SPECIES DIVERSITY, PHYLOGENY, AND APPLICATIONS OF SELECTED PLEUROTOID MUSHROOMS FROM LAOS AND THAILAND

MONTHIEN PHONEMANY

DOCTOR OF PHILOSOPHY
IN
BIOLOGICAL SCIENCE

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THIS DISSERTATION IS A PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BIOLOGICAL SCIENCE

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Monthien Phonemany

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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 discolorous 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 transtucent-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. *fuscopruinosu*s 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. *fuscopruinosu*s 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 ± 0.2% of carbohydrates content, 25.00 ± 1.5 (g/100 g) of protein content, 30.25 ± 0.5% of moisture content, 19.10 ± 0.9% of fibre content, 7.59 ± 1.0% of ash content, and 2.07 ± 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 ± 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 ± 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 ± 7.60 µg/ml) and colorectal cancer cells (SW480, IC_{50} = 382.03 ± 4.55 µg/ml), compared to the standard positive control doxorubicin with an IC_{50} value of 14.96 ± 1.58 µg/mL. Moderate cytotoxicity was observed in RAW 264.7 macrophages (IC_{50} = 213.08 ± 4.08 µg/ml). Antidiabetic potential was demonstrated through α-glucosidase inhibition, with the hot water extract (IC_{50} = 582.91 ± 3.0 µg/ml) showing the highest activity, followed by ethyl acetate (IC_{50} = 473.87 ± 1.4 µg/ml) and methanolic (IC_{50} = 357.63 ± 3.3 µg/ml), comparable to the standard positive control acarbose (IC_{50} = 635.70 ± 4.9 µg/ml). Glucose uptake in 3T3-L1 adipocytes was enhanced (IC_{50} = 582.91 ± 3.0 µg/ml) but lower than the standard positive control metformin (IC_{50} = 99.58 ± 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 ± 0.34 mm/day) and sawdust (9.02 ± 0.40 mm/day). Fruiting bodies appeared 14 days after unsealing the substrate bag, with the highest fresh weight yields from sawdust (180.77 ± 44.41 g) and sawdust +

corn cob (176.60 ± 17.65 g) over 60 days. Among the three casing formulas, F1 produced the highest yield (278.54 ± 89.04 g). Nutritional analysis per 100 g of dried sample revealed 61.32 ± 0.0% carbohydrates, 20.32 ± 0.8 g protein, 15.6 ± 0.5% moisture, 14.28 ± 0.3% fibre, 5.61 ± 0.9% ash, and 2.98 ± 1.58 g fat. Ethyl acetate extract showed antioxidant activity with DPPH (IC_{50} = 743.78 ± 6.02 µg/mL) and ABTS (IC_{50} = 704.82 ± 2.28 µg/mL). It exhibited cytotoxicity against colorectal cancer cell SW480 (IC_{50} = 149.11 ± 5.31 µg/mL) and lung cancer cell A549 (IC_{50} = 302.67 ± 11.71 µg/mL) compared to standard positive control doxorubicin (IC_{50} = 14.96 ± 1.58 µg/mL), with IC_{50} = 165.49 ± 2.92 µg/mL for RAW 264.7 cells. Antidiabetic potential was demonstrated via α-glucosidase inhibition, with hot water extract (IC_{50} = 583.04 ± 4.1 µg/mL) showing the highest activity. Glucose consumption in 3T3-L1 cells was 340.78 ± 5.32 µg/mL for hot water extract, compared to standard positive control metformin (IC_{50} = 99.58 ± 0.59 µg/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

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ABBREVIATIONS AND SYMBOLS

–	The inaccessibility of the sequence
*	New species
**	New records
%	Percentage
&	And
°C	Degree centigrade
avl	Average length
avw	Average width
$C_4H_8O_2$	ethyl acetate
CK	Chiang Khan
cm	Centimeter
diam.	Diameter
DL	Dong Luang district
DP	Doi Pui
DW	Dry weight
e.g.	For example
et al.	And other
FW	fresh weight
g	Gram
H_2O	Water
HK	Huay Kaeo village
HM	Huay Mae Suk
IC_{50}	Inhibitory Concentration 50%
KC	Kroung Ching
KP	Khao Phanom District
KS	Khlong Sok subdistrict
L	Liter
l	Length

ABBREVIATIONS AND SYMBOLS

µL	Microliter
µm	Micrometer
MeOH	methanol
MF	Mae Feak village
MFU	Mae Fah Lung University
mg	Milligram
mL	Milliliter
mm	Millimeter
MS	Mae Sai
MT	Mae Toe village
N/A	No data available
NL	Nang Lae Nai Village
No.	Number
nov.	Novum (Latin for new thing)
OF	Percentage of occurrence frequency
PD	Pa Deng village
PK	Pox Kaeo village
PN	Pha Ngae village
PT	Phouthong Temple
Q	Quotient
Qav	Average quotient
R	Replication
sp.	Species
spp.	Species (plural)
SV	Sirivong subdistricts
T1	Formula 1
T2	Formula 2
T3	Formula 3

ABBREVIATIONS AND SYMBOLS

TP	Tha Pha village
v/v	Volume/volume
w	Width
w/w	Weight/weight
α	Alpha
β	Beta
μg	Microgram

CHAPTER 1

GENERAL INTRODUCTION

1.1 Basidiomycota

Basidiomycota is a large group in the kingdom of fungi, which include many familiar mushrooms and toadstools, bracket fungi, earth balls, earth stars, false truffles, jelly fungi, puffballs, and stinkhorns (Webster & Weber, 2007). The classification of Basidiomycota was approximately four subphylums, 18 classes, 68 orders, 241 families, and 1928 genera (He et al., 2019). Basidiomycota plays an important role in forest ecosystems, especially in biodiversity, due to its mycorrhizal relationship with other plant species and can be found as ectomycorrhizas, beneficial (edible species or medicine), decomposers (saprotrophic species) and harmful (pathogenic) (de Mattos-Shipley et al., 2016; Hyde et al., 2019). Moreover, it can be a food source and income for local people worldwide, some edible species can be grown with agricultural waste, making production to improve the market value (Boa, 2008; Hyde et al., 2019).

1.2 Pleurotoid Mushrooms

Pleurotoid mushrooms (Basidiomycota) are agaric forms that typically have a fan-shaped fruiting body, the presence of lamellae, and lateral stem attachment. In some species, the stipe can be reduced or absent. The pleurotoid form is well-represented in the genus *Pleurotus*. The pleurotoid form is not phylogenetic informative. The habit has evolved and diverged many times in some genera. Pleurotoid species can be found in many different genera and families, but in some genera, not all species are pleurotoid, such as *Arrhenia* Fr., *Deconica* (W.G. Sm.) P. Karst., *Entoloma* (Fr.) P. Kumm., *Gloiocephala* Massee, and *Lactifluus* (Pers.) Roussel (Laessoe & Petersen, 2019). Pleurotoid (Greek.: pleurē + ōtos + -oid, literally 'side-ear form' or 'having the likenes of *Pleurotus* ssp.') is described as mushrooms with laterally attached fruiting bodies

with gills (Thorn et al., 2000). Some genera of pleurotoids, e.g., *Panus* Fr., *Neofavolus* Sotome & T. Hatt., and older basidiomata of *Lentinellus* P. Karst. and *Pleurotus* (Fr.) P. Kumm., have extraordinarily tough basidiomata. A few genera, such as *Hohenbuehelia* Schulzer, *Sarcomyxa* P. Karst., and *Schizophyllum* Fr., contain gelatinous or stretchy flesh (Gulde, 2011; Seelan, 2015). There are some forms of the fruiting body that are at times similar to pleurotoid forms, such as clitocyboid, cyphelloid, and lentinoid; clitocyboids differ from pleurotoid by having a central stipe (Laessoe & Petersen, 2019). The cytophile differs by having small basidiomata without lamellae, hardly any stipe, and being attached to the upper side of the basidiomata (Sulzbacher et al., 2009; Laessoe & Petersen, 2019). Lentinoids, on the other hand, are different from pleurotoids in that they have a leathery texture and consist of tough to fbasidiospores are white to cream; some pleurotoid species have rose-to-brown basidiospores (Laessoe & Petersen, 2019). *Pleurotus* species can even have greyish violet spores (e.g., *Pleurotus ostreatus* (Jacq.) P. Kumm.) (Laessoe & Petersen, 2019). The spore colour of pleurotoid mushrooms is often considered to divide groups with completely different phylogenetic relationships (Thorn et al., 2000; Seelan, 2015; Laessoe & Petersen, 2019). Five orders, 16 families, 25 genera, and one pleurotoid species are listed based on the colour of the spores in Table 1.1 (Laessoe & Petersen, 2019).

The majority of pleurotoid mushrooms are decomposers that can be found in decaying wood, stump trees, the bark of living trees, or herbaceous stems (Laessoe & Petersen, 2019). A few species are ectomycorrhizal fungi, e.g. members of *Lactifluus* (Pers.) Roussel (Wang et al., 2012; Morozova et al., 2013; De Crop et al., 2018). Pleurotoid mushrooms include various genera such as *Campanella* Henn., *Clitopilus* (Fr. ex Rabenh.) P. Kumm., *Crepidotus* (Fr.) Staude, *Deconica* (W.G. Sm.) P. Karst., *Entoloma* (Fr.) P. Kumm., *Gloiocephala* Massee, *Hohenbuehelia*, and *Lentinellus* are decomposers (Laessoe & Petersen, 2019). Some pleurotoid mushrooms have been widely consumed, especially members of *Pleurotus*. *Pleurotus ostreatus* (Jacq.) P. Kumm. (oyster mushroom) is the most famous species cultivated worldwide (Sánchez, 2010; Cohen et al., 2002; Khan & Tania, 2012). Additionally, other commercially cultivated *Pleurotus* species are *P. citrinopileatus* Singer (yellow oyster mushroom or golden oyster mushroom), *P. cornucopiae* (Paulet) Rolland, and *P. cystidiosus* O.K.

Mill. (abalone mushroom or maple oyster mushroom), *P. djamor* (Rumph. ex Fr.) Boedijn (pink oyster mushroom), *P. floridanus* Singer (white oyster mushroom), *P. flabellatus* Sacc. (red oyster mushroom), *P. giganteus* (Berk.) Karun. & K.D. Hyde (giant oyster mushroom), *P. pulmonarius* (Fr.) Quél. (Indian oyster or Italian oyster), and *P. tuber-regium* (Fr.) Singer (king tuber mushroom) (Samsudin & Abdullah, 2019). *Pleurotus* species are not only edible but also have notable pharmacological properties such as anti-aging, hepatoprotective, hyperglycemic, hypotensive, anti-inflammatory, anti-lipidemic, antimicrobial, antineoplastic, antiviral, antioxidant, antitumour, and antimutagenic activities (Phan et al., 2019). These activities have been reported for various extracts and isolated compounds, such as polysaccharides, polysaccharide-protein complexes, proteoglycans, proteins and DNA from oyster mushroom fermentation broth, mycelia, or basidiomes (Kumar et al., 2020; Krishnakumar et al., 2023). In particular, polysaccharides appear to be potent antitumour and immunomodulatory substances, besides possessing other beneficial activities (Gregori et al., 2007; Patel et al., 2012).

Laessoe and Petersen (2019) listed 26 genera of pleurotoid mushrooms from temperate Europe (Table 1.1 and Figure 1.1). Ten species of pleurotoid mushrooms were reported from Laos (Læssøe, 2018; Rattanamalee & Rattanamalee, 2012, Sysouphanthong et al., 2017; Łuczaj et al., 2021; Phonemany & Thongklang, 2023), and 29 species from Thailand (Baroni et al., 2001; Karunarathna et al., 2011a; Jatuwong et al., 2017; Sakonrak, 2017; De Crop et al., 2018; Sandargo et al., 2018a; Kumla et al., 2019; Suwannarach et al., 2020; 2022; Phonemany & Thongklang, 2023; Sysouphanthong et al., 2023). However, more studies are needed on pleurotoid mushrooms to clarify their taxonomy, and more new species could be discovered in both regions. This study provides information on the taxonomic classification and distribution of pleurotoid mushrooms in Laos and Thailand. The cultivation of some important species, including the substrate used and biological efficiency, was also discussed. In addition, nutritional analyses of edible species and secondary metabolites of pleurotoid species with their bioactivities were also determined.

Table 1.1 The divisions genera of pleurotoid by spore colour based on the study of Laessoe & Petersen (2019)

Genera/ species	Spore colour			
	Brown	**Greyish violet**	**White to cream**	**Rose to pinkish**
Arrhenia Fr.			+	
Campanella Henn.			+	
Chaetocalathus Singer			+	
Cheimonophylum Singer			+	
Clitopilus (Fr. ex Rabenh.) P. Kumm.				+
Crepidotus (Fr.) Staude	+			
Deconica (W.G. Sm.) P. Karst.	+			
Entoloma P. Kumm.	+			+
Gloiocephala Massee			+	
Hohenbuehelia Schulzer			+	
Lentinellus P. Karst.			+	
Neofavolus Sotome & T. Hatt.			+	
Neolentinus Redhead & Ginns			+	
Panellus P. Karst.			+	
Panus Fr.			+	
Phyllotopsis E.-J. Gilbert & Donk ex Singer				+
Pleurocybella Singer			+	
Pleuroflammula Singer	+		+	
Pleurotus (Fr.) P. Kumm.				
Pleurotus ostreatus (Jacq.) p. Kumm		+		
Plicatura Peck			+	
Resupinatus Nees ex Gray			+	
Sarcomyxa P. Karst.				
Schizophyllum Fr.			+	
Tapinella E.-J. Gilbert	+			
Tectella Earle			+	

Note (+) means the spore colour of the genus.

Source Læssøe & Petersen (2019)

Figure 1.1 The major morphological characteristics of pleurotoid genera in temperate Europe

1.3 Cultivation of Pleurotoid Mushrooms

Mushroom cultivation is a simple microbiological method for large-scale commercial agro-waste recycling (Raman et al., 2021). The cultivation of pleurotoid mushrooms is preferred using many agricultural and forest wastes. It is easy to cultivate using low-cost wood, wood by-products, and agricultural waste (Girmay et al., 2016; Thongklang & Luangharn, 2016; Balan et al., 2022). Sawdust is the main substrate used for the cultivation of pleurotoid mushrooms cultivation; it can be used mixed with agricultural wastes, and some species can grow on composted and non-composted agricultural wastes (Phonemany et al., 2023). The nutritional supplements for high

yields depended on the type of pleurotoid species. Several edible pleurotoid mushrooms have been cultivated, *Pleurotus* ssp. is the most cultivated worldwide; however, in Thailand, eight species of *Pleurotus* are listed (Chandrasrikul et al., 2011), and some species have been cultivated, such as *P. citrinopileatus*, *P. djamor*, *P. eryngii*, *P. floridanus*, *P. giganteus*, *P. ostreatus* var. *columbinus*, *P. ostreatus*, and *P. pulmanarius* (Kumla et al., 2013; Thawthong et al., 2014; Thongklang & Luangharn, 2016).

The nutrition of pleurotoid mushrooms has high nutritional value, their rich protein, providing low amounts of fat, vitamins (Vitamin C and B complex), carotenoids and minerals such as Phosphorus (P), Potassium (K), Nickel (Ni), and Iron (Fe) (Singdevsachan et al., 2013). The nutritional properties of the mushroom species can be compared to foods such as eggs, milk (Ho et al., 2020; Niego et al., 2021). The *Pleurotus* species were considered famous for healthy foods because they have a high nutrient content, such as proteins, carbohydrates, fibres, minerals, and vitamins (Maftoun et al., 2015; Lesa et al., 2022). There are several important elements included in cultivations, food applications, and the value of medicine (Krakowska et al., 2020; Juárez-Hernández et al., 2023). Some pleurotoid mushroom species had proximate nutritional values and compared the nutritional value of the pleurotoid species that have been reported. There are 18 pleurotoid species and included ash, carbohydrate, fat, fibre, moisture, protein, reducing sugar, and energy (Phonemany et al., 2023).

