on morpho-molecular identification techniques. However, this approach has been largely been overtaken by culture-independent high-throughput sequencing (HTS) methods, which are now widely used in global soil fungal studies. Both culture-dependent and culture-independent methods are significantly important in understanding fungal taxonomy and estimating the total fungal number of fungal species in soil. This study provides a comprehensive discussion on the contributions of both approaches, emphasizing the continued relevance of culture-dependent methods in modern taxonomy and advocating for a complementary approach to enhance the recovery of fungal taxa and the fungal numbers in global soils. While each method has its own advantages and disadvantages, HTS has revealed numerous unculturable soil taxa and provided valuable insights into global fungal numbers. However, HTS also have several limitations, particularly in identifying species due to short read lengths and the inability to link physical specimens with species identifications. Therefore, morpho-molecular identifications are important to study fungal species. There are several culture-dependent methods available, namely the soil dilution series method, baiting method, soil plate method, heat treatment method, and soil steaming method. Among these, the dilution series method is widely used in many taxonomic studies. This study compiled a detailed account of twelve soil-fungal isolation methods, each described with the steps, advantages, disadvantages, uses and importance. This compilation will be helpful for taxonomists, researchers, and students engaged in soil-related research. Combining both culture-

dependent and -independent methods could represent a significant step towards the fungal taxonomic investigations within soil ecosystems.

Soil-based ecosystems in the world can be categorized as disturbed (e.g., agricultural and urbanized environments) or undisturbed (e.g., forests), each with distinct biological, chemical, and physical factors that influence fungal distribution. Ascomycota is the most abundant fungal phylum in global soils, with Sordariomycetes and Dothideomycetes being the dominant classes found. They play vital roles in ecosystem functioning in various edaphic habitats. In general, the ascomycete taxa present in soil can be categorized as common or uncommon genera/species. There are several genera commonly found in the soil, such as Acremonium, Alternaria, Aspergillus, Cladosporium, Curvularia, Fusarium, Penicillium and Trichoderma. These common groups have high adaptability and fast-growing nature, and can be easily detected and isolated from culturing and HTS methods. There are also rare species in the soil that have received little attention in scientific studies. However, these uncommon fungi have a greater share of the biodiversity in edaphic habitats. Most of the uncommon species are slow-growing and highly adapted to the soil micro-climatic conditions, which make it difficult to isolate them in the artificial environments. Although some uncommon fungal groups can possibly be detected from the HTS methods, identifying smaller or low-abundance communities is a challenge. Therefore, isolating and detecting these uncommon soil fungi are important to uncover new taxa and the hidden fungal diversity in soil ecosystems.

Over the past two decades, numerous studies have investigated soil-dwelling Ascomycota species worldwide, generating a significant amount of taxonomic information. Further, the information derived from HTS methods is compiled, but much of the information from culture-dependent methods remains scattered across various publications. To address this, an updated taxonomic outline was compiled, with comprehensive notes and line drawings for 270 ascomycete genera reported in soils worldwide. The majority of these compiled genera belong to Sordariomycetes (45%), followed by Dothideomycetes (28%) and Eurotiomycetes. Based on this compilation, the distribution of each class varies across different soil habitats, which can primarily be influenced by their ecological adaptations and nutritional requirements. While some habitats have been well studied globally, many ecological niches remain unexplored. Most of the studies have conducted in the forests,

cultivated lands, and urban soils. Based on the available data, forest soils are considered to harbor high Ascomycota diversity. However, a thorough investigation of unexplored or poorly studied habitats is essential to fully understand the true diversity of soil Ascomycota. Thereby, this study serves as a key resource for taxonomists and researchers studying soil fungi, offering a comprehensive compilation of Ascomycota genera and species. It provides valuable insights into their diversity and ecological roles, while also emphasizing the importance of studying Ascomycota on understudied environments in global soils.

