

<b>Dissertation Title</b>	Species Diversity, Phylogeny, and Applications of Selected Pleurotoid Mushrooms from Laos and Thailand
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## ABSTRACT

Pleurotoid mushrooms are characterised by their fan-shaped basidiomata with or without stipe. They have a widespread distribution in many habitats worldwide. These mushrooms are diverse, and some species are edible and cultivable. Some cultivable varieties have drawn interest from local and international communities due to their potential culinary and economic value. Apart from their culinary uses, pleurotoid mushrooms have pharmacological properties and environmental significance. Some pleurotoid mushrooms produce bioactive compounds that can be used in medicine and biotechnology. The bioactive properties and culinary appeal of these mushrooms encourage their production, consumption and various applications. In this study, a comprehensive exploration of the distribution of pleurotoid mushrooms is carried out in Laos and Thailand, which offers insight into their prevalence in these regions. It covers the diversity of pleurotoid species that have been recorded, some of which are popular culinary choices due to their unique taste profiles.

Although pleurotoid mushrooms are recognised as edible, their abundance remains largely overlooked in Thailand. This study analyses the distribution, diversity and morphology of pleurotoid mushrooms in 19 community forests in eight provinces from 2019 to 2023 during the rainy season. 250 collections were collected, representing 70 species within thirteen genera from ten families. The most diverse species were *Hohenbuehelia tristis* ( $H' = 2.33$ ), *Schizophyllum commune* ( $H' = 2.20$ ), and *Campanella* sp. 1 ( $H' = 1.94$ ). The highest species diversity index was estimated

in Pa Deng village ( $H' = 3.34$ ), followed by Pox Kaeo ( $H' = 3.05$ ), and Huay Kaeo ( $H' = 2.91$ ). The most common species observed were *Hohenbuehelia tristis* (10.44%) and *Crepidotus* sp. 10 (5.62%). Pleurotaceae presented the greatest alpha diversity with *Hohenbuehelia*, *Pleurotus*, and *Resupinatus*. The largest number of species was found in *Crepidotus* (30), followed by *Hohenbuehelia* (8), and *Pleurotus* (7). This research highlights the importance of raising awareness among local communities about the conservation of mushroom resources, aligning with the United Nations Sustainable Development Goals, particularly SDG 12 (Responsible Consumption and Production) and SDG 15 (Life on Land).

Six new species and two new records of *Crepidotus* are described from Thailand. Macroscopic and microscopic descriptions with photoplates, as well as a multigene phylogeny are provided. *Crepidotus Chiangraiensis* sp. nov. is recognised as densely velutinous with white hairs longer near the point of attachment, not striate margin and not hygrophanous. *Crepidotus flavocarpus* sp. nov. is recognised by yellow to yellowish pileus basidiomata, margin discolourous paler yellow, densely velutinous with white hairs near the point of attachment, smooth globose basidiospores, and inverse hymenophoral trama. *Crepidotus thailandicus* sp. nov. is recognised by a tomentose surface with white hair when primordial to young stages and then disappearing with age, present long transtuent-striate reward to the point of attachment becoming fade away at central. *Crepidotus roseocarpus* sp. nov. is recognised by large basidiomata, pileus, lamellae, and context are pastel pink, concolorous darker pink at margin, hymenophoral trama convergent, with cylindrical terminal elements. *Crepidotus ungulatus* sp. nov. is recognised by ungulate basidiomata, brownish orange near the point of attachment, the colour of the basidiomata changed to light brown in 3 hours and the margin is concolorous to the surface of the pileus. *Crepidotus viscidus* sp. nov. is recognised by white semicircular pileus, translucent-striate with grey to dark grey margin, lamellae pale orange to orange - white, glutinous context, smooth basidiospore, and hymenophoral trama present in regular form. Additionally, two newly recorded species are *C. lateralipes* and *C. striatus*.