1.4 Bioactivities of Pleurotoid Mushrooms

The bioactive and medicinal properties of pleurotoid species have been investigated *in vitro* and *in vivo*, such as antioxidant, antifungal, anti-inflammatory, anti-cancer, anti-diabetic, anti-dengue, hepatoprotective activity, neurite outgrowth stimulating activity and Genoprotection (Phan et al., 2019). Furthermore, many compounds have been isolated from extracts of *P. giganteus*, for example alkaloids, fatty acids, organic acids, phenolics, sterols and triterpenoids (Phan et al., 2019; Yadav et al., 2020). Many studies of *P. giganteus* reported various biological activities and medicinal properties, optimal medium, PH, temperature, and optimal cultivation (Klomklung et al., 2012; Kumla et al., 2013; Klomklung et al., 2014; Soytong & Asue,

2014; Phan et al., 2019; Yadav et al., 2020). *Pleurotus* species have several bioactivities such as anticancer, antimicrobial, antiviral, antineoplastic, antioxidant, anti-inflammatory, anti-ageing, anti-tumour, antimutagenic, antilipidemic, hepatoprotective, hyperglycemic, and hypotensive properties (Juárez-Hernández et al., 2023). For instance, *Pleurotus* species was able to inhibit Gramme-negative bacteria such as *Escherichia coli*, *Pseudomonas* sp., and *Vibrio cholera*. *Pleurotus ostreatus* (oyster mushroom) species were ranked as the second world mushroom market for consumption and industry worldwide (Zhai & Han, 2018). In Thailand, studies have investigated the bioactivities of four genera such as *Clitopilus*, *Hohenbuehelia*, *Lentinus*, and *Pleurotus*. For example, research on *Pleurotus* species has reported antioxidant, anticancer, and antidiabetic properties, while *Lentinus* spp. has demonstrated antibacterial and immunomodulatory effects. *Hohenbuehelia* spp. has shown potential antifungal and nematicidal activities, whereas *Clitopilus* spp. has been explored for its antimicrobial compounds. These findings highlight the diverse medicinal potential of these fungal genera, warranting further investigation into their bioactive compounds and therapeutic applications.

1.5 Research Objectives

1.5.1 To investigate species diversity and phylogeny of pleurotoid mushrooms from Laos and Thailand.

1.5.2 To cultivate edible wild strains of *Pleurotus* and determine their nutritional contents and cytotoxicity.

1.5.3 To evaluate the biological activities of wild strains of *Pleurotus*.

1.6 Scope of Research

1.6.1 This study is focused on pleurotoid mushrooms from Laos and Thailand, with an emphasis on their species diversity in selected community forests in Thailand. The analysis used Shannon's diversity index to assess species diversity and distribution.

1.6.2 The taxonomy and phylogeny of pleurotoid mushrooms, macroscopic and microscopic descriptions with photoplates, and a multigene phylogeny are studied.

1.6.3 Two wild strains of *Pleurotus* were selected for cultivation using different methods. The cultivation of *P. giganteus* is focused on optimising spawning production, evaluating agricultural waste as an alternative substrate for fruiting body development, assessing the effect of soil casing on yield, and analysing the nutritional composition of cultivated mushrooms. For *P. djamor* var. *fuscopruinosus*, this study represents the first attempt at its cultivation, including optimising spawn production, evaluating the impact of different agricultural waste substrates on mycelium growth, and assessing fruiting body development. Additionally, the nutritional composition of the cultivated mushrooms was analysed, including ash, carbohydrate, fat, fibre, moisture, and protein content.

1.6.4 The mycelium extract of two cultivated *Pleurotus* strains were tested for their biological activities including antioxidant (2,2-Diphenyl-1-picrylhydrazyl radical-scavenging activity and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical cation scavenging activity), anticancer (against lung cancer A549 and colorectal cancer SW480), antidiabetic (α-glucosidase assay and glucose consumption assay), and the cytotoxicity assay was using raw cells (264.7).

1.7 Research Contents

This thesis includes nine chapters as follows:

1.7.1 Chapter 1 (General introduction)

Chapter 1 is a general introduction, providing background information on Basidiomycota, pleurotoid mushrooms, their cultivation, nutritional content, and bioactivities. It also includes the research objectives, scope, and content of this thesis.

1.7.2 Chapter 2 (Literature review)

Chapter 2 provides the topography of Laos and Thailand, check list studies of pleurotoid mushrooms in Laos and Thailand, check list studies of pleurotoid mushroom cultivation in Laos and Thailand, check list studies of nutrition and check list studies of bioactive compounds from pleurotoid mushrooms in Laos and Thailand.

1.7.3 Chapter 3 (General material and methodology)

Chapter 3 provides an overview of the material and methodology of the research, collection sites, sample collection and morphological identification, data analysis of diversity of pleurotoid mushrooms, DNA extraction and sequencing, sequence alignment and phylogenetic analysis of pleurotoid, sequence alignment and phylogenetic analyses of *Crepidotus*, *Hohenbuehelia*, and *Pleurotus* species, *P. djamor* var. *fuscopruinosus*, *P. tuber-regium*, cultivation of wild *Pleurotus* strains: *P. djamor* var. *fuscopruinosus* and *P. giganteus* with statistical analysis. In addition, nutritional analysis and biological activity of two strains of *P. djamor* var. *fuscopruinosus* and *P. giganteus* were provided.

1.7.4 Chapter 4 (Species diversity of pleurotoid mushrooms (Agaricales) in selected community forests in Thailand)

Chapter 4 provides the diversity of pleurotoid mushrooms in the different collection sites, the diversity of the species of pleurotoid mushrooms in all the collecting sites, the percentage of frequency of occurrence (%) of pleurotoid species, and the diversity of the pleurotoid genus in Thailand.

1.7.5 Chapter 5 (Taxonomy and phylogeny of *Crepidotus* collected from Thailand)

Chapter 5 provides the taxonomy and phylogeny of six new species and two new records of *Crepidotus* from Thailand.

1.7.6 Chapter 6 (Taxonomy and phylogeny of *Hohenbuehelia* collected from Thailand)

Chapter 6 also provides the taxonomy and phylogeny of two new species and a species noted on *H. titris*.

1.7.7 Chapter 7 (Taxonomy and phylogeny of *Pleurotus* collected from Laos and Thailand)

Chapter 7 also provides the taxonomy and phylogeny of three new records of *Pleurotus* from Laos, and a new record of *P. djamor* var. *fuscopruinosus* from Thailand.

1.7.8 Chapter 8 (Cultivation, nutritional value, and biological activities of two wild *Pleurotus* strains)

Chapter 8 provides the cultivation of the wild *P. giganteus* strain that included the effect of grain media on spawning production, the effect of agricultural waste on

fruit production, and the effect of soil casing. The cultivation of wild *P. djamor* var. *fuscopruinosus* strain included the effect of grain media on spawning production, the effect of different agricultural wastes on mycelium growth, and a fruiting test is also provided. Also provides the determination of the nutritional content of two cultivated *Pleurotus* species; biological activity of two wild *Pleurotus* species included antioxidant (DPPH scavenging activities and ABTS scavenging activity), anticancer (lung cancer cell A549 and colorectal cancer cells SW480), Cytotoxicity (raw cell 264.7), and antidiabetic (α-glucosidase and glucose consumption assay).

1.7.9 Chapter 9 (Conclusions)

This section of the thesis presents conclusions on the diversity of pleurotoid mushrooms in Laos and Thailand, their cultivation, nutritional composition and biological activities. Furthermore, it discusses the advantages and limitations of the study, proposes suggestions for future research, and includes a list of my related publications.

CHAPTER 2

LITERATURE REVIEW

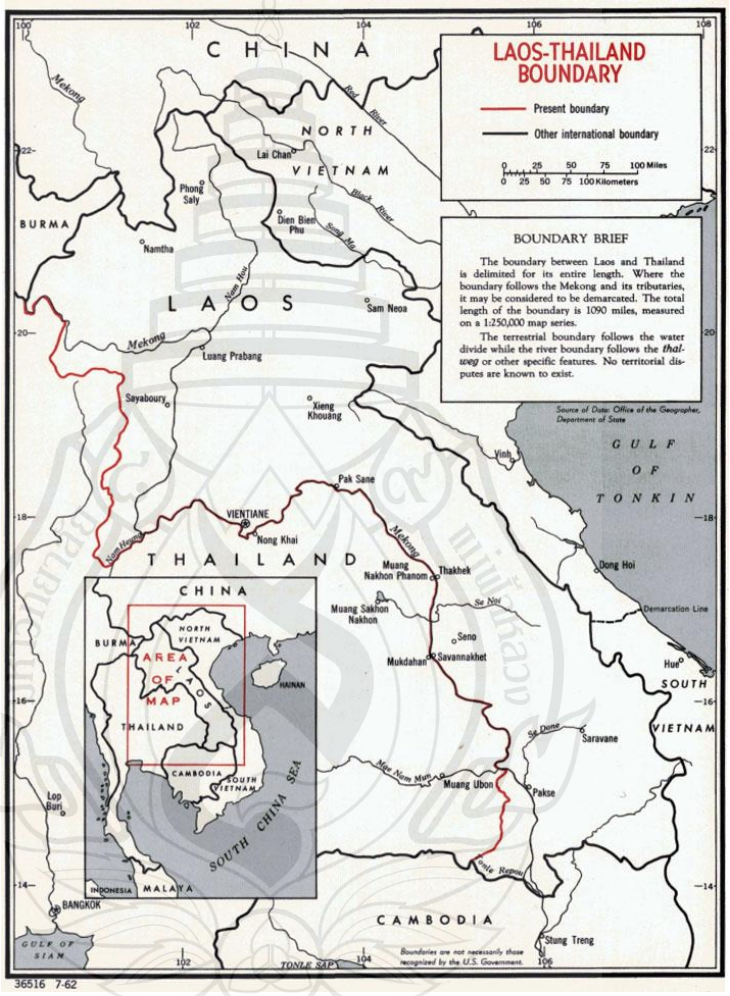
2.1 Topography of Laos and Thailand

Laos is a tropical country located in the Indochinese peninsula in Southeast Asia. A total of area 236,800 square kilometres covered 47% of the forest and 80% mountainous. Laos has a good landscape with highly mountainous areas covered with thick forests and has the highest mountain, the 2,800-meter Phou Bia in Xieng Khouang province, and three large plateaus. Laos has many rivers and streams, of which the Mekong River is the largest river that flows through 1,835 km of the country from north to south and shares with a part of Thailand (Byung-wook et al., 2004). Laos has been divided into three parts, the north, middle, and south, with three seasons as northern Thailand. there are borders with six countries, Cambodia, China, Myanmar, Thailand, and Vietnam (Stuart-Fox, 1998; Duckworth et al., 1999). Laos is country rich in natural resources and abundant biodiversity (Uitto 2011; Mijović, 2014; UNESCAP, 2005).

Thailand is located at a strategic crossroads in Southeast Asia. A total area of 513,120 square kilometres with a water area of 2,230 square kilometres and a land area of 510,890 square kilometres (Lepoer, 1987; Baimai, 2010). Approximately the forest area covers at least 33% of the total area of the country. Thailand is rich in biological diversity with abundant plants and animals. North of the part is well known for hill tribe cultures with lush forests; there are 15 mountain ranges, and the highest point location is Doi Inthanon, 2,576 m, with the lowest point location being the Gulf of Thailand (Office of Natural Resources and Environmental Policy and Planning, 2009). There are five geographical regions: northern, north-eastern, central, eastern, western, and southern. Thailand shares a border with Cambodia, Laos, Myanmar, and Malaysia (Gardner & Sidisunthorn, 2000). The climate is tropical, with two seasons influenced by the southwest monsoon and the northeast monsoon; therefore, the climate of the north region has three seasons (a cool dry season, a hot dry season, and a warm wet

season), while in the south region, there is a tropical rainforest climate with only two seasons (dry and rainy) (Gardner & Sidisunthorn, 2000).

According to Laos and Thailand share a long border and also together under the Greater Mekong Subregion border (Figure. 2.1). Furthermore, the climates of the north region are similar climates of Laos. Consequently, the diversity of pleurotoid mushrooms in these regions is interesting.



Source Wikimedia Commons contributors (n.d.)

Figure 2.1 Sharing a border between Laos and Thailand and together under the border of the Greater Mekong Sub-region

2.2 Diversity of Pleurotoid Mushrooms in Laos and Thailand.

Pleurotoids are one of the most diverse and large groups of basidomycetes comprising many important edible and medicinal species. Studies on mushrooms were lacking in Laos. Some edible pleurotoid species are commonly sold in local markets but have not been scientifically identified. The two pleurotoid species, *Pleurotus ostreatus* and *P. giganteus*, were first recorded in central Laos (Sysouphanthong et al., 2017). However, these specimens were identified only on the basis of their macromorphological characteristics. Later, Læssøe et al. (2018) produced a field guide of edible, poisonous, and medicinal mushrooms from northern Laos. They reported five edible pleurotoid species, including *Lentinus polychrous* Lev., *L. squarrosulus* Mont., and *L. sajor-caju* (Fr.) Fr., *Pleurotus ostreatus* f. *ostreatus* (Jacq.) P. Kumm., *P. giganteus*, and *Schizophyllum commune* Fr. Studies on mushrooms in Laos have increased since 2018 due to the publication of numerous new species and records (Læssøe et al., 2018; Eberhardt et al., 2020; Phonemany et al., 2021b; Sysouphanthong et al., 2020). In recent years, Phonemany et al. (2021b) discovered *P. djamor* (Rumph. ex Fr.) Boedijn for the first time in this country. Łuczaj et al. (2021) reported five pleurotoid species in north Laos (Luang Prabang province). In total, 12 species of pleurotoid mushrooms belonging to three genera and three families are recorded in Laos (Table 2.2). According to the literature review of the pleurotoid group in Laos, found that the study of pleurotoid is limited in number, only few species reported were reported. Therefore, the pleurotoids group is interesting and there is still no checklist of diversity of this group.

Thailand has a rich diversity of mushroom species (Thongbai et al., 2018; Vadthanarat et al., 2021). Many new basidiomycetes species have been observed in the region, and many studies on the biodiversity of pleurotoid species have been reported (Sakonrak, 2017; De Crop et al., 2018; Suwannarach et al., 2022; Phonemany & Thongklang, 2023; Sysouphanthong et al., 2023). Around 1,978 species of mushrooms, including 59 pleurotoid species in different families, were listed in Thailand in 2010, according to the checklist of Chandrasrikul et al. (2011). However, these species did not provide detailed morphological descriptions or molecular data to confirm their

identities. Therefore, the lists of pleurotoid species reported by Chandrasrikul et al. (2011) should be reviewed and updated again. Several genera of pleurotoid were reported, and some new species were introduced in subsequent years. Karunarathna et al. (2011a) introduced new species of *Lentinus roseus* Karun., K.D. Hyde & Zhu L. Yang [current name as *Panus roseus* (Karun., K.D. Hyde & Zhu L. Yang) N. Vinjusha & T.K.A. Kumar], *L. concentricus* Karun., K.D. Hyde & Zhu L. Yang, and *L. megacystidiatus* Karun., K.D. Hyde & Zhu L. Yang. Sysouphanthong et al. (2023) reported five species: *Lentinus*, *L. arcularius* (Batsch) Zmitr., *L. badius* (Berk.) Berk., *L. polychrous*, *L. sajor-caju* and *L. squarrosulus*. Three other species of *Clitopilus* have been reported in Thailand, such as *Clitopilus doimaesalongensis* Jatuwong, Karun. & K.D. Hyde, *C. chalybescens* T.J. Baroni & Desjardin, and *C. lampangensis* J. Kumla, N. Suwannarach & S. Lumyong (Jatuwong et al., 2017; Kumla et al., 2019). *Crepidotus asiaticus* Guzm-Dáv., C.K. Pradeep & T.J. Baroni was also first recorded in Thailand (Guzmán-Dávalos et al., 2017). Another new species, *Pleurotus sirindhorniae* Suwannar., Kumla & Lumyong was introduced in this country (Suwannarach et al., 2022). Currently, a new species, *H. nakhonphanomensis* Somrith., Sommai, Khamsuntorn & Pinruan, has been recorded (Somrithipol et al., 2025). In total, 30 species of pleurotoid mushrooms, belonging to eight families and 11 genera have been documented in Thailand (Table 2.3).