This study further focuses on investigating the uncommon Ascomycota species from the different soil ecosystems in Thailand. Being a tropical country with high biodiversity, Thailand has limited studies on soil fungi. Previous research has mainly focused on agricultural and forest soils, with around 185 species, belonging to 79 genera, reported over the past two decades. Most of the species are commonly found. To expand this knowledge, the current study investigated Ascomycota species from three distinct soil ecosystems in Thailand. Samples were collected from caves, forests and urban areas to capture a variety of genera present. In the current research, soil dilution series and the baiting methods have been employed to isolate a diverse range of Ascomycota, including the uncommon taxa, followed by a polyphasic approach for species identification, which included morphological characterization and phylogenetic analyses using maximum likelihood and Bayesian methods. The study reveals 39 species, including 11 new species and 28 host and geographical records from Thai soils. Among these, *Beltraniella fertilis*, *Chloridium gonytrichii*, *Cordana terrestris*, *Curvularia dactylocteniicola*, *Kionochaeta microspora*, *Neopestalotiopsis terricola*, *Neoroussoella entadae*, *Paraconiothyrium cyclothyrioides*, *Paraleptospora thailandica*, *Pararoussoella lincangensis*, *Pseudopithomyces chartarum*, *Pochonia chlamydosporia*, *Pyrenochaetopsis tropicalis* and *Roussoella terrestris*, *Neptunomyces soli*, and and, *Verruconis soli* and *V. thailandica* were reported from forests soils. Species such as *Anteaglonium soli*, *Acrocalymma medicaginis*, *Currahmyces indicus*, *Diaporthosporella soli*, *Hansfordia pruni*, *H. pulvinata*, *Microascus croci*, *Neoceratosperma soli*, *Neopyrenochaeta telephoni*, *Remotididymella ageratinae*, *Wardomycopsis fusca* and *Yunnania penicillata* were isolated from disturbed caves. Five species were found in undisturbed caves, including *Amorocoelophoma soli*, *Cumuliphoma indica*, *Nigrograna speluncae*, *Microascus croci*, and *Wardomycopsis fusca*, totaling 15

species isolated from cave habitats across 14 genera. We have isolated *Microascus croci* and *Wardomycopsis fusca* from both disturbed and undisturbed cave soils. *Curvularia suttoniae*, *Ectophoma multirostrata*, *Epicoccum catenisporum*, *Paraconiothyrium zingiberacearum*, *Pyrenochaetopsis orizicolz*, *Remotididymella ageratinae*, *Setophoma thailandica* and *Stachybotrys phaeophialis* were recorded from urban soils. This study also explored cellulophilic and keratinophilic taxa in tropical forest soils, leading to the identification of a new species, *Neptunomyces soli* (Didymosphaeriaceae), and a new habitat record for *Curvularia dactylocteniicola* (Pleosporaceae). The potential lingo-cellulophilic and keratinophilic characteristics of these taxa are discussed in detail.

The GlobalFungi website reports Asia as the second most studied region in the world. However, compared to neighboring countries, soil fungal studies in Thailand remain limited, particularly in caves, urban areas, and hot springs. Thai caves, which hold unexploited fungal diversity, and urban soils, which require further exploration due to their ecological roles and potential health risks, represent underexplored habitats for fungal research. Expanding investigations across these diverse habitats will significantly enhance our understanding of soil Ascomycota and their ecological significance. By focusing on these less-explored habitats, we can uncover new species, gain a better understanding of the fungal ecology, and contribute to the global knowledge of soil fungi.

The study expanded the data availability on soil Ascomycota by digitizing a database that includes genera and species isolated from the culture-based methods. While most recent studies and databases on soil Ascomycota rely on high-throughput techniques, typically focusing on higher taxonomic ranks, detailed information at the genus and species levels remains scattered and inaccessible. To address this gap, we developed the Soil Fungi website (https://soilfun.org/), an online platform designed to compile soil-associated fungal species with comprehensive taxonomic descriptions. This platform is designed to be continuously updated to reflect the latest classifications. The Soil Fungi website aggregates scattered information from publications into a centralized, user-friendly database. It provides detailed descriptions of soil-associated fungal species, their taxonomic classifications, and other relevant information. The platform allows users to easily access and retrieve data, making it an essential resource for researchers, students, and anyone interested in soil mycology. The website also includes a guide outlining its objectives,

functions, and instructions for data access and retrieval. By serving as a central repository for soil fungal taxonomy, the Soil Fungi website aims to support and advance research, education, and global collaboration in this field.

Keywords: *Ascomycota*, Culture-dependent Methods, *Dothideomycetes*, Soil Fungi, *Sordariomycetes*