Two new species and the first geographical record of *Hohenbuehelia* are described from Thailand. Macroscopic and microscopic descriptions with

photoplates as well as a multigene phylogeny are provided. *Hohenbuehelia flabelliformis* sp. nov. is recognised by large flabelliform basidiomata, densely villose yellowish-white pileus with white hairs near the point of attachment, basidiospores that mostly are ellipsoid in front view and phaseoliform in side view, the absence of cheilocystidia, and a trichoderm pileipellis. *Hohenbuehelia lageniformis* sp. nov. is characterised by fleshy basidiomata, velutinous pileus with whitish hairs near the point of attachment and the margin, elsewhere pale greyish-yellow and with only sparse white hairs, pale brown to light brown and mucilaginous context, subglobose basidiospores, lageniform cheilocystidia, an ixotrichoderm pileipellis, and the absence of pileoleptocystidia. *Hohenbuehelia tristis* is characterised by small creamy white, spathuliform basidiomata that are larger than the type subspecies, minutely pubescent pileus with tiny greyish hairs that disappear when mature, leaving the surface glutinous, faintly translucent and shiny, ellipsoid to sub-ellipsoid basidiospores, lecythiform to sublageniform cheilocystidia, and an ixotrichoderm pileipellis. *Hohenbuehelia tristis* is recorded from Thailand for the first time. On the basis of the polymorphism observed in part of nuclear large subunit ribosomal RNA (nrLSU), the presence of two divergent lineages within *H. tristis* is discussed.

*Pleurotus* is an edible mushroom, also known as oyster mushroom, that has been consumed and cultivated worldwide. In addition to being widely consumed, some species have medical, biotechnological, and environmental applications owing to their bioactivities. In this study, we report on three species of *Pleurotus* from Laos. *Pleurotus djamor* and *P. tuber-regium* are reported for the first time from Laos, while *P. giganteus*, a common species in Laos, is also reported. *Pleurotus djamor* var. *fuscopruinosus* is a new record in Thailand also provided. Molecular phylogenetic analyses of the nuclear ribosomal internal transcribed spacer region (ITS) were performed in addition to the morphological analysis to confirm its identification. Full descriptions, colour photo plates, micrographs, and taxonomic comparisons are provided.

The mycelium of *P. djamor* var. *fuscopruinosus* strain MFLUCC24-0056 was grown on agar medium, and the *Sorghum bicolor* (sorghum) medium showed the best spawn production. In the production trial, sawdust is suitable for the growth

of the mycelium of *P. djamor* var. *fuscopruinosus*. The wild strain of *P. djamor* var. *fuscopruinosus* produced fruiting bodies at 25–28°C and 70–80% humidity. The primordia formed four weeks after the mycelia fully colonised the substrate and illuminated the conditions for the development of a mature fruit body in 4–5 days. Nutritional analysis of 100 g of dried sample from *P. djamor* var. *fuscopruinosus* showed  $15.99 \pm 0.2\%$  of carbohydrates content,  $25.00 \pm 1.5$  (g/100 g) of protein content,  $30.25 \pm 0.5\%$  of moisture content,  $19.10 \pm 0.9\%$  of fibre content,  $7.59 \pm 1.0\%$  of ash content, and  $2.07 \pm 0.5$  (g/100 g) of fat content.