Table 2.1 Pleurotoid mushrooms checklist in Thailand based on studies of Chandrasrikul et al. (2011)

Family	Genera/Species	Common name (English)
Auriscalpiaceae	***Lentinellus*** P. Karst.	
	L. cochleatus (Pers. Ex Fr.) Karsten	Aniseed cockleshell
Crepidotaceae	***Crepidotus*** (Fr.) Staude	
	C. applanatus (Pers.) P. Kumm.	Little shell, Flat oyster ling
	C. citrinus P. Larsen	N/A
	C. crocophyllus (Berk.) Sacc.	N/A
	C. herbarum Peck	Little white crep
	C. maculans Hesler & Smith	N/A
	C. mollis (Schaeff. ex Fr.) Stande	Peeling oysterling
	C. boninensis (Hongo) E. Horak & Desjardin	Pink Crepidotus
	C. variabilis (Pers. ex Fr.) Kummer	Variable oysterling, Varied Slipper, White cap

Table 2.1 (continued)

Family	Genera/Species	Common name (English)
	C. versutus (Peck) Saccardo	N/A
Entolomataceae	***Clitopilus*** (Fr. ex Rabenh.) P. Kumm.	
	C. apalus (Berk. & Br.) Petch	N/A
	C. chalybescens T.J. Baroni & Desjardin	N/A
	C. prunulus (Scop. ex Fr.) Kummer	Sweetbread mushroom
Marasmiaceae	***Campanella*** Henn.	
	C. junghuhnii (Mont.) Singer	N/A
	C. simulans (Pat.) Singer	N/A
	Chaetocalathus Singer	
	C. africanus (Pat.) Singer	N/A
	C. liliputianus (Mont.) Sing	N/A
Mycenaceae	***Panellus*** P. Karst.	
	P. pusillus (Pers. ex Lév.) Burds. & O.K. Mill.	N/A
	P. stipticus (Bull. ex Fr.) Karsten	Bitter oysterling
Phyllotopsidaceae	***Pleurocybella*** Singer	
	P. porrigens (Pers.) Singer	Angel's wings
Pleurotaceae	***Hohenbuehelia*** Schulzer	
	H. petaloides (Bull. ex Fr.) Schulzer	Leaf like oyster, Shoehorn oyster mushroom
	H. reniformis (G. Mever & Fr.) Sing.	N/A
	Pleurotus (Fr.) P. Kumm.	
	P. cystidiosus O.K. Miller	Abalone mushroom
	P. djamor (Fr.) Boedijn.	N/A
	P. dryinus (Pers. ex Fr.) Kummer	Veiled oyster mushroom
	P. flabellatus (Berk. & Br.) Sacc	Fat fungus
	P. ostreatus (Jacq. ex Fr.) Kummer	Oyster mushroom
	P. pulmonarius (Fr.) Quél.	Pale oyster
Gloeophyllaceae	***Neolentinus*** Redhead & Ginns	
	N. lepideus (Buxb. ex Fr.) Redhead & Ginns	Scaly sawgill
Panaceae	***Panus*** Fr.	
	P. ciliatus (Lév.) T.W. May & A.E. Wood	N/A

Table 2.1 (continued)

Family	Genera/Species	Common name (English)
	P. conchatus (Bull.) Fries	Lilac oysterling
	P. luteolus Massee	N/A
	P. tephroleucus (Mont.) T.W. May & A.E. Wood	N/A
Schizophyllaceae	***Schizophyllum*** Fr.	
	S. commune Fr	Common split gill
Tapinellaceae	***Tapinella*** E.-J. Gilbert	
	T. atrotomentosa (Batsch) Šutara	Velvet rollrim

Note N/A means no data available.

Table 2.2 Checklist of pleurotoid mushrooms in Laos

Family	Genera/ Species	Common name (English)	References
Pleurotaceae	***Pleurotus*** (Fr.) P.Kumm.		
	P. djamor (Rumph. ex Fr.) Boedijn	Pink oyster mushroom	Phonemany et al. (2021b)
	P. eryngii (DC.) Quél.	Eryngi, King Oyster Mushroom, King Brown Mushroom	Łuczaj et al. (2021)
	P. giganteus Berk.) Karun. & K.D. Hyde	Giant oyster mushroom	Sysouphanthong (2017)
	P. ostreatus (Jacq.) P. Kumm.)	Oyster mushroom	Sysouphanthong (2017)
	P. aff. *ferulaginis* Zervakis, Venturella & Cattar	Oyster mushroom	Łuczaj et al. (2021)
Polyporaceae	***Lentinus*** Fr.		
	L. polychrous Lev.	N/A	Rattanamalee and Rattanamalee (2012), Łuczaj et al. (2021)
	L. sajor-caju (Fr.) Fr.	N/A	Læssøe et al. (2018)
	L. squarrosulus Mont.	N/A	Læssøe et al. (2018), Łuczaj et al. (2021)
Schizophyllaceae	***Schizophyllum*** Fr.		

Table 2.2 (continued)

Family	Genera/ Species	Common name (English)	References
	S. commune	cockle mushroom	Læssøe et al. (2018), Łuczaj et al. (2021)

Note N/A means no data available.

Table 2.3 Additional checklist of pleurotoid mushrooms in Thailand

Family	Genera/Species	Common name (English)	References
Crepidotaceae	***Crepidotus*** (Fr.) Staude		
	Crepidotus asiaticus Guzm.-Dáv., C.K. Pradeep & T.J. Baroni	Little shell, flat oysterling	Guzmán-Dávalos et al. (2017)
Entolomataceae	***Clitopilus*** (Fr. ex Rabenh.) P. Kumm.		
	C. chalybescens T.J. Baroni & Desjardin	N/A	Baroni et al. (2001), Jatuwong et al. (2017)
	C. doimaesalongensis Jatuwong, Karun. & K.D. Hyde	N/A	Jatuwong et al. (2017)
	C. peri (Berk. & Broome)	N/A	Raj and Manimohan (2018)
	C. lampangensis J. Kumla, N. Suwannarach & S. Lumyong	N/A	Jatuwong et al. (2017), Kumla et al. (2019)
Marasmiaceae	***Marasmius*** Fr.		
	M. tenuissimus (Jungh.) Singer	N/A	Sakonrak (2017)
Omphalotaceae	***Anthracophyllum*** Ces.		
	A. nigritum (Lév.) Kalchbr.	Grape-coloured fan	Sakonrak (2017)
Pleurotaceae	***Hohenbuehelia*** Schulzer		
	H. flabelliformis Phonemany & Raspé	N/A	Phonemany et al. (2023)
	H. grisea (Massee) E. Horak	N/A	Sandargo et al. (2018b)
	H. lageniformis Phonemany & Raspé	N/A	Phonemany et al. (2023)
	H. nakhonphanomensis Somrith., Sommai. Khamsuntorn & Pinruan	N/A	Somrithipol et al. (2025)

Table 2.3 (continued)

Family	Genera/Species	Common name (English)	References
	H. reniformis (G.Mever & Fr.) Sing.	N/A	Phonemany et al. (2023)
	H. tristis G. Stev.	N/A	Phonemany et al. (2023)
	Pleurotus (Fr.) P. Kumm.		
	P. giganteus (Berk.) Karun. & K.D. Hyde	Giant oyster mushroom	Phonemany and Thongklang (2023)
	P. sirindhorniae Suwannar., Kumla & Lumyong	N/A	Suwannarach et al. (2022)
Polyporaceae	***Favolus*** P. Beauv.		
	F. grammocephalus (Berk.) Imazeki	palm leaf fan	Sakonrak (2017)
	Lentinus Fr.		
	L. arcularius (Batsch) Zmitr.	N/A	Sysouphanthong et al. (2023)
	L. badius (Berk.) Berk.	N/A	Sysouphanthong et al. (2023)
	L. concentricus Karun., K.D. Hyde & Zhu L. Yang	N/A	Karunarathna et al. (2011a)
	L. megacystidiatus Karun., K.D. Hyde & Zhu L. Yang	N/A	Karunarathna et al. (2011a)
	L. polychrous Lev.	N/A	Rattanamalee and Rattanamalee (2012), Sysouphanthong et al. (2023)
	L. roseus Karun., K.D. Hyde & Zhu L. Yang	N/A	Karunarathna et al. (2011a)
	L. sajor–caju (Fr.) Fr.	N/A	Sysouphanthong et al. (2023)
	L. squarrosulus Mont.	N/A	Sysouphanthong et al. (2023)
Panaceae	***Panus*** Fr.		

Table 2.3 (continued)

Family	Genera/Species	Common name (English)	References
	Pa. sribuabanensis J. Kumla, N. Suwannarach & S., Lumyong	N/A	Kumla et al. (2023)
Russulaceae	***Lactifluus*** (Pers.) Roussel		
	La. auriculiformis Verbeken & F. Hampe	N/A	De Crop et al. (2018)
	La.s raspei Verbeken & De Crop	N/A	De Crop et al. (2018)
	La. uyedae (Singer) Verbeken	N/A	De Crop et al. (2018)
Schizophyllaceae	***Schizophyllum*** Fr.		
	S. commune Fr.	Common split gill	Phuket et al. (2019)

Note N/A means no data available.

2.3 Cultivation of Pleurotoid Mushrooms in Laos and Thailand

Mushroom cultivation is a viable alternative to traditional employment, it is profitable for growers and essential to an economic system, since it can be produced from a variety of agricultural wastes readily available around the world, and it can aid in the environmentally friendly recycling of agricultural waste and its transformation into high protein foods (Okuda, 2022). Nowadays, cultivation techniques have been developed as a result of many studies; there are many pleurotoid species that have been considered edible species; and some species have been cultivated such as *Hohenbuehelia* species (*H. karrara* Grgur. and *H. petaloides* (Bull.) Schulzer), *Lentinus* spp. (*L. squarrosulus*), *Neolentinus* spp. (*N. ponderosus* (O.K. Mill.) Redhead & Ginns), *Panellus* spp. (*P. stipticus* (Bull.) P. Karst.), *Pleurotus* spp. (*P. ostreatus*), *Pleurocybella* spp (*P. porrigens* (Pers.) Singer.), *Panus* spp. (*P. conchatus* (Bull.) Fr.), and *Schizophyllum* spp. (*S. commune*) (Barrett et al., 2009; Jang et al., 2010; Suzuki et al., 2011; Dasanayaka & Wijeyaratne, 2017; Woo et al., 2018; Raman et al., 2021; Dedousi et al., 2023; Sysouphanthong et al., 2023).

The primary substrate for the cultivation of pleurotoid is sawdust; which can be used alone or mixed with other materials such as paper waste (Girmay et al., 2016);

pulp sludge, cereals, and several agricultural wastes such as cocoa shell waste (Bermúdez et al., 2001), corn, corn cobs (Dias et al., 2003; Naraian et al., 2009), cotton waste (Chang et al., 1981), and cottonseed (Girmay et al., 2016). Other ingredients can be diverse plant leaves such as banana leaves (de Carvalho et al., 2012), grass plants (Girmay et al., 2016), straw (Mehta et al., 1990), sugarcane bagasse (Ragunathan et al., 1996), wheat straw (Zhang et al., 2002), and coffee residues (Sözbir et al., 2015; Ramalho et al., 2018). Depending on the type of pleurotoid mushrooms that could be used for cultivation on both composted and non-composted agricultural wastes, for example, cultivation on composted substrates such as *P. ostreatus* can be done on fermented rice straw or a short-term composted substrate (Jang et al., 2016; Yang et al., 2022). For cultivation on non-composted substrates, genera such as *Panus*, *Pleurotus*, *Lentinus*, and *Schizophyllum* are commonly utilized due to their ability to efficiently degrade lignocellulosic materials and thrive on untreated organic matter (Philippoussis & Diamantopoulou, 2011; Suwannarach et al., 2022). The yield of mushroom species depends on the availability of nutrients in their environment. The additives used for pleurotoid cultivation include rice bran, wheat bran, sugar, calcium carbonate ($CaCO_3$), and calcium sulphate ($CaSO_4$) (Nadeem et al., 2014). The composition of ingredients varies depending on the type of mushroom species. Among pleurotoid species, *Pleurotus* is the most cultivated mushroom for food purposes (Cohen et al., 2002). Ten available *Pleurotus* species reported for commercial use include *P. citrinopileatus*, *P. cystidiosus*, *P. cornucopiae*, *P. djamor* var. *djamor* (Rumph. ex Fr.) Boedijn, *P. eous* (Berk.) Sacc., *P. djamor* var. *roseus* Corner, *P. eryngii*, *P. ostreatus*, *P. pulmonarius*, and *P. tuber-regium* (Samsudin & Abdullah, 2019; Raman et al., 2021).

Currently, there is no scientific report on the production of pleurotoid mushrooms in Laos. However, there are four species of these mushrooms that have been grown for consumption or sale in local markets such as *Lentinus polychrous* (Lao name as Het Bot), *L. squarrosulus* (Lao name as Het Khao), *Pleurotus* aff. *ferulaginis*, *P. pulmonarius* (Lao name is Het Nang Lom). In addition, some pleurotoid species were cultivated in Thailand, such as *Lentinus* species and *Pleurotus* species, which included the best growing spawn, the suitable substrate, and the suitable conditions for the growth of the fruiting body.

2.3.1 Cultivation of *Lentinus*

Some *Lentinus* species were successfully cultivated such as *L. arcularius*, *L. crinitus*, *L. levis*, *L. polychrous*, *L. sajor-caju*, *L. swartzii*, *L. tigrinus*, and *L. squarrosulus* (Lechner & Albertó, 2007; Hussein et al., 2017; Lau & Abdullah, 2017; Meniqueti et al., 2021; Fabros et al., 2022; Sysouphanthong et al., 2023). The growth of these species has been optimised in suitable media, pH, and temperature for optimum yield (Okhuoya et al., 2005; Lechner & Papinutti, 2006; Lechner & Albertó, 2007; Salmones & Gutiérrez-Lecuona, 2008; Ratanapongleka & Phetsom, 2014; de Araújo Conceição et al., 2017; Hussein et al., 2017). The mycelium of *Lentinus* can grow at a pH of 6.5–7.0 and a temperature of 30±2°C (Dulay et al., 2012; Dulay et al., 2017). Sorghum is commonly used for spawning in Thailand, and other materials can also be used for spawning preparation, e.g., millet, rice paddy, rice berry, sawdust, straw, and wheat (Phonemany et al., 2021a). Another suitable medium for the growth of *L. polychrous* mycelium was sweet potato dextrose broth (Kupradit et al., 2020). In addition, the additives for *Lentinus* species can grow well in culture media such as ammonium chloride, fructose, malt extract, starch, sucrose and yeast extract with a C/N ratio of 10:1, 10:1, and 40:1 (Dulay et al., 2020). Two species of *Lentinus* species are cultivated and widely consumed in northern Thailand, such as *L. polychrous* and *L. squarrosulus* (Petcharat, 1995; Kupradi et al., 2017; Kupradi et al., 2020). Rubber sawdust (*Hevea brasiliensis*) was used as the main substrate to grow most *Lentinus* species (Sysouphanthong et al., 2023). There are four wild strains such as two strains of *L. arcularius*, *L. squarrosulus*, and *L. sajor-caju* were grown on a laboratory scale on sawdust substrates and the yields of *L. squarrosulus* were higher than other strains that have been observed to produce fruiting body, fresh weight and biological efficiency (Sysouphanthong et al., 2023). Furthermore, sawdust can be mixed with other agricultural waste, such as cassava bagasse (25%). The sawdust substrate can be used to cultivate *L. squarrosulus*, and from 20 bags of substrate, the mushrooms yielded 1.151 kg (Kupradi et al., 2017). Some studies on the cultivation of *Lentinus* species in Thailand are listed in Table 2.4.

2.3.2 Cultivation of *Pleurotus*

Among the mushrooms in the pleurotoid group, *Pleurotus* species have been known to be cultivated in local communities of both Laos and Thailand (Kang et al., 2004; Thawthong et al., 2014). The commercial species are *P. ostreatus* and *P. eryngii* (Melanouri et al., 2022). An attempt was made to isolate and culture a wild strain of *P. giganteus*. Later, Kumla et al. (2013) discovered *P. giganteus* in northern Thailand and tested the *in vitro* cultivation conditions that provide the information to cultivate this species for the first time in Thailand. Soytong and Asue (2014) used garden soil supplemented with 1% nano-KS1 to maintain moisture and trigger the growth of fruiting bodies to obtain a higher yield for *P. giganteus*. Phonemany and Thongklang (2023) investigated some agricultural waste that can be used as substrates to grow *P. giganteus*, such as corn cobs, rice straw, and sugarcane bagasse mixed with sawdust as the main substrate. Sawdust mixed corn cobs obtained a high yield compared with the yields from sawdust substrates. Furthermore, the impact of soil casing on the development of fruiting bodies was examined and the results obtained a higher fresh mushroom weight when using 25% humus soil, 35% lime sand, 25% calcium carbonate and 15% peat (Phonemany & Thongklang, 2023). Some agricultural wastes were also used to produce *P. ostreatus*, providing a new alternative substrate for its cultivation (Thongklang & Luangharn, 2016). The studies on the cultivation of *Pleurotus* species in Thailand are listed in Table 2.4.

Table 2.4 Cultivable pleurotoid species in Thailand on different substrates

Species	Substrates	Biological Efficiency (%)	Wet yields (g/kg)	References
L. arcularius	sawdust	50.90 ± 5.48	42.50 ± 00	Sysouphanthong et al. (2023)
L. sajor–caju	sawdust	29.94 ± 20.00	25 ± 00	Sysouphanthong et al. (2023)
L. squarrosulus	sawdust	59.88 ± 10.00	50 ± 00	Sysouphanthong et al. (2023)
	sawdust (75%) + cassava bagasse (25%)	N/A	1.515 + 00	Kupradi et al. (2017)
P. giganteus	sawdust	11.40 ± 5.46 (primordia)	N/A	Kumla et al. (2013)
	sawdust	42.20 ± 10.36	N/A	Phonemany and Thongklang (2023)
	sawdust + corn cobs	45.45 ± 4.54	N/A	Phonemany and Thongklang (2023)
	sawdust + bagasse	10.55 ± 3.45	N/A	Phonemany and Thongklang (2023)
	sawdust + rice straw	11.51 ± 4.61	N/A	Phonemany and Thongklang (2023)
P. ostreatus	sawdust	N/A	162.50 ± 28.72	Thongklang and Luangharn (2016)
	sawdust (75%) + cassava bagasse (25%)	N/A	2.670 + 00	Kupradi et al. (2017)
	sawdust + corn cobs	N/A	175.00 ± 64.55	Thongklang and Luangharn (2016)
	sawdust + rice straw	N/A	262.50 ± 45.73	Thongklang and Luangharn (2016)

Note N/A means no data available.

