Dissertation Title	Taxonomy, Phylogeny, Cultivation, and Biologically Active Compounds of Selected *Agaricus* and Bolete Genera from Northern Thailand
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ABSTRACT

Thailand, especially the northern part, has an amazingly high diversity of mushrooms. Those mushrooms can either be saprobic, ectomycorrhizal (EcM) or parasitic. All of these play important roles in ecosystems. Many species can be cultivated and studied for biotechnological applications such as the production of mushrooms for food or nutraceuticals, which may lead to drug development. This thesis included several aspects of basic and applied research on Thai Basidiomycota, focusing on their taxonomy, phylogeny, cultivation, and secondary metabolite production.

The Agaricomycetidae (Agaricomycetes) are the most morphologically diverse Basidiomycota and they comprise two well-known orders, Agaricales and Boletales. The taxonomy, phylogeny, diversity, and relationship of these two orders have been extensively studied. In this work, one genus from Agaricales, *Agaricus*, and five genera from Boletales, *Hourangia*, *Phlebopus*, *Phylloporus*, *Retiboletus*, and *Xerocomus* were studied. *Agaricus* (mainly *A. bisporus*) is widely consumed. Moreover, this genus

produces many biologically active compounds with antioxidant, antibacterial, anti-inflammatory, and antitumor properties. *Hourangia*, *Phlebopus*, *Phylloporus*, *Retiboletus*, and *Xerocomus* are ectomycorrhizal genera that contain small numbers of species compared to *Agaricus*. This study included multi-gene phylogenetic analysis of *Agaricus, Hourangia, Phlebopus, Phylloporus, Retiboletus, and Xerocomus* from diverse habitats in northern Thailand, using nuclear ribosomal (ITS), mitochondrial (ATP6) as well as nuclear protein-coding genes (RPB2, TEF1). New species were described and illustrated. For the utilization of Agaricomycetidae, *Agaricus* was selected for cultivation due to their life cycle and nutrition mode as saprotrophs can be mimicked. Also, morphological and phylogenetic evidence were used to classify the species (with a focus on edible sections of *Agaricus*). Mushrooms were collected, identified and isolated by tissue culture. The optimal condition of mycelia growth including medium, pH and temperature of new *Agaricus* species were investigated. After that, spawn was produced by inoculating optimal substrate with pure cultures. The inoculum was transferred to compost for mushrooms production. Further, the selected *Agaricus* and *Phlebopus* spp. (both on agar media and basidiomata) were extracted for biological assay. The bioactive compounds were analyzed by High-performance liquid chromatography technique (HPLC) and chemical structure elucidation by Nuclear magnetic resonance spectroscopy (NMR).

Morphological characters and multi-gene phylogenetic analyses were used to identify specimens collected in northern Thailand. Three new *Phylloporus* species, *P. pusillus, P. subbacillisporus* and *P. subrubeolus*, from southwestern China and northern Thailand were introduced with macro- and micro-descriptions and illustrations. They are found mostly in forests dominated by Fagaceae or Dipterocarpaceae trees. *Phylloporus pusillus* and *P. subrubeolus* are morphologically similar, but the former can be separated by very small basidiomata, lamellae becoming deeper yellow to reddish with age and less densely encrusted walls of pileipellis hyphae. *Phylloporus subbacillisporus* can be easily distinguished from other species by shallowly

intervenose lamellae and subbacilliform basidiospores. Two new geographical record of *Phylloporus*, *P*. *attenuatus* and *P*. *gajari* were also reported from Thailand.

Retiboletus brevibasidiatus was described as new to science, whereas *R*. *fuscus* and *R. nigrogriseus* were reported for the first time from Thailand. *Retiboletus brevibasidiatus* produces medium-sized basidiomes, with a dark blonde to clay pileus and densely reticulate stipe mostly on the upper part with pale yellow to chrome yellow basal mycelium. It is difficult to separate *R. brevibasidiatus* from other closely related species on the basis of macroscopic characters. However, the new species can be distinguished by microscopic characters, mostly the shorter basidia. The macro- and micro-morphology of the *R. fuscus* and *R. nigrogriseus* collections from Thailand fit well with the previous descriptions of materials from China and Japan.

Three novel species and two first records, *Hourangia microcarpa* and *H. nigropunctata*, were reported for *Hourangia* from Thailand. Moreover, two novel species and one first record, *Xerocomus microcarpoides*, were described from Thailand.

The genus *Agaricus* contains many edible and medicinal mushrooms and some of them are used for commercial purposes. Most *Agaricus* species have been described from temperate geographic areas, and their occurrence in the tropics is still underexplored. In this study, *Agaricus campestroides* and *A. flocculosipes* were selected for cultivation. *Agaricus campestroides* has been formally described as infrageneric taxon belongs to "Tropical clade b" and known as wild edible species from Ethiopia. *Agaricus flocculosipes* is also an edible species belonging to section *Arvenses*. The present study describes the successful cultivation of two wild strains of *A. flocculopsipes* and *A. campestroides* from Thailand, for the first time. The optimal conditions for mycelial growth have been evaluated for both strains, formation of basidiomata on compost could only be observed for *A. campestroides.* In the present study, mycelial growth and mushroom production conditions were optimized. The

temperature range of 25–30 °C with 80–90% humidity was assessed as preferred conditions for primordia and basidiomata formation in a compost rice straw medium with sandy-soil casing layer. Blazeispirols (blazeispirol A and D), a family of triterpenoids that is known to act as selective agonists of Liver X receptor alpha with beneficial effects in vivo in a murine model, were detected in crude extracts from submerged cultures of *A. campestroides* and *A. flocculosipes*.

Submerged cultures of the edible mushroom-forming species *Phlebopus portentosus* and *Phlebopus spongiosus* were screened for their secondary metabolites by HPLC-UV/Vis and HR-LC-ESI-MS. Two new compounds, 9′-hydroxyphenyl pulvinone (1), containing an unusual pulvinone structure, and phlebopyron (2), together with the seven known pigments, atromentic acid (3), xerocomic acid (4), variegatic acid (5), methyl atromentate (6), methyl isoxerocomate (7), methyl variegatate (8), and variegatorubin (9) were isolated from the cultures. Their structures were assigned on the basis of extensive 1D/2D NMR spectroscopic analyses, as well as HR-ESI-MS, and HR-ESI-MS/MS measurements. Furthermore, the isolated compounds were evaluated for their antimicrobial and cytotoxic properties. 9′-hydroxyphenyl pulvinone (1), xerocomic acid (4), and methyl variegatate (8) exhibited weak to moderate cytotoxic activities against several tumor cell lines. The present paper provides a comprehensive characterization of pigments from the class of pulvinic acids that are present in the basidiomes of many edible bolete species.

Keywords: Agaricales, Asia, Boletales, Edible Mushrooms, Systematics