Among the three different extractants, ethyl acetate, methanol, and hot water showed antioxidant activities of 50% (volume per volume; v/v). The ethyl acetate extract of *P. djamor* var. *fuscopruinosus* mycelium exhibited DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity with an  $IC_{50}$  of  $694.47 \pm 3.92$  µg/mL, compared to ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation scavenging activity, which had an  $IC_{50}$  of  $652.92 \pm 2.53$  µg/mL. *In vitro* anticancer effects were evaluated using the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The ethyl acetate extract of *P. djamor* var. *fuscopruinosus* mycelium exhibited cytotoxicity against lung cancer (A549,  $IC_{50} = 245.73 \pm 7.60$  µg/ml) and colorectal cancer cells (SW480,  $IC_{50} = 382.03 \pm 4.55$  µg/ml), compared to the standard positive control doxorubicin with an  $IC_{50}$  value of  $14.96 \pm 1.58$  µg/mL. Moderate cytotoxicity was observed in RAW 264.7 macrophages ( $IC_{50} = 213.08 \pm 4.08$  µg/ml). Antidiabetic potential was demonstrated through  $\alpha$ -glucosidase inhibition, with the hot water extract ( $IC_{50} = 582.91 \pm 3.0$  µg/ml) showing the highest activity, followed by ethyl acetate ( $IC_{50} = 473.87 \pm 1.4$  µg/ml) and methanolic ( $IC_{50} = 357.63 \pm 3.3$  µg/ml), comparable to the standard positive control acarbose ( $IC_{50} = 635.70 \pm 4.9$  µg/ml). Glucose uptake in 3T3-L1 adipocytes was enhanced ( $IC_{50} = 582.91 \pm 3.0$  µg/ml) but lower than the standard positive control metformin ( $IC_{50} = 99.58 \pm 0.59$  µg/ml).

*Pleurotus giganteus* is a wild edible mushroom successfully domesticated in Thailand. In this study, the strain MFLUCC23-0016 was isolated on PDA agar, with optimal spawn growth observed in millet ( $11.10 \pm 0.34$  mm/day) and sawdust ( $9.02 \pm 0.40$  mm/day). Fruiting bodies appeared 14 days after unsealing the substrate bag, with the highest fresh weight yields from sawdust ( $180.77 \pm 44.41$  g) and sawdust +

corn cob ( $176.60 \pm 17.65$  g) over 60 days. Among the three casing formulas, F1 produced the highest yield ( $278.54 \pm 89.04$  g). Nutritional analysis per 100 g of dried sample revealed  $61.32 \pm 0.0\%$  carbohydrates,  $20.32 \pm 0.8$  g protein,  $15.6 \pm 0.5\%$  moisture,  $14.28 \pm 0.3\%$  fibre,  $5.61 \pm 0.9\%$  ash, and  $2.98 \pm 1.58$  g fat. Ethyl acetate extract showed antioxidant activity with DPPH ( $IC_{50} = 743.78 \pm 6.02$   $\mu$ g/mL) and ABTS ( $IC_{50} = 704.82 \pm 2.28$   $\mu$ g/mL). It exhibited cytotoxicity against colorectal cancer cell SW480 ( $IC_{50} = 149.11 \pm 5.31$   $\mu$ g/mL) and lung cancer cell A549 ( $IC_{50} = 302.67 \pm 11.71$   $\mu$ g/mL) compared to standard positive control doxorubicin ( $IC_{50} = 14.96 \pm 1.58$   $\mu$ g/mL), with  $IC_{50} = 165.49 \pm 2.92$   $\mu$ g/mL for RAW 264.7 cells. Antidiabetic potential was demonstrated via  $\alpha$ -glucosidase inhibition, with hot water extract ( $IC_{50} = 583.04 \pm 4.1$   $\mu$ g/mL) showing the highest activity. Glucose consumption in 3T3-L1 cells was  $340.78 \pm 5.32$   $\mu$ g/mL for hot water extract, compared to standard positive control metformin ( $IC_{50} = 99.58 \pm 0.59$   $\mu$ g/mL). The bioactivity varied with the solvent type. This study provided diversity, taxonomy, and phylogeny of cultivation, as well as nutritional analysis from the fruiting bodies of two wild strains of *Pleurotus*. The study not only contributes to understanding the medicinal properties of pleurotoid mushrooms, but also underscores the importance of further research to explore their mechanisms and optimise their application in clinical contexts. Moreover, extracts from both *Pleurotus* species have been shown to be non-toxic to normal cells, underscoring their potential for safe therapeutic applications and contributing to the advancement of SDG 3 (Good Health and Well-being) through the development of sustainable, health-promoting bioresources.

**Keywords:** Biological Activities, Cultivation, Nutritional Value, Taxonomy, Phylogeny