2.4 Nutrition and Therapeutic Efficacy from Pleurotoid Mushrooms in Laos and Thailand

More than 2,000 edible mushrooms species are widely accepted for human consumption and have been documented to be used as food (Li et al., 2021), and there are many studies that have been analysing the nutritional composition of edible pleurotoid species and some species were done are shown in Table 2.5. An edible pleurotoid species not only have a high nutritional value but also have shown a number of bioactive compounds such as medicinal properties which included antiallergic, antibacterial, antifungal, anti-inflammatory, antioxidative, antiviral, immunomodulating, antidepressive, antihyperlipidemic, antidiabetic, digestive, hepatoprotective, neuroprotective, nephroprotective, osteoprotective, and hypotensive activities.

The bioactive of pleurotoid mushrooms were tested *in vitro* and *in vivo* (Venturella et al., 2021). For example, some well-known species of pleurotoid is the *Pleurotus* species, which is considered a functional food as it has a positive effect on humans in several ways, studies on *Pleurotus* are important not only because they are edible and easily cultivated but also because they produce valuable primary and secondary metabolites (Renuga Devi & Krishnakumari, 2015) with bioactivities such as hepatoprotective (Zhang et al., 2016a), anti-oxidant (Jayakumar et al., 2011; Zhang et al., 2016b), antimicrobial (Schillaci et al., 2013), antiviral, anti-cancer, anti-inflammatory, anti-ageing, anti-tumour, anti-mutagenic, anti-lipidemic, hepatoprotective, anti-hyperglycaemic, hypotensive, and immunostimulant properties with nutraceutical and pharmaceutical applications (Gregori et al. 2007; Khan & Tania, 2012; Wahab et al., 2014). Moreover, *P. djamor* was able to inhibit Gram-negative bacteria such as *Vibrio cholera, Escherichia coli,* and *Pseudomonas* sp. (Suresh et al., 2017), *Pleurotus eryngii* var. *eryngii*, *P. eryngii* var. *ferulae, P. eryngii* var. *elaeoselini*, and *P. nebrodensis* were inhibited activity of some bacterial strains of medical relevance such as *Staphylococcus aureus, S. epidermidis, Pseudomonas aeruginosa*, and *Escherichia coli*. (Schillaci et al., 2013). Furthermore, β-glucans in *P. ostreatus* have been clinically identified to possess immunostimulant properties (Morris et al.,

2011). *Pleurotus giganteus* was considered to be a nutraceutical that can be developed for the mitigation of neurodegenerative diseases according to its bioactive compounds contained (Phan et al., 2012). Several species of the genus *Pleurotus* have been used in traditional medicine for approximately 35 disorders or diseases (Guzman, 2000). Therefore, the properties of pleurotoid species are beneficial for humans, and they are becoming increasingly attractive as sources for the development of new drugs and the development of functional foods.

The study of nutrition and bioactive compounds from pleurotoid mushrooms in Laos was lacking and there is no report yet in this country. The bioactive compounds of some pleurotoid species were investigated in Thailand. There are some pleurotoid species reported that were done. For example, extraction from *Hohenbuehelia grisea* strain MFLUCC 12-0451 was to isolate the new derivatives 1−3 with a decrease in cytotoxicity and antimicrobial activities (Sandargo et al., 2018a), and the 4-hydroxypleurogrisein isolate from *H. grisea* that can inhibit hepatitis C virus infectivity in mammalian liver cells (Sandargo et al., 2018b). The extraction of *Lentinus polychrous*, *L. squarrosulus*, *Pleurotus ostreatus*, and *P. pulmonarius* were investigated for its antibacterial activity against food-borne pathogenic bacteria, i.e. *Bacillus cereus*, *Enterobacter aerogenes*, *Escherichia coli*, *Micrococcus luteus*, *Proteus vulgaris*, *Salmonella typhimurium* and *Staphylococcus aureus* (Chaiharn et al., 2018). In addition the extraction of *P. eryngii* was tested for the killing of larvae and the attraction of adult mosquito vectors, and found that the extraction can attract adult mosquitoes (Chaiphongpachara et al., 2018) and the crude mycelial extracts of *C. chalybescens* have a potential for further development in antibacterial and antioxidant applications (Jatuwong et al., 2016). However, limited studies have been done on the bioactivities of pleurotoid species. According to the literature review this is a large group, which in some family is still lacking a study.

Clitopilus species were known to produce physiologically active chemicals in their basidiomata. For example, pleuromutilin was a diterpene synthesised from *C. passeckerianus* (Pilát) Singer that served as the main component of the antibiotic retapamulin® (Alberti et al., 2017; Gargano et al., 2017). There are four species of *Clitopilus* reported in Thailand (Baroni et al., 2001; Jatuwong et al., 2017; Raj & Manimohan, 2018; Kumla et al., 2019; Suwannarach et al., 2022). *Clitopilus*

chalybescens was first described from Lampang province by Baroni et al. (2001), and this Thai strain MFLUCC 13–0809 of *C. chalybescens* has been investigated for its bioactivities by Jatuwong et al. (2016), who found that mycelial extracts of this species have the potential to be further developed for use in antibacterial and antioxidant drugs.

Polysaccharides and polyphenols were found in the mycelium extract of *Hohenbuehelia* species (Wang et al., 2018; Wang et al., 2019). These bioactive compounds have been found to exhibit antioxidant activity in addition to having anticancer and antiviral properties (Ji et al., 2012; Sandargo et al. 2018a, b). The extract of *H. grisea* strain MFLUCC 12-0451 produced the new derivative 1−3 with decreased cytotoxicity and antimicrobial activities (Sandargo et al., 2018a). Another compound, 4-hydroxypleurogrisein, isolated from *H. grisea*, inhibits the infectivity of the hepatitis C virus in mammalian liver cells (Sandargo et al., 2018b).

Many species of *Lentinus* have been cultivated for consumption because of their medicinal properties (Okhuoya et al., 2005; Adesina et al., 2011). There are eight species of *Lentinus* reported in Thailand (Karunarathna et al., 2011; Sysouphanthong et al., 2023). *Lentinus* species offer high nutritional value and medicinal properties, such as antioxidant, antibacterial, antitumor, antiviral, immune-modulating, and cholesterol-regulating effects (Dulay et al., 2014; Sevindik, 2018; López-Legarda, et al., 2020; Phonemany & Thongklang, 2023). Sysouphanthong et al. (2023) reported that *L. sajor-caju* and *L. squarrosulus* have biological activity to inhibit alpha-glucosidase enzyme activity.

Table 2.5 Comparison of the proximate nutritional values of some pleurotoid species

Species name	Ash (%)	Carbohydrate (g of/100 g DW)	Fat (g of/100 g DW)	Fibre (%)	Moisture (g/100 g FW or DW)	Protein (g of/100 g DW)	Reducing sugar (g of/100 g DW)	Energy (kcal/100 g)	References
Clitopilus prunulus	30.19 ± 2.50	50.66 ± 2.21	1.01 ± 0.06	N/ A	89.78 ± 1.46 (FW)	18.13 ± 0.37	N/ A	N/ A	Grangeia et al. (2011)
L. sajor-caju.	N/ A	3.58 ± 0 .67	6.14 ± 0.70	34.03 ± 0.30	N/ A	12.26 ± 0.07	N/ A	N/ A	Sysouphanthong et al. (2023)
L. squarrosulus	N/ A	4.18 ± 0 .15	5.73 ± 0.14	9.48±0.04	N/ A	14.03 ± 0.27	N/ A	N/ A	Sysouphanthong et al. (2023)
L. squarrosulus var. *squarrosulus*	3.12 ± .2	19.14 ± .01	N/ A	6.1 ± .1	86.2 ± 0.01 FW; 13.8 ± 0.01 DW	18.77 ± .02	5.39 ± .08	N/ A	Ao and Deb (2019)
L. tigrinus	3.41 ± .2	52.4 ± 0.5	2.1 ± 0.4	17.4 ± 0.5	73.7 ± 0.04 FW; 26.3 ± 0.04 DW	31.85 ± .03	30.1 ± 0.3	N/ A	Dulay et al. (2014), Ao and Deb (2019)
Pleurotus cornucopiae	6.58 ± 0.0	71.05 ± 0.0	1.30 ± 0.0	N/ A	9.07 ± 0.0	11.08 ± 0.0	N/ A	N/ A	Landingin et al. (2021)
P. cystidiosus	6.30 ± 0.00	N/ A	2.05 ± 0.0	20.05 ± 0.0	91.13 ± 0.0	15.68 ± 0.0	N/ A	N/ A	Hoa and Wang (2015)
P. djamor	0.87 ± 0.22	N/ A	0.17 ± 0.01	3.10 ± 0.24	90.15 ± 0.27	0.12 ± 0.0	N/ A	N/ A	Zurbano et al. (2017)
P. eryngii	5.99 ± 0.08	N/ A	3.4 ± 0.1	N/ A	9.47 ± 0.08	16.2 ± 0.3	N/ A	N/ A	Rodrigues et al. (2015)
P. floridanus	8.69 ± 0.09	42.83 ± 2.54	2.4 ± 0.40	12.25 ± 0.30	89.23 ± 0.10	27.89 ± 0.23	N/ A	N/ A	Alam et al. (2008)
P. giganteus	5.61 ± 0.9	61.32 ± 0.0	2.98 ± 1.5	14.28 ± 0.3	15.6 ± 0.5	20.31 ± 0.8	N/ A	N/ A	Phonemany and Thongklang (2023)

Table 2.5 (continued)

Species name	Ash (%)	Carbohydrate (g of/100 g DW)	Fat (g of/100 g DW)	Fibre (%)	Moisture (g/100 g FW or DW)	Protein (g of/100 g DW)	Reducing sugar (g of/100 g DW)	Energy (kcal/100 g)	References
P. ostreatus	9.71±1.09	45.82±0.45	4.77 ± 0.08	11.65±1.52	11.96±1.44	22.45±1.15	N/ A	316.01±4.75	Igile et al. (2020)
P. tuberregium	9.66±0.02	66.13±0.33	2.24±0.02	6.44±0.02	8.88±0.01	6.65±0.35	N/ A	311.28±0.34	Onuoha and Obi-Adumanya (2010)
Schizophyllum commune	6.02 ± 0.6	5.31 ± 0.01	N/ A	N/ A	69.8 ± 0.02 (FW)	24.42 ± 0.02	N/ A	N/ A	Ao and Deb (2019)

Note FW = fresh weight; DW = dry weight; N/A = no data available.

Table 2.6 Secondary metabolites of pleurotoid species from Thailand with their bioactivities

Species	Active ingredients	Biological activities	References
Clitopilus chalybescens	The mycelial extracts	Antibacterial, Antioxidant	Jatuwong et al. (2016)
Hohenbuehelia grisea	Dihydropleurotinic acid 4-hydroxypleurogrisein Pleurotin Pleurothiazole Thiopleurotinic acid A Thiopleurotinic acid B	Antimicrobial, Hepatitis C virus	Sandargo et al. (2018a, b)
Lentinus sajor-caju	Extracts	Inhibition of alpha-glucosidase enzyme activity	Sysouphanthong et al. (2023)
L. squarrosulus	Extracts	Inhibition of alpha-glucosidase enzyme activity	Sysouphanthong et al. (2023)
Pleurotus eryngii	Extracts	Killing larvae and attracting adult mosquito vectors (*Aedes aegypti* and *Culex sitiens*)	Chaiphongpachara et al. (2018)
P. ostreatus	Extracts	Antibacterial	Chaiharn et al. (2018)
P. pulmonarius	Water extracts	Antibacterial (*Bacillus cereus*, *Enterobacter aerogenes*, *Escherichia coli*, *Micrococcus luteus*, *Proteus vulgaris*, *Salmonella typhimurium*, and *Staphylococcus aureus*)	Chaiharn et al. (2018)

CHAPTER 3

GENERAL MATERIAL AND METHODOLOGY

3.1 Sample Collection and Morphological Identification

Dried specimens from Laos were loaned from the Herbarium National of Laos (HNL) at the Biotechnology and Ecology Institute, Ministry of Science and Technology, Laos, and the collections were carried out from north to south in all parts of Laos (Figure 3.1, Table 3.1). Thai specimens were collected in three parts including northeast, northern and southern Thailand from 2019 to 2023, The map and details of each location site are shown in Figure 3.1 and Table 3.1. The fresh basidiomata were photographed in the field; date, location, habit, and habitat type were recorded. The samples were wrapped in aluminium foil and kept in the sample boxes, and those samples were notes on macromorphological characteristics in the laboratory. The outside of freshly collected specimens was cleaned to prevent contamination from other fungi. Pure cultures were isolated from fresh basidiomata by cutting context tissues using sterile forceps plated on Petri dishes with potato dextrose agar (PDA) and incubated at room temperature for 2 weeks. Pure cultures were depositedat the Mae Fah Luang University.

The macromorphological descriptions of the fresh specimens included the shape and size of the basidiomata, pileus, lamellae, stipe, context, and spore print (Figure 3.2). The technical terms followed the glossary of Vellinga and Noordeloos (2001). The colour codes were given following the colour charts of Kornerup and Wanscher (1978). The samples were dried in a hot air dryer for 24 hours at 50^{o}C until the samples were completely dried and kept separately in zip-lock plastic bags. Thai specimens were deposited in the herbarium of Mae Fah Luang University (MFLU). Lao specimens were loaned from the National Herbarium of Laos (HNL) for this study. Micromorphological characters were observed from the dried specimens including basidiospores, basidia, pleurocystidia, and cheilocystidia of the lamellae and the structure of the pileus covering (Figure 3.3). A razor blade was used to make thin sections of the specimens,

and these were mounted on slides in water (H_2O) and 3–10% of potassium hydroxide (KOH), or 1% ammoniacal Congo red. The chemical reactions were observed using Melzer's reagent and Cresyl Blue as staining reagents. The microscopic characters were studied and photographed using a compound microscope Nikon Eclipse Ni. Freehand drawings were made for the microscopic features and plates were assembled using Adobe Photoshop CS6. Fifty basidiospores per basidioma were measured inside view. The notation [x/y/z] denotes the number of basidiospores (x) measured from the number of basidiomata (y) of the number of collections (z). At least 25 basidia, pleurocystidia, cheilocystidia, and pileipellis hyphae, were observed and measured. The dimensions of microscopic structures are presented in the following format: (a–)b–c–d(−e), in which c represents the average, b the 5th percentile, d the 95th percentile, and minimum and maximum values a and e are shown in parentheses. Q, the length/width ratio, is presented in the same format. Faces of Fungi numbers (Jayasiri et al., 2015) and MycoBank (2024) numbers are provided for each new species.

3.2 Data Analysis of the Diversity of Pleurotoid Mushrooms

The percentage of occurrence frequency (% OF) for each species was calculated using the following formula.

$$\% \text{ OF} = \text{OSa} \times 100/\text{TN}$$

Notes: "OSa" is the occurrence of species A and TN is the total number of all species.

The total number of species was calculated and the final numbers were compared to determine the number of species for each locality. The species (section) diversity was calculated using Shannon's diversity index, *H´* (Shannon & Weaver, 1963).

$$H' = \sum_{i=l}^{n} P_i \, log_e \, P_i$$

Notes: $P_i = \frac{N_i}{n}$, where N_i is individual number of *i* species, *N* is individual number of all species, P_i is the proportion of *i* species, and *n* is the number of species.

Source Geology.com (n.d.)

Figure 3.1 Map of collecting sites from Laos and Thailand

Figure 3.2 Macro-morphological characteristic. 1 pileus, 2 lamellae, 3 stipes, 4 attachments, 5 margin

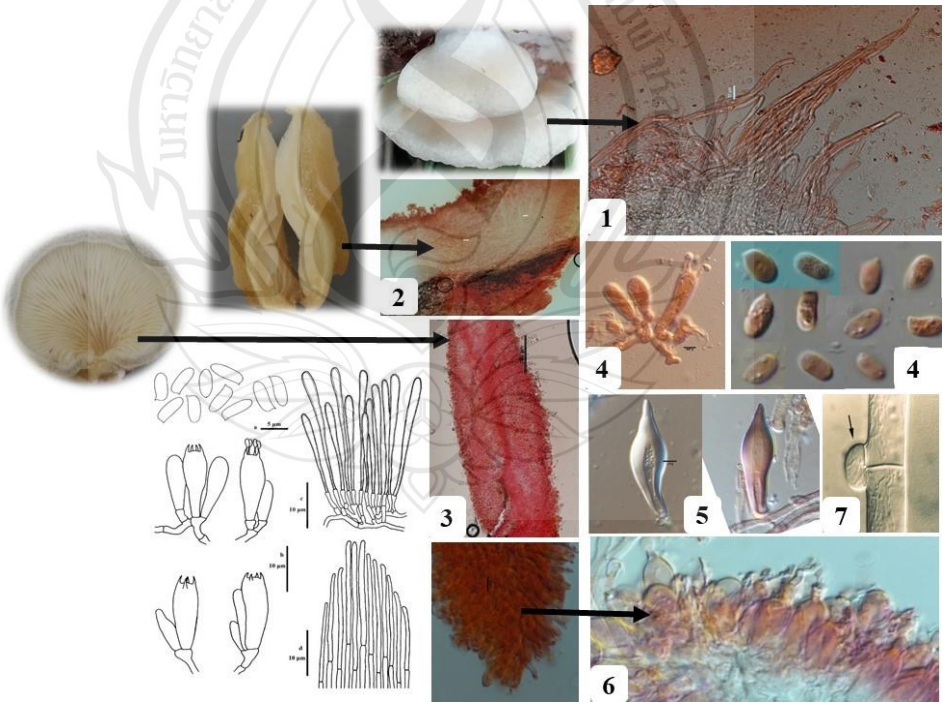


Figure 3.3 Micro-morphological characteristic. 1 Pileipellis, 2 Gelatinous layer, 3 Trama, 4 Basidia and basidiospores, 5, Pleurocystidia, 6 Cheilocystidia, 7 Clamp connections

Table 3.1 Collection sites in this study

Collection sites	
I. Chiang Mai province, northern Thailand	
1. Mae Feak village, Mae Taeng District (MF)	Mixed forest dominated by *bambusa* spp., *Dipterocarpus tuberculatus*, *Lithocarpus* spp.
2. Pha Deng village, Mae Taeng District (PD)	Deciduous mixed rain forest dominated by *Castanopsis armata*, *Castanopsis* sp., *Lithocarpus* spp. etc.
3. Tha Pha village, Mae Taeng District (TP)	Deciduous rain forest dominated by *Lithocarpus* spp., *Dipterocarpus tuberculatus*, *Dipterocarpus* sp., *Bamboo*, etc.
4. Pox Kaeo village, Mae On District (PK)	Mixed forest dominated by *Dipterocarpus* sp., *Lithocarpus polystachyus*, and other trees.
5. Huay Kaeo village, Mae On District (HK)	Mixed forest dominated by *Dipterocarpus* sp., *Lithocarpus polystachyus*, and other trees.
II. Chiang Rai province, northern Thailand	
6. Mae Fah Luang University, Muang District (MFU)	Forest dominated by *Ficus* spp.
7. Pha Ngae village, Pa Daed District (PN)	Mixed forest dominated by *Castanopsis* spp., *Lithocarpus polystachyus*, and other trees.
8. Nang Lae Nai Village, Muang District (NL)	Mixed forest, deciduous forest dominated by *Tectona grandis*, *Bamboo*, etc.
9. Mae Toe village, Mae Sa long Nok subdistrict, Mae Fah Luang District (MT)	Mixed forest dominated by *Pinus* sp., *Pinus kesiya* and coffee.
10. Huay Mae Suk, Mae Yao District (HM)	Mixed rain forest with various tree species.
11. Doi Pui, Mae Yao District (DP)	Deciduous rain forest dominated by *Lithocarpus* spp., *Dipterocarpus tuberculatus*, *Dipterocarpus* sp.
12. Sala Choeng Doi, Mae Sai District (MS)	Mixed forest, deciduous forest dominated by *Tectona* sp., *Bamboo* etc.
III. Mukdahan province, northeast Thailand	
13. Na Lak village, Dong Luang District (DL)	Deciduous rain forest dominated by *Lithocarpus* spp., *Dipterocarpus tuberculatus*, *Dipterocarpus* sp.

Table 3.1 (continued)

Collection sites	
VI. Roi Et province, northeast Thailand	
14. Phou Thong Temple, Phou Khao Thong village, Pho Chai District (PT)	Burned forest dominated by *Castanopsis* spp., *Dipterocarpus* sp., *Lithocarpus polystachyus*, and other trees.
V. Loie Province, northeast Thailand	
15. Chiang khan south (CK)	Deciduous rain forest dominated by *Lithocarpus* spp., *Dipterocarpus tuberculatus*, *Dipterocarpus* sp., *Bamboo*, etc.
VI. Surat Thani province, southern Thailand	
16. Krung Ching, Nopphitam District (KC)	Mixed forests are dominated by *bambusa* spp., palm, Rubber Garden.
17. Khlong Sok subdistrict, Phanom District (KS)	Mixed forest dominated by *Bamboo* and unknown tree species.
VII. Nakhon Si Thammarat province, southern Thailand	
18. Sirivong, Lansaka District (SV)	Mixed forests are dominated by unknown tree species.
VIII. Krabi province, southern Thailand	
19. Khao Phanom District (KP)	Mixed forests are dominated by unknown tree species

3.3 DNA Extraction and Sequencing

Genomic DNA was extracted from the dried herbarium specimens using the Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology, Hangzhou, China), following the manufacturer's instructions. The ITS as it has ITS1 and ITS2 and parts of the nrLSU and *tef*1 genes were amplified by polymerase chain reaction (PCR) and sequenced. The following primers were used: ITS1-F and ITS4 for ITS (White et al., 1990; Gardes & Bruns, 1993), LR0R and LR5 for nrLSU (Vilgalys & Hester, 1990; White et al., 1990), and EF1-983F and EF1-1567R for *tef*1 (Rehner & Buckley, 2005).

Table 3.2, showed the PCR cycling for ITS and LSU was as follows: 3 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 52°C, 1 min at 72°C; 10 min at 72°C. For *tef*1 the following program was used: 5 min at 95°C; 35 cycles of 1 min at 94°C, 2 min at 52°C, 1.5 min at 72°C; 10 min at 72°C. The PCR products were purified and sequenced using the same forward and reverse primers by Sangon Biological Engineering Technology & Services (Shanghai).

3.4 Sequence Alignment and Phylogenetic Analyses

Sequence reads were checked using Bioedit Sequence Alignment Editor version 7.0.9.0 and assembled using SeqMan (DNAstar, Madison, WI, USA). Each sequence was blasted using the Basic Local Alignment Search Tool (BLAST) against National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/genbank/) to verify sample identification, as well as to find the closest matches. The newly generated sequences were deposited in GenBank. All sequences including the outgroup, were retrieved and aligned using MAFFT v.7 (Katoh et al., 2017) on the online server (http://mafft.cbrc.jp/alignment/server/). For *tef*1 sequences, introns were delimited by comparing with the amino acid sequence of a reference sequence and locating the GT/AG motifs of the splicing sites and removed before further analyses. The ITS and nrLSU alignments were trimmed separately using TrimAl to eliminate ambiguously aligned positions (Capella-Gutiérrez et al., 2009). After checking for supported conflicts (BS ≥ 70%) between single-gene maximum likelihood (ML) phylogenies, a concatenated three-gene dataset was assembled.

All phylogenetic analyses were done on CIPRES science Gateway version 3.3 web server (Miller et al., 2010), accessed at https://www.phylo.org/. For both maximum likelihood and Bayesian analyses, a mixed-model (partitioned) scheme was used, with the alignment divided.

Maximum likelihood phylogenetic inference was performed using RAxML-HPC2 version 8.2.12 (Stamatakis, 2006) on XSEDE. For Bayesian analysis, the best-fit substitution models were selected from jModelTest2 version 2.1.6 (Darriba et al., 2012) on XSEDE. Bayesian analysis was performed in MrBayes version 3.2.7a

(Ronquist et al., 2012). The phylogenetic tree was visualised and edited in Figtree version 1.4.4 (Rambaut, 2018), and then exported and further edited in Adobe Illustrator CS3. The final alignment of the new species was deposited in TreeBASE (https://treebase.org/treebaseweb/user/submissionList.html).

Table 3.2 Primers, PCR amplification conditions, and partial gene regions used in this study

Gene	Primer		PCR condition					Reference
	Forward	Reverse	Initial Denaturation	Denaturation	Annealing	Extension	Final extension	
ITS	ITS1	ITS4	95°C, 3 min 1 cycle	94°C, 40 sec	54°C, 40 sec 35 cycles	72°C, 1 min	72°C, 08 min 1 cycle	White et al. (1990)
LSU	LROR	LR5	95°C, 3 min 1 cycle	94°C, 40 sec	50°C, 40 sec 35 cycles	72°C, 2 min	72°C, 08 min 1 cycle	Gardes and Brruns 1993, Rehner and Samuels (1994) Vilgalys and Hester (1990)
Tef1-α	EF1-983F	EF1-1567R	95°C, 3 min 1 cycle	94°C, 40 sec	54°C, 40 sec 35 cycles	72°C, 1 min	72°C, 08 min 1 cycle	Rehner and Buckley (2005)

3.5 Cultivation of Wild *Pleurotus giganteus*

3.5.1 Collecting, Isolation and Identification

Mushroom collecting and isolation same in section 3.1. Figure 3.4 showed the isolation steps. Identification was used molecular analysis to confirm the species.

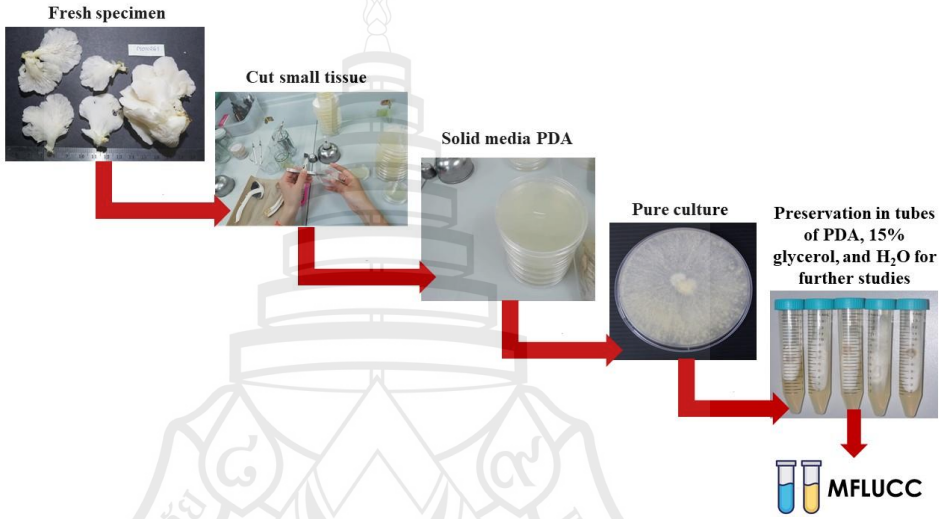


Figure 3.4 Process of cultural isolation from a fresh specimen

3.5.2 Effect of Spawn Production

Various materials including corncobs (*Zea mays* L.), coconut residue (*Cocos nucifera* (L.)), sugarcane bagasse (*Saccharum officinarum* L.), millet (*Panicum miliaceum L.*), wheat (*Triticum aestivum* L.), paddy rice (*Oryza sativa* L. var. *glutinosa*), rice berry (*Oryza sativa* L.), and sorghum (*Sorghum bicolor*) were used for optimal spawning. The grains were placed in vitro and sterilised at 121°C for 15 minutes. The tubes containing the grains were inoculated with a mycelium plug (approximately 0.5 cm diam), and the culture was incubated at 30°C in the dark. The experiment was carried out in five replicates. The length of the liner mycelium was measured and recorded every other day for 14 days.

3.5.3 Agricultural Residues for Cultivation

Four different formulas were used in this study, which is shown in (Table 3.3), corn cobs (*Zea mays* L.), rice straw (*Oryza sativa* L. var. *glutinosa*), and sugarcane bagasse (*Saccharum officinarum* L) were mixed with rubber sawdust (*Hevea*

brasiliensis Müll.Arg.) with a ratio of 1:1 (Thongklang & Luangharn, 2016) and rubber sawdust alone was used as a control. Rice bran, pumice sulphate, gypsum ($CaSO_4.2H_2O$), yeast, calcium carbonate ($CaCO_3$), molasses and EM (Effective Microorganism) were also added. The substrate bags (800 g per bag) were sterilised for 1 hour at temperatures of 121°C. The bags were left to cool, then approximately 50 g of the spawn was inoculated and incubated under dark conditions at 28 ± 1.09°C until the mycelia had grown to completely fill the bags. Watering was carried out twice daily in the morning and evening using a tap water sprayer until the fruiting bodies had fully developed. The results were harvested manually and recorded daily. The experiment was carried out with 15 repetitions for each substrate and the experiment was carried out for 60 days.

Table 3.3 The ratio of experimental substrates (w/w) for the cultivation of *P. giganteus*.

Treatment	The composition of the bag (w/w)
Sawdust	
Sawdust + corn cobs	1:1
Sawdust + rice straw	1:1
Sawdust + sugarcane bagasse	1:1

Note 1:1 means the ratio of experimental substrates (w/w).

3.5.4 Casing and Nutrient Supplementation

The full mycelium in the substrate bag was opened after 30 days of incubation and transferred to the tray. The bag was placed in the centre of the tray and then covered with soil approximately 3 cm deep. Humus soil was used as the main capillary material for growth with three different soil capillary formulas, as shown in (Table 3.4). The first treatment (T1) used 25% humus soil, 35% lime sand, 25% calcium carbonated, and 15% peat. The second treatment (T2) used 50% lime sand, 25% calcium carbonate and 25% peat. The third treatment (T3) used 100% humus soil and removed tough parts before use (Table 3.4). After applying the soil casing, the plates were incubated in a mushroom house at 30 °C, 90% humidity, and the plates were watered every week. The moisture of the trays was maintained by spraying water outside the trays two to three times per day. The experiment was carried out on five replicated trays. The mushroom

products that developed were harvested when the cap started to curl. Data were collected each day for 60 days.

Table 3.4 The ratio of the soil casing formula.

Treatment	The composition of the soil casing formula (%)			
	Humus soil	**Lime sand**	**Calcium carbonated**	**Peat**
T1	25	35	25	25
T2		50	25	15
T3	100			

Note T1 **=** first treatment, T2 = second treatment, T3 = third treatment.

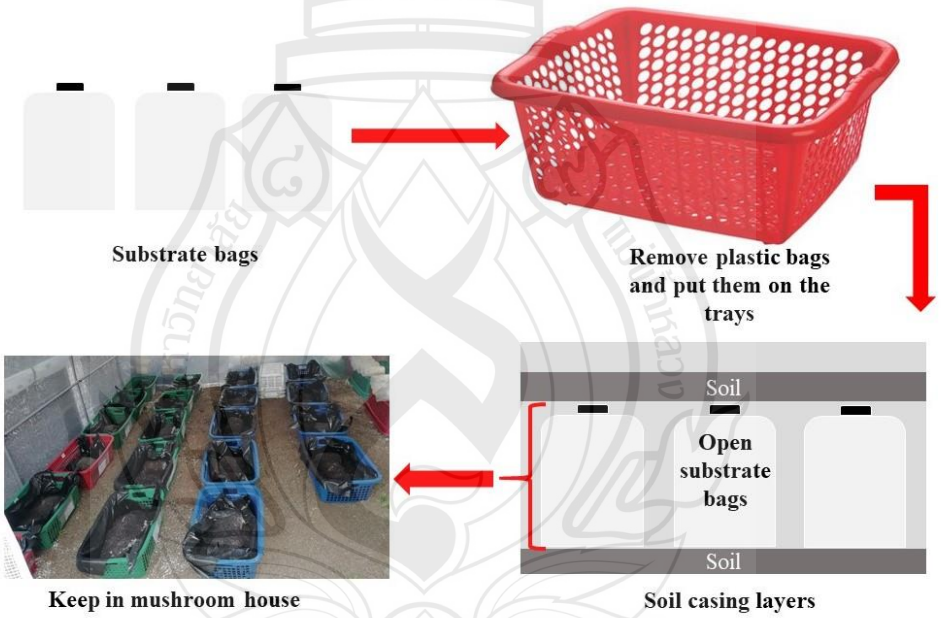


Figure 3.5 Process of the soil casing

3.6 Cultivation of Wild *Pleurotus djamor* var. *fuscopruinosus*

3.6.1 Effect of Grain Media for Spawn Production

The six different grains were used to find the best spawn production: *Hordeum vulgare* (barley), *Zea mays* (corn), *Oryza sativa* (paddy rice), *Pennisetum glaucum* (L.) R. Br. (millet), *Oryza sativa* L. (rice berry), *Sorghum bicolor* (sorghum). Each spawn

grain will be washed and soaked overnight, the water will be drained, the mixture will boil for 10-15 minutes and leave to cool. The grains were placed in 30 × 140 mm test tubes to approximately 120 cm depth, autoclaved at 121°C for 15 minutes, and left to cool .Tubes containing the same grain length were inoculated with five mycelial plugs of approximately 5 mm diam. of the mycelia colony on PDA plates under aseptic conditions and the test tubes were incubated at 28°C under dark conditions. The length of the linear mycelium was measured every 2 days for 10 days to calculate the growth rate. All analyses were carried out in five replicates.

3.6.2 Effect of Different Agricultural Residues on Mycelium Growth

Ten agricultural wastes including banana leaves, sugarcane bagasse, coconuts sell fibre, corn cob, coffee ground, corn husks, mixed leaves, pine apple, rice husks, rice straw, sawdust, substrate material was mixed with 9% rice bran, 1% sugar, 1% calcium carbonate, 0.03% ammonium chloride, 0.03% magnesium sulphate and 0.036% monopotassium phosphate. Mix the substrate with the supplements and fill with distilled water. Each petri dish was placed with 20 g of a mixed substrate with 5 replications per substrate. Sterilised in an autoclave at 121°C for 1 hour and left to cool, the Petri dish of each substrate was inoculated with a 10 mm mycelium disc under aseptic conditions and incubated at 28°C under dark conditions. Diameter measurements for mycelium proliferation every 2 days for 10 days. The protocol follows Hoa and Wang (2015), Nguyen and Ranamukhaarachchi (2019).

3.6.3 Fruiting Test

The best substrate from the effect of agricultural waste on the growth test of mycelium was selected for the fruiting test. Rubber sawdust was used as the main substrate mixed (w/w) with 9 % rice bran, 1% sugar, 1% calcium carbonate, 0.03% ammonium chloride, 0.03% magnesium sulphate, and 0.036% monopotassium phosphate (Nguyen & Ranamukhaarachchi, 2019). All substrate supplements were mixed manually with 70% moisture. The mixed substrate (800 g) was packed in polypropylene bags and then capped with a plastic ring and lid. The sawdust bags were sterilised at 121 °C for 1 hour. After the temperature cooled, the spawn was inoculated in the sawdust bags and, appropriately, 50 g of mushroom spawn were inoculated in the sawdust bags under aseptic conditions. The bags were incubated in darkness at 25±1 °C, and 60–75% relative humidity. All analyses were carried out from five replicates.

After the mycelial had completely colonised the bag substrates, they were removed from the shelves and opened at the ends. The bags were kept in the mushroom house with 70 to 80% humidity. Watering was carried out two times per day using a tap water until the fruiting bodies had fully developed.

3.7 Statistical Analysis

Statistical significance for the mycelium growth rate of spawn production, the total wet weight of mature fruiting bodies harvested, the average wet weight, and data analysis were determined by means variance using Tukey's test with significance of $P < 0.05$.

For the fruiting bodies, *Pluerotus* species were manually harvested, counted, and weighed daily. Yield data was defined as the total weight of fresh mushrooms per kilogramme of substrate (Thongklang et al., 2014). The biological efficiency (B.E.) was recorded. B.E. means the total weight of fresh mushrooms/weight of dry substrate) x 100% (Liang et al., 2019; Thongklang et al., 2020).

3.8 Determination of the Nutritional Content of Cultivated Species

Proximate analysis was performed on the basidiomata of *Pleurotus* species. Fresh basidiomata were dried at 50ºC for 24 hours or until completely dried and powdered using a blender.

3.8.1 Determination of Ash Content

Ash refers to the inorganic matter remaining after the complete oxidation of organic matter in mushroom samples. Ash content analysis was done by the dry ashing method following (Jame, 1995). Six crucibles were preheated at 525ºC for 24 hours. Three grammes of mushroom powdered sample were placed in each crucible and weighed accurately. The crucibles were put to a muffle furnace and samples were dried at 525 °C for 4 hours. After drying, the samples were stored in a desiccator and weighed accurately. The percentage of ash on the wet weight basis (wwb) and the dry weight basis (dwb) were calculated as follows:

$$\% \text{ Ash (wwb)} = \frac{\text{wt of ash}}{\text{wt of sample}} \times 100$$

$$\% \text{ Ash (wwb)} = \frac{(\text{wt of ashed sample} + \text{wt of crucible}) \text{ - } (\text{wt of crucible})}{(\text{wt of wet sample} + \text{crucible}) \text{ - } (\text{wt of crucible})} \times 100$$

$$\% \text{ Ash (dwb)} = \frac{\% \text{ ash (wwb)}}{(100 - \% \text{ moisture})} \times 100$$

3.8.2 Determination of Fat Content

The dried samples were ground, 2 g of samples on paper and placed in the thimble, place the samples and thimble in a Soxhlet method using organic solvent following (Nielsen et al., 2017), record weight pre-dried extraction cub, place extraction cup in a Soxhlet extractor, 70 ml of petroleum ether was added using a dispenser, set-up programme and follow instruction (Fat was extracted, continuously, with an organic solvent. The solvent was headed and volatized and then condensed above the sample. The solvent continues to drip through the sample to extract the fat). After finished, heat cub in the hot air oven at 105℃ for 2 hours, leave it cool down in desiccator and fat content is measured by weight loss of sample or weight of fat in solvent. Calculate percentage fat (wwb and dwb) were calculated as follows:

$$\% \text{ Fat (wwb)} = \frac{(\text{wt of cup} + \text{fat}) \text{ - } (\text{wt of cup})}{(\text{wt of wet sample})} \times 100$$

$$\% \text{ Fat (dwb)} = \frac{\% \text{ fat (wwb)}}{(100 - \% \text{ moisture})} \times 100$$

3.8.3 Determination of Fibre Content

The crude fibre in pleurotoid mushroom samples was determined as follows (Nielsen, 2017a). Crude fibre is composed of cellulose, hemicellulose, and lignin, the residue after chemical digestion with hot sulfuric acid (1.25% w/v) and hot sodium hydroxide (1.25% w/v). The crucibles to be used will be pre-dried (W_1) and weighed. The powdered sample (1 g) was accurately weighed and placed in the crucible. The crucible was placed in the Fibertec cold extraction unit. Exactly 25 ml of acetone was added to the crucible and left for 10 min to filter. The process was repeated three times

and washed with water. The crucible was placed in the Fibertec hot extraction unit and added with 150 ml of hot 1.25% w/v sulfuric acid, diatomaceous earth, and filter aid. Four drops of n-octanol were added to prevent foaming and heated to boiling for 30 minutes. The acid was filtered and washed three times with hot distilled water. The crucible was again placed in the Fibertec hot extraction unit and added with 150 ml of hot 1.25% w/v sodium hydroxide and followed the previous process with sulfuric acid. The crucible was placed in the Fibertec cold extraction unit and filled with 25 ml of acetone, then filtered for 10 min, repeatedly 3 times. The solvent was evaporated and the crucible was dried at 130ºC for 2 hours. The crucible was cooled in the desiccator and accurately weighed (W2). The sample in the crucible is ashed at 525±25ºC for 4 hours. Leave it cooled in the desiccator and weigh it (W3). The calculation for the percentage of crude fibre (wwb) is as follows:

$$\% \text{ Crude fiber (wwb)} = \frac{(W2 - W3)}{W1} \times 100$$

Where:

W1 = Sample weight (g)
W2 = Weight of crucible + residue (g)
W3 = Weight of crucible + ash (g)

3.8.4 Determination of Moisture Content

Moisture content analysis was performed by the oven drying method following (Nielsen, 2010). Pre-dried 6 disposable aluminium pans at 100ºC for 24 hours. Approximately 3 g of powdered mushroom samples were placed in each pan and accurately weighed. The samples were placed in an oven and dried at 103 ± 2ºC for 18 hours. After drying, the samples were placed in the desiccator to lower the temperature and weighed. The percentage of moisture and dry matter was calculated as follows:

$$\% \text{ Moisture} = \frac{\text{wt of } H_2O \text{ in sample}}{\text{wt of wet sample}} \times 100$$

$$\% \text{ Moisture} = \frac{(\text{wt of wet sample} + \text{pan}) - (\text{wt of dried sample} + \text{pan})}{(\text{wt of wet sample} + \text{pan}) - (\text{wt of pan})} \times 100$$

$$\% \text{ Dry matter} = 100 - \% \text{ moisture}$$

3.8.5 Determination of Protein Content

The protein content of the mushroom samples was determined using the Kjeldahl method (Nielsen, 2017b). Digestion was started by turning on the digestion block and setting the temperature to 420ºC. Six digestion tubes were prepared. Approximately 1g of sample was weighed and recorded, then placed in each digestion tube. Exactly 5g of catalyst and 12ml of concentrated sulphuric acid were placed in each tube with the sample. The digestion tubes were arranged on the rack and placed in the digestion block; then the exhaust system was turned on. The digestion process was completed in about 45 minutes or until the samples were clear. The samples were taken from the digestion block and cooled. The samples were diluted with 20ml of distilled water.

Distillation processes were done following the manual of the distillation equipment. The appropriate volume of boric acid (25ml) was dispensed into the receiving flask. The receiving flask was placed on the distillation system and submerged in the boric acid solution. In the distillation process, the NaOH solution (50ml) was delivered to the tube. The steam generator was set at 4 minutes to distill the sample. The colour of boric acid then changed from red to green. The same procedures were applied to all six tube samples. The titration process was performed using a standardised HCl solution in 6 replicates of samples in tubes and 1 blank. The normalities of HCl were recorded. A methyl red indicator was added five drops for each tube, then titrated with the standardised HCl solution. The colour changed from green to pink. The volume of HCl titrant used was recorded. The percent nitrogen and the percent protein were calculated using the formula below. The conversion factor that would be used for nitrogen to protein was 6.25.

$$\% \text{ N (wwb)} = \frac{\text{Normality HCl}}{1000} \times \frac{\text{Corrected acid vol. (ml)}}{\text{wt of sample (g)}} \times 14 \left(\frac{\text{g of N}}{\text{mol}} \right) \times 100$$

$$\% \text{ Protein (wwb)} = \% \text{ N} \times \text{Protein Factor}$$

Remark:

Corrected acid vol.= (ml std. acid used for sample) – (ml std. acid used for blank)

3.8.6 Determination of Carbohydrate Content

Carbohydrate was calculated using the following equation (Raghuramulu et al., 2003):

$$\text{Carbohydrate (g/100 g sample)} = 100 - \frac{\text{(Moisture + Fat + Protein + Ash + Crude + Fibre) g}}{\text{100 g}}$$

3.9 Therapeutic Efficacy of *Pleurotus djamor* var. *fuscopruinosus* and *Pleurotus giganteus*

3.9.1 Mushroom Extract

The mycelium of *P. djamor* var. *fuscopruinosus* was subcultured on potato dextrose agar using agar plugs. The cultures were grown in 100 plates and incubated at 28°C for four weeks or until the mycelium covered the entire plate media. Mycelium cultures were scraped from the agar plates and then soaked in 30 mL of HPLC grade ethyl acetate (EtOAc), methanol, and water using a homogenizer (Ultra-turraxP®). This extraction process was conducted in three replicates. The mycelium samples were soaked for three days for the first extraction using each solvent. The solid and liquid phases were then separated. The solid layer was subjected to further extractions with the same solvents, each for three days, resulting in second and third extractions. After the last extraction, the solid layer was discarded. The collected liquid layers (EtOAc extracts) were combined and transferred to a pre-weighed vial. The solution was allowed to air dry, evaporating the liquid components and leaving behind a solid

(powdery) crude extract. This crude extract will be used for subsequent laboratory analyses (Xiao et al. 2020).

3.9.2 Antioxidant Assay

3.9.2.1 2,2-Diphenyl-1-picrylhydrazyl radical-scavenging activity (DPPH)

The DPPH radicals were followed by the protocol of Xiao et al. (2020). In a test tube, a 1,000 µL DPPH solution was combined with 800 µL of Tris-HCl buffer (pH 7.4). Subsequently, 200 µL of the sample solution was initially prepared as a crude extract at 1000 mg/mL (w/v). The sample was tested at various concentrations following a 2-fold dilution series (500, 250, 125, and 62.5 mg/mL). was added and rapidly mixed. The mixture was incubated at room temperature for 30 minutes, after which its absorbance was measured at 517 nm. A control solution (A_0) was prepared by mixing 1,000 µL of DPPH solution, 800 µL of Tris-HCl buffer (pH 7.4), and 200 µL of ethanol (instead of the sample extract). Ascorbic acid and Trolox were used as positive controls. The percentage inhibition of DPPH was calculated using the following formula:

$$[(A_0 - A_1) / A_0] \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the samples or positive control. The concentration required to reduce DPPH absorbance by 50% (IC_{50}) was determined graphically.

3.9.2.2 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical cation scavenging activity (ABTS)

ABTS radical scavenging activity was determined following the protocol described by Xiao et al. (2020). In a 96-well plate, 200 µL of ABTS working solution was combined with 10 µL of the sample solution, which was initially prepared as a crude extract at 1,000 mg/mL (w/v). The sample was tested at various concentrations following a 2-fold serial dilution (500, 250, 125, and 62.5 mg/mL). The mixture was thoroughly mixed and incubated in the dark for 7 minutes before the absorbance was measured at 734 nm. A control solution (A_0) was prepared by mixing 200 µL of ABTS working solution with 10 µL of ethanol (in place of the sample solution). Ascorbic acid

and Trolox were used as positive controls. The percentage inhibition of ABTS was calculated using the following formula:

$$[(A_0 - A_1) / A_0] \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample or positive control. The concentration required to reduce ABTS absorbance by 50% (IC_{50}) was determined graphically.

3.9.3 Antidiabetic Assay

3.9.3.1 Screening α-glucosidase Assay

The α-glucosidase inhibitory assay procedure followed a previously described method (Phukhatmuen et al., 2020) with modifications .The sample solutions at 200 µm/ml were dissolved with 100 %dimethyl sulfoxide (DMSO) in phosphate buffer (pH 6.8), and 50 µl of each sample was pipetted and mixed with 100 µl of alpha-glucosidase enzyme (0.35 unit/ml) in vitro. After pre-incubation at 37℃ for 10 min, 100 µl of 1.5 mM p-NPG was added and the sample was further incubated at 37℃ for 20 min, then 1000 µl of Na_2CO_3 (1M) was added to terminate the reaction. Acarbose was used as a positive control .The absorbance was measured at 405 nm with a microplate reader (PerkinElmer, lnc., USA).

3.9.3.2 Glucose Consumption Assay

The glucose consumption assay utilized the 3T3-L1 cell line (ATCC: CL-173), kindly supported by the Medicinal Plant Innovation Center of Mae Fah Luang University Thailand, following a method described previously (Phukhatmuen et al., 2020). 3T3-L1 cells were seeded at a density of 4×10^5 cells per well in 96-well plates, with 100 µL of culture medium. The plates were incubated for 24 h at 5% CO_2, and 37°C to allow cells to adhere until they reached more than 80% confluence. The crude extract of *P. djamor* var. *fuscopruinosus* was dissolved in an appropriate solvent (10% DMSO), and 100 µL of the extract was introduced into the 96-well plates, which were left for an additional 24 h. Subsequently, 10 µL of supernatant from each well was transferred to a new 96-well plate, and 40 µL of enzymes (GOD (enzyme 25 Kv) + POD (enzyme 10 Kv) + Buffer) was added. Absorbance was measured at 510 nm using a microplate reader (INFINITE M NANO). Metformin was prepared as a 10 mM stock

solution in sterile phosphate-buffered saline (PBS), then diluted in DMEM to a final concentration of 100 µg/mLusing a 2-fold dilution series (50, 25, 12.5, and 6.25 mg/mL), and used as a positive control. MTT assay was conducted by adding 100 µL of 0.5% MTT to the 96-well plates, which were incubated for 4 h. Finally, sterile DMSO (100 µL) was added, and the absorbance was measured at 570 nm. Cytotoxicity was assessed by determining the concentration of extracts that inhibited cell growth by 50% (IC_{50}).

3.9.4 Anticancer Assay

Lung cancer (A549, ATCC® CCL-185™) and colorectal cancer (SW480, ATCC® CCL-228™) cell lines were kindly provided by the Medicinal Plant Innovation Center, Mae Fah Luang University, Thailand, and were used in the anticancer assay. Cytotoxicity procedures against these cancer cells followed a previously described method in the literature that utilised the resazurin reduction test on 96-well tissue culture plates (Ma et al., 2013). Cancer cells were seeded at a density of 4×10^5 cells per well in 100 µL of culture medium. The plates were incubated for 24 h at 5% CO_2 and 37°C until cell growth exceeded 80%. The crude extract of *P. djamor* var. *fuscopruinosus* MFLUCC24-0056 was prepared in an appropriate solvent (10% DMSO), and 100 µL of the extract was added to the 96-well plates. The plates were incubated for an additional 24 h. Doxorubicin was dissolved in sterile phosphate buffered saline (PBS) to prepare a 1 mM stock solution and further diluted in culture medium to a final concentration of 100 µg/ml using a series of 2-fold dilutions (50, 25, 12.5, and 6.25 mg / ml), and used as a positive control. Following this incubation, 100 µL of 0.5% MTT was added to each well. The plates were then incubated for 4 h. After incubation, sterile DMSO (100 µL) was added and the absorbance was measured at 570 nm using a microplate reader (INFINITE M NANO). Cytotoxicity was quantified as the concentration of extracts that inhibited cell growth by 50% (IC_{50}). The IC_{50} values were calculated based on a trend line derived from varying concentrations of extracts. A percentage of inhibition less than 50% was indicated as inactive.

3.9.5 Cytotoxicity Assay

RAW 264.7 (ATCC: TIB-71) cells obtained from the Medicinal Plant Innovation Center of Mae Fah Luang University Thailand and used in the cytotoxicity of the extract from *P. djamor* var. *fuscopruinosus*. The cytotoxicity test was conducted

through the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan. One hundred microliters of Raw 264.7 cells were seeded in a 96-well plate at a density of 4×10^5 cells per well. The cells were allowed to adhere for 24 h in an incubator maintained at 5% CO_2 and 37°C, until they reached more than 80% confluence. After the adherence period, the mushroom extract was dissolved in the appropriate solvent (10% DMSO), and 100 µL of the extract was added to the 96-well plates. The plates were incubated for an additional 24 h. Subsequently, 100 µL of MTT solution (0.5 mg/mL) was added to each well, and the plates were incubated for 4 h. Following this incubation, sterile DMSO (100 µL) was added to dissolve the formazan crystals. The absorbance was then measured at 570 nm using a microplate reader (INFINITE M NANO). Cytotoxicity was quantified as the concentration of extracts that inhibited cell growth by 50% (IC_{50}). The IC_{50} values were calculated from a trend line derived from varying concentrations used in the assays. Each test included an appropriate range of concentrations for precise assessments.

CHAPTER 4

SPECIES DIVERSITY OF PLEUROTOID MUSHROOMS (AGARICALES) IN SELECTED COMMUNITY FORESTS IN THAILAND

4.1 Introduction

In general, biological diversity refers to the variety of organisms that exist in all habitats, including terrestrial, marine, and other aquatics, including a wide range of insects, plants, animals, pests, fungi, and bacteria (Tsioumani & Tsioumanis 2020). Knowledge of biodiversity that has not yet been fully explored may be helpful in both positive and negative elements of existence (Aerts et al., 2018; Ostfeld & Keesing, 2017). Fungi are incredibly diverse, enigmatic organisms that come together to form intricateand dynamic communities. Most of the time, fungi are hard to identify since they grow as a network of thin filaments on the substrate (soil, wood, insect guts, living plant parts, etc.). (Money, 2016). Although their fruiting times can be brief and their fructifications transient, species that generate structures that bear seeds can be commonly found (Branco, 2011). Currently, more than 100,000 species of fungi are officially recognised and there are probably hundreds of thousands more that have not been described (Hawksworth & Lücking, 2017; Laessoe & Petersen, 2019). Current fungal diversity estimates in various environments outline general methods of identification and highlight the importance of culture-dependent and culture-independent methods in fungal identification, and Gautam et al. (2022) reported a comprehensive review of fungi aspects.). General information that pleurotoid mushrooms (Basidiomycota) are agaric forms that typically have a fan-shaped fruiting body, the presence of lamellae, and lateral stem attachment. In some species, the stipe can be reduced or absent (Seelan, 2015; Læssøe & Petersen, 2019). The pleurotoid form is well represented in the genus *Pleurotus*. The pleurotoid form is not phylogenetic informative. The habit has evolved and diverged many times in some genera. In (2019) Laessoe and Petersen divided 26 genera of pleurotoid mushrooms based on the colous

deposit of spores, and the different spore colours reflect the fact that the species concerned have completely different phylogenies. Consequently, to identify whether mushrooms are pleurotoid or another group, it should be based on morphological characteristics using molecular analysis for confirmation of the exact genera or species.

Forests are a source of water, land for cultivation, food and medicine, as well as essential for human survival. Especially tropical forests, which are a source of biodiversity (Putz et al., 2001; Aju et al., 2015). In Thailand, more than 12,000 community forest areas have been established, covering a total around 1.2 million hectares (Khongswasdi, 2022). The North has the largest community forest area, followed by the Northeast, Central, and South, respectively (Thailand Forests, 2021). Thailand is abundant in various forests and has a high diversity of mushrooms, and many new species have yet to be discovered (Thongbai et al., 2018; Vadthanarat et al., 2021). Some studies have been recorded on the diversity of pleurotoid species and many novel types of basidiomycetes have been found in Thailand (Baimai, 2010; Chandrasrikul et al., 2011; Sakonrak et al., 2017; De Crop et al., 2018; Suwannarach et al., 2022; Phonemany & Thongklang, 2023; Sysouphanthong et al., 2023). However, some of the pleurotoid species should be reviewed and updated again. Pleurotoid mushrooms (Agaricales) are well known for their edibility. However, studies on the diversity of this group are not well represented in Thailand. Thus, this study aims to survey and provide an overview of the diversity of pleurotoid mushrooms in Thailand. Overall, the data obtained provide insight into a better understanding of the distribution and diversity of pleurotoid mushrooms in Thailand.

4.2 Material and Methods

4.2.1 Collecting Sites and Mushroom Sampling

The fresh specimens were collected from a number 19 localities see the collecting sites in Table 3.1 in chapter 3, and sample collection and morphological identification the same in section 3.1 in chapter 3.

4.2.2 Data Analysis of the Diversity of Pleurotoid Mushrooms

Terms and formula analysis are the same in section 3.2 in chapter 3.

4.3 Results

4.3.1 Species Diversity of Pleurotoid Mushrooms in Thailand

The results of species richness (S), number of mushroom samples, Shannon's diversity index (*H'*), and Evenness_e^H/S are shown in all analysis. According to the diversity of pleurotoid species in the 19 sites, 250 collections of pleurotoid mushrooms were identified based on morphological characteristics and 70 species were found. Shannon's diversity index (*H'*) showed five highest distributions, *Hohenbuehelia tristis* with an *H'* of 2.33, followed by *Schizophyllum commune* with an *H'* of 2.20, and *Hohenbeuhelia* sp. 3 with an *H'* of 2.10, *Campanella* sp. 1 with an *H'* of 1.94, and *Crepidotus* sp. 10 with an *H'* of 1.94, respectively. For other pleurotoid species, there is a lower diversity from 1.94 to 0.60, respectively, shown in Table 4.1. Figure 4.1 shows the diversity of the species of pleurotoid mushrooms of the highest diversity. The percentage of frequency of occurrence (%) of the highest diversity of pleurotoid species is shown in Figure 4, the highest is *Hohenbuehelia tristis* with 10.44%, followed by *Crepidotus* sp. 10 with 5.62%, and *Crepidotus* sp. 15 with 5.22%, respectively; the lower are *Campanella* sp., and *Crepidotus thialandicus* with 4.41%, 4.01%, respectively; followed by *Crepidotus flavocarpus* with 3.21%; the lowest is *Hohenbeuhelia* sp. 3, *Clitopilus* sp. 1, *Crepidotus* sp. 8, *Schizophyllum commune*, *Crepidotus* sp. 16, *Pleurotus giganteus*, *Pleurotus* sp. 1, *Resupinatus* sp. 1 with 2.4 to 2, respectively.

A summary of the distribution of pleurotoid species collected from 19 sites in Thailand during 2019–2023. Table 4.2, showed that the highest diversity of pleurotoid mushrooms was found in Pa Deng village, where seven families were found, with 45 collections and 25 species; followed by Pox Kaeo village, where six families were found, with 52 collections and 23 species; Huay Kaeo village, where five families were found, with 18 collections and 14 species; Tha Pha village, where four families were found, with 13 collections and 11 species; Pa Ngae village, where four families were found, with 11 collections and 10 species, and other collecting sites showed the lowest diversity, respectively (see Table 4.2). According to Shannon's diversity index (*H'*), Pa Deng village contained the highest species diversity with an *H'* of 3.34, followed by Pox Kaeo village with an *H'* of 3.05, Huay Kaeo village with an *H'* of 2.91, Tha Pha

village with an *H′* of 2.73, and Pa Ngae village with an *H′* of 2.68, respectively. Lower diversities of pleurotoid mushrooms were found in Krung Ching (Nopphitam district) with an *H′* of 0, Khlong Sok subdistrict (Phanom district) with an *H′* of 0.8, Chiang khan south with an *H′* of 0.94, Khao Phanom district with an *H′* of 0.94, and Huay Mae Suk, Mae Yao districts with an *H′* of 0.94, respectively.

Division of the diversity of pleurotoid mushrooms found a diversity of pleurotoid mushroom families collected from 19 sites found ten families, such as Entolomataceae, Marasmiaceae, Crepidotaceae, Cyphellaceae, Panaceae, Phyllotopsidaceae, Pleurotaceae, Polyporaceae, Schizophyllaceae, and Tapinellaceae. According to the results of Table 4.2, the family has the highest diversity with three genera consisting of *Hohenbuehelia*, *Pleurotus*, and *Resupinatus*; followed by Entolomataceae with two genera consisting of *Entoloma* and *Clitopilus*; and lower diversity Crepidotaceae, Schizophyllaceae, Marasmiaceae, Panaceae, Polyporaceae, Cyphellaceae, respectively; for Phyllotopsidaceae, and Tapinellaceae shows no diversity. The genera consisting of the highest species of pleurotoid mushrooms, *Crepidotus*, have the highest diversity, consisting of 30 species, followed by *Hohenbuehelia*, consisting of 8 species, *Pleurotus*, consisting of 7 species, and *Panus*, consisting of 6 species, respectively. Other genera are shown lower than 1 to 3 species. A key for the genera of pleurotoid mushrooms identified in this study is provided below:

Key for Genera of Pleurotoid Mushrooms

1a. Mushroom with a well-developed, visible stem……..**2**

1b. Mushroom without a distinct stem (sessile or with a very reduced base) ……...**5**

2a. Lamellae serrated, split or deeply notched……....*Schizophyllum*

2b. Lamellae not split or serrated …..……..…… **3**

3a. Spore print white.…… ……..…… **4**

3b. Spore print pinkish to reddish ..…… ***Clitopilus***

4a. Grows on wood, gills crowded, fruiting body tough and leathery.……***Lentinus***

4b. Grows on decaying wood, soft, fragile fruiting body …..……...***Pleurocybella***

5a. Spore print pinkish to reddish ……..……....**6**

5b. Spore print white to cream or brownish ……..……**7**

6a. Lamellae decurrent, soft fruiting body ……..……. ***Entoloma***

6b. Lamellae well-spaced, small delicate mushroom……...***Clitopilus***

7a. Basidiomata tiny, gills widely spaced or nearly absent, growing in clusters…… ***Campanella***

7b. Lamellae well-developed, not widely spaced …...……...…...…….....**8**

8a. Pileus small, thin, delicate, grows on decayed wood ….....…...***Cheimonophyllum***

8b. Pileus larger, fleshy, tough or gelatinous.……...……...……...……...**9**

9a. Pileus surface velvety or hairy, tough texture.....……...……...…...***Panus***

9b. Pileus smooth or slightly slimy, soft to firm texture ……...……..**10**

10a. Pileus gelatinous or slimy, soft when touched…....…….....***Hohenbuehelia***

10b. Pileus not gelatinous, firm but not slimy …..……...……...…...……...**11**

11a. Lamellae running down the stem, spore print white, thick fleshy fruiting body…….***Pleurotus***

11b. Lamellae running down the stem, spore print brown, thick but soft fruiting body……...……...……...……...…….....***Tapinella***

Figure 4.1 Diversity of the highest diversity species of pleurotoid mushrooms. **a** *Hohenbuehelia tristis*, **b** *Schizophyllum commune*, **c** *Hohenbeuhelia* sp. 3, **d** *Campanella* sp. 1, **e** *Crepidotus* sp. 10, **f** *Crepidotus* sp. 15, **g** *Hohenbuehelia lageniformis*, **h** *Lentinus squarrosulus*, and **i** *Crepidotus flavocarpus*

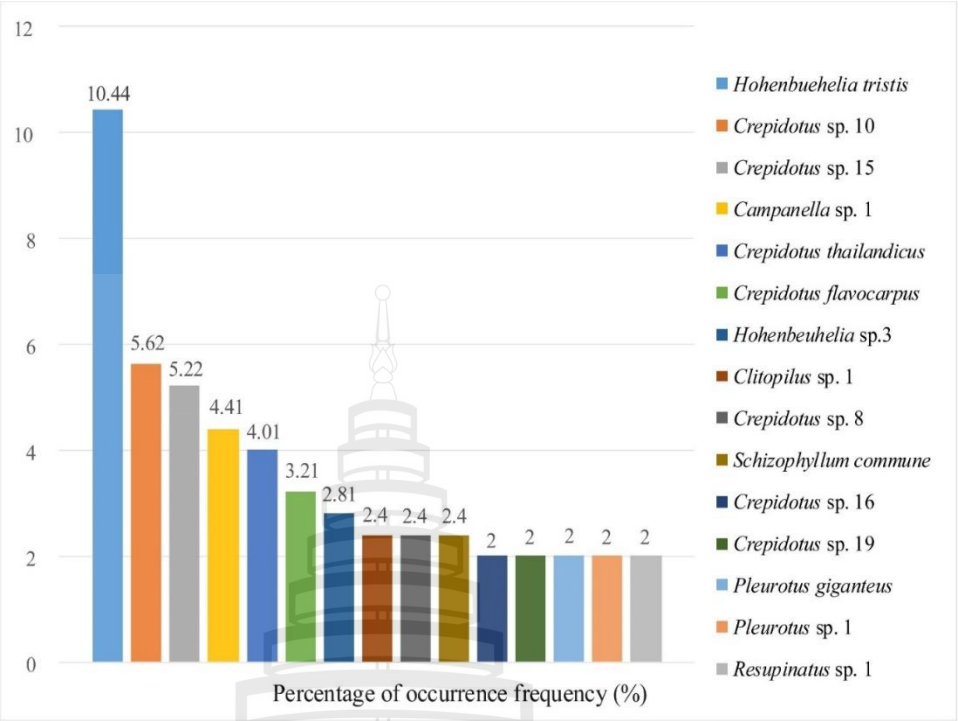


Figure 4.2 The 15 highest percentage of frequency of occurrence (%) of pleurotoid species

Table 4.1 Thirty highest diversity of pleurotoid mushrooms

Species	**Species richness (S)**	**Number of mushroom samples**	**Shannon's diversity index (*H'*)**	**Evenness _e^H/S**
Hohenbuehelia tristis	10	26	2.33	1.03
Schizophyllum commune	6	6	2.20	1.51
Hohenbeuhelia sp. 3	6	7	2.10	1.36
Campanella sp. 1	6	11	1.94	1.16
Crepidotus sp. 10	7	14	1.94	0.99
Crepidotus sp. 15	6	13	1.77	0.98
Hohenbuehelia lageniformis	4	4	1.76	1.45
Lentinus squarrosulus	3	3	1.43	1.39
Crepidotus flavocarpus	4	8	1.40	1.01

Table 4.1 (continued)

Species	Species richness (S)	Number of mushroom samples	Shannon's diversity index (*H'*)	Evenness _e^H/S
Crepidotus striatus	3	4	1.29	1.21
Entoloma sp.	3	4	1.29	1.21
Crepidotus sp. 16	3	5	1.25	1.16
Pleurotus giganteus	3	5	1.25	1.16
Clitopilus sp. 1	3	6	1.17	1.08
Cheimonophyllum sp. 1	2	2	0.94	1.28
Cheimonophyllum sp. 2	2	2	0.94	1.28
Crepidotus sp. 11	2	2	0.94	1.28
Crepidotus sp. 13	2	2	0.94	1.28
Crepidotus sp. 7	2	2	0.94	1.28
Crepidotus ungulatus	2	2	0.94	1.28
Hohenbuehelia flabelliformis	2	2	0.94	1.28
Panus sp. 1	2	2	0.94	1.28
Resupinatus sp. 2	2	2	0.94	1.28
Clitopilus chalybescens	2	4	0.81	1.13
Crepidotus asiaticus	2	4	0.81	1.13
Crepidotus sp. 17	2	4	0.81	1.13
Crepidotus chiangraiensis	2	3	0.80	1.11
Crepidotus sp. 5	2	3	0.80	1.11
Lentinus sp. 1	2	3	0.80	1.11
Panus fasciatus	2	3	0.80	1.11

Table 4.2 Distribution of pleurotoid species collected from Thailand during 2019–2023

Family /Genera /Species	PK	HK	PD	TP	MF	ML	NL	MFU	HM	DP	PN	MS	KC	KS	KP	SV	PT	DL	CK	Total	OF
Crepidotaceae																					
Crepidotus asiaticus	2					2														4	1.6
Cr. chiangraiensis						1												2		3	1.2
Cr. flavocarpus	4	2									1					1				8	3.2
Cr. lateralipes			1																	1	0.4
Cr. mollis		1																		1	0.4
Cr. palodensis	4																			4	1.6
Cr. roseocarpus												2								2	0.8
Crepidotus sp. 1	2																			2	0.8
Crepidotus sp. 2	1																			1	0.4
Crepidotus sp. 3	1																			1	0.4
Crepidotus sp. 4								1												1	0.4
Crepidotus sp. 5			2					1												3	1.2

Table 4.2 (continued)

Family /Genera /Species	PK	HK	PD	TP	MF	ML	NL	MFU	HM	DP	PN	MS	KC	KS	KP	SV	PT	DL	CK	Total	OF
Crepidotus sp. 6				2																2	0.8
Crepidotus sp. 7				1							1									2	0.8
Crepidotus sp. 8			4													2				6	2.4
Crepidotus sp. 9																1				1	0.4
Crepidotus sp. 10	5		2				3			1	1						1	1		14	5.6
Crepidotus sp. 11	1		1																	2	0.8
Crepidotus sp. 12	1																			1	0.4
Crepidotus sp. 13		1								1										2	0.8
Crepidotus sp. 14										1										1	0.4
Crepidotus sp. 15	2	1	5	1		1												3		13	5.2
Crepidotus sp. 16	2		1	2																5	2
Crepidotus sp. 17	2		2																	4	1.6
Crepidotus sp. 18			2																	2	0.8

Table 4.2 (continued)

Family /Genera /Species	PK	HK	PD	TP	MF	ML	NL	MFU	HM	DP	PN	MS	KC	KS	KP	SV	PT	DL	CK	Total	OF
Crepidotaceae																					
Crepidotus sp. 19								2	3											5	2
Cr. striatus	1			2	1															4	1.6
Cr. thailandicus	10																			10	4
Cr. ungulatus					1	1														2	0.8
Cr. viscidus											1						3			4	1.6
Cyphellaceae																					
Cheimonophyllum sp. 1				1								1								2	0.8
Cheimonophyllum sp. 2				1								1								2	0.8
Cheimonophyllum sp. 3		1																		1	0.4
Entolomaceae																					
Entoloma sp.	1			1								2								4	1.6
Clitopilus chalybescens									2										2	4	1.6

Table 4.2 (continued)

Family /Genera /Species	PK	HK	PD	TP	MF	ML	NL	MFU	HM	DP	PN	MS	KC	KS	KP	SV	PT	DL	CK	Total	OF
Clitopilus sp. 1			2			1				3										6	2.4
Clitopilus sp. 2				1																1	0.4
Clitopilus sp. 3																			1	1	0.4
Marasmiaceae																					
Campanella sp. 1			3					1						2	1	2		2		11	4.4
Campanella sp. 2																1				1	0.4
Panaceae																					
Panus fasciatus		2	1																	3	1.2
Panus sp. 1		1																	1	2	0.8
Panus sp. 2			1																	1	0.4
Panus sp. 3																	2			2	0.8
Panus sp. 4									1								1	1		3	1.2
Panus sp. 5		1																		1	0.4

Table 4.2 (continued)

Family /Genera /Species	PK	HK	PD	TP	MF	ML	NL	MFU	HM	DP	PN	MS	KC	KS	KP	SV	PT	DL	CK	Total	OF
Phyllotopsidaceae																					
Pleurocybella sp.								3												3	1.2
Pleurotaceae																					
Hohenbeuhelia sp. 1			2																	2	0.8
Hohenbeuhelia sp. 2			1																	1	0.4
Hohenbuehelia sp. 3				1	1					2		1	1			1				7	2.8
Hohenbuehelia sp. 4	2																			2	0.8
Hohenbuehelia sp. 5	1																			1	0.4
H. flabelliformis			1								1									2	0.8
H. lageniformis	1							1	1		1									4	1.6
H. tristis	4	3	2	1			2	5		3	1					1		4		26	10.4
Pleurotus djamor								2												2	0.8

Table 4.2 (continued)

Family /Genera /Species	PK	HK	PD	TP	MF	ML	NL	MFU	HM	DP	PN	MS	KC	KS	KP	SV	PT	DL	CK	Total	OF
P. djamor var. *fuscopruinosus*																2				2	0.8
P. giganteus	2		1													2				5	2
Pleurotus sp. 1					4				1											5	2
Pleurotus sp. 2							2													2	0.8
Pleurotus sp. 3					1															1	0.4
Pleurotus sp. 4			3				1													4	1.6
Resupinatus sp. 1				1						4										5	2
Resupinatus sp. 2							1											1		2	0.8
Polyporaceae																					
Lentinus sp. 1			2	1																3	1.2
Lentinus sp. 2		1																		1	0.4
Lentinus squarrosulus	1		1		1															3	1.2
Schizophyllaceae																					

Table 4.2 (continued)

Family /Genera /Species	PK	HK	PD	TP	MF	ML	NL	MFU	HM	DP	PN	MS	KC	KS	KP	SV	PT	DL	CK	Total	OF
Schizophyllum commune		1				1			1				1		1	1				6	2.4
Shicophylum sp. 1													4							4	1.6
Tapinellaceae																					
Tapinella sp.	1																			1	0.4
Species richness (S)	23	14	25	11	3	3	5	12	2	8	10	3	1	2	2	8	4	8	2	250	
Number of mushroom samples	52	18	45	13	6	4	10	23	2	16	11	4	4	3	2	11	7	16	2		
Shannon's diversity index (*H'*)	3.05	2.91	3.34	2.73	1.03	1.29	1.75	2.55	0.94	2.14	2.68	1.29	0	0.8	0.94	2.33	1.49	2.17	0.94		
Evenness_e^H/S	0.92	1.31	1.13	1.4	0.93	1.21	1.15	1.07	1.28	1.06	1.46	1.21	1	1.11	1.28	1.29	1.11	1.1	1.28		

Notes. PK = Pox Kaeo village; HK = Huay Kaeo village; PD = Pa Deng village; TP = Tha Pha village; MF = Mae Feak village; MT = Mae Toe village; NL = Nang Lae Nai Village; MFU = Mae Fah Lung University; HM = Huay Mae Suk; DP = Doi Pui; PN = Pha Ngae village; MS = Mae Sai; KC = Kroung Ching; KS = Khlong Sok subdistrict; KP = Khao Phanom District; SV = Sirivong subdistricts; PT = Phouthong Temple; DL = Dong Luang district; CK = Chiang Khan; OF = Percentage of occurrence frequency (%); S = Species richness.

4.4 Discussion

Some forms of fruiting bodies are sometimes similar to pleurotoid forms, such as clitocyboid, cyphelloid, and lentinoid; clitocyboids differ from pleurotoid by having a central stipe (Laessoe & Petersen, 2019). This study found that some families included genera with various morphology characteristics, for example, genera of Entolomaceae, Marasmiaceae, and Polyporaceae. The members of these families could be many shapes, and only some species are pleurotoid shape (Co-David et al., 2009; Laessoe & Petersen, 2019). Furthermore, *P. giganteus* and *P. tuberrigium* have forms similar with lentinoid (Corner, 1981), and only a few species were collected in this study and are identified as *Lentinus* and *Panus*.

In total, collections were collected at 19 sites in eight provinces during 2019–2023. The appearance of pleurotoid mushrooms throughout the rainy season found that 250 collections included 70 species in ten families, and thirteen genera of pleurotoid mushrooms. The highest diversity at all sites is *Hohenbuehelia tristis*, *Schizophyllum commune*, *Hohenbeuhelia* sp. 3, *Campanella* sp. 1, and *Crepidotus* sp. 10, respectively. The highest diversity of pleurotoid mushrooms was found in Pa Deng village, Pox Kaeo village, Huay Kaeo village, Tha Pha village, and Pa Ngae village, respectively. Furthermore, the diversity of the pleurotoid mushroom families was found at Mae Fah Luang University, and the highest Pleurotaceae are diversity that includes three genera found at several collecting sites. The genera consisting of the highest species are *Crepidotus*, *Hohenbuehelia*, *Pleurotus*, and *Panus*.

There are 29 species of pleurotoid mushrooms belonging to eleventh genera and eight families are recorded (Baroni et al., 2001; Karunarathna et al., 2011; Rattanamalee & Rattanamalee, 2012; Guzmán-Dávalos et al., 2017; Jatuwong et al., 2017; Sakonrak, 2017; De Crop et al., 2018; Raj & Manimohan, 2018; Sandargo et al., 2018; Kumla et al., 2019; Phuket et al., 2019; Suwannarach et al., 2022; Kumla et al., 2023; Phonemany et al., 2023; Phonemany & Thongklang, 2023; Sysouphanthong et al., 2023; Phonemany et al., 2024). The comparison of the diversity of pleurotoid mushrooms reported in Thailand with pleurotoid mushrooms in this study found that the diversity of families is almost similar to the previous study. However, this study found a higher

number of species. Based on this study, *H. tristis* is highly diverse, as this species is commonly found in several collecting sites and has a high % OF at 10.44%. This agrees with Phonemany et al. (2023), who reported new species of *Hohenbuehelia* species and noted on *H. tristis*. Other commonly found species are *Crepidotus* sp. 10, and *Crepidotus* sp. 15 (OF at 5.62%, 5.22%).

During the rainy season, pleurotoid mushrooms appeared in decayed wood, dead branches, dead bamboo, tree stumps, underground buried leaves, herbaceous stems, and sometimes on the bark of living trees. Among the 19 collecting sites, the five highest diversities of pleurotoid mushrooms are found in Pa Deng village, Pox Kaew village, Huay Kaeo village, Tha Pha village, and Pa Ngae village. Those collecting sites have an abundance of forests, such as mixed forest and deciduous rainforest dominated by *Lithocarpus* spp., *Dipterocarpus tuberculatus, Dipterocarpus* sp., *Castanopsis armata*, *Castanopsis* sp., *Bambusa* spp., and other trees. The lowest diversities of pleurotoid mushrooms are found in Krung Ching (Nopphitam district); these collecting sites have mixed forests dominated by *Bambusa* spp., palm, and rubber gardens. However, the number of diversities of pleurotoid mushrooms in this study depended on collecting time; some pleurotoid species appeared only a few days and rotted over time (for example, *Crepidotus* sp., *Resupinatus* sp., and *Entoloma* sp.) and the environmental conditions of the forests. Environmental stressors can also change the phenotype of mushrooms for their adaptive advantage (Hewitt et al., 2016).

CHAPTER 5

TAXONOMY AND PHYLOGENY OF *CREPIDOTUS* COLLECTED FROM THAILAND

5.1 Introduction to *Crepidotus*

Crepidotaceae (S. Imai) Singer (Agaricales, Agaricomycetes) is a gill mushroom that consists of six genera, namely *Crepidotus* (Fr.) Staude, *Episphaeria* Donk, *Nanstelocephala* Oberw. & R.H. Petersen, *Pellidiscus* Donk, *Pleuroflammula* Singer, and *Simocybe* P. Karst (He et al., 2019). *Crepidotus* is a member of the Crepidotaceae, originally described as *Agaricus* trib. *Crepidotus* Fr., later Staude (1857), described and transferred it to *Crepidotus*. In 1947, mycologist Rolf Singer published a seminal monograph on *Crepidotus*, establishing the first infrageneric classification system based primarily on microscopic characteristics, a significant departure from earlier systems that relied on macroscopic morphology. Pilát (1950) and subsequent researchers expanded Singer's framework, describing numerous new species, though many were later reclassified into other genera due to overlapping morphological traits. Despite these efforts, the infrasubgeneric taxonomy of *Crepidotus* remains challenging, as the genus exhibits remarkable phenotypic plasticity; observed morphological variations often reflect environmental adaptations rather than phylogenetic divergence. Thus, modern molecular studies have become critical in clarifying evolutionary relationships within this group. Currently, the most widely accepted infrasubgeneric classification follows Senn-Irlet (1995), which divides the genus into sections *Crepidotus*, *Velutini*, and *Sclerodermatini* based on integrative morphological and ecological criteria.

Currently, 319 taxa are listed under *Crepidotus* in the Index Fungorum (2024), but 200 species are accepted, and *Cr. mollis* (Schaeff.) Staude is the type species (He et al., 2019). The main characteristics of this genus are having pleurotoid basidiomata, smooth or ornamented with brown to yellowish basidiospores, which are mostly absent stipe or pseudo-stipe, and currently some species present stipe (e.g., *Cr. asiaticus*

Guzm.-Dáv., C.K. Pradeep & T.J.Baroni, *Cr. iqbalii* A. Izhar, Usman & Khalid) (Guzmán-Dávalos et al., 2017; Izhar et al., 2021). *Crepidotus* species are saprotrophic and can be found in temperate and tropical regions (Laessoe & Petersen, 2019), mostly growing mainly on decaying wood; some species are found on soil, often on buried substrate, and only a species is biotrophic in basidiomata, e.g., *Craterellus lutescens* (Fr.) Fr. (Singer, 1986). Bioactive compounds from some species of *Crepidotus* have been investigated, such as Strobilurin E, a new antibiotic of the (E)-β-methoxyacrylate was isolated from *Cr. fulvotomentosus* that can inhibit fungal respiration (Weber et al., 1990).

Thailand is rich in biodiversity. Many new species of fungi have been reported in Thailand, but many new species have yet to be discovered (Hyde et al., 2018; Vadthanarat et al., 2021). Nine species have been reported in Thailand: *Cr. applanatus* (Pers. Ex Pers.) Kummer [current name: *Cr. applanatus* var. *applanatus* (Pers.)], *Cr. citrinus* Petch, *Cr. crocophyllus* (Berk.) Saccardo, *Cr. herbarum* (Peck) Saccardo [current name: *Cr. epibryus* (Fr.) Quél.], *Cr. maculans* Hesler & Smith, *Cr. mollis* (Schaeff. ex Fr.) Stande, *Cr. roseus* var. *boninense* Hongo [current name: *Cr. boninensis* (Hongo) E. Horak & Desjardin] P. Kumm.], *Cr. variabilis* (Pers. ex Fr.) Kummer, and *Cr. versutus* (Peck) Saccardo (Chandrasrikul et al., 2011). However, most of these species have been reported without detailed morphological descriptions or molecular data to confirm their identifications. Consequently, it is necessary to analyse and update the lists of *Crepidotus* species published by Chandrasrikul et al. (2011). *Crepidotus asiaticus* Guzm.-Dáv., C.K. Pradeep & T.J.Baroni is a new *stipitate* species recorded in the country (Guzmán-Dávalos et al., 2017). In this study, during the pleurotoid mushroom survey in Thailand, several collections of *Crepidotus* were collected and studied. Based on morphological and phylogenetic results, six novel species and two new records were identified and are described herein.

5.2 Material and Methods

5.2.1 Sample Collection and Morphological Study

All mushroom samples used in this study were collected from selected sites of Thailand (Table 3.1). The specimens were morphologically characterised as described in Section 3.1 of Chapter 3.

5.2.2 DNA Extraction and Sequencing

DNA extraction, primers used for PCR conditions and sequencing were the same as in Section 3.2 (Table 3.2) of Chapter 3.

5.2.3 Sequence Alignment and Phylogenetic Analyses

Sequences were checked using Bioedit Sequence Alignment Editor version 7.0.9.0 and assembled using SeqMan (DNAstar, Madison, WI, USA). All sequences were blasted using the Basic Local Alignment Search Tool (BLAST) against the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/genbank/) to find the closest matches. New sequences were deposited at GenBank. The reference sequences are shown in Table 5.1 including the outgroup, were retrieved and aligned using MAFFT v.7 (Katoh et al., 2017) on the online server (http://mafft.cbrc.jp/alignment/server/). ITS and LSU alignments were trimmed separately using TrimAl to eliminate ambiguously aligned positions (Capella-Gutiérrez et al. 2009). After checking for supported conflicts (BS ≥ 70%) between single-gene maximum likelihood phylogenies, a concatenated two-gene dataset was assembled.

All phylogenetic analyses were performed on the CIPRES Science Gateway version 3.3 web server (Miller et al., 2010), accessed at https://www.phylo.org/. A mixed-model (partitioned) scheme was used for both maximum likelihood and Bayesian analyses, with the alignment divided into the following two-character sets: ITS1+ITS2, LSU. Maximum likelihood (ML) phylogenetic inference was performed using RAxML-HPC2 on XSEDE version 8.2.12 (Stamatakis, 2006). Three species of *Neopaxillus* were used as an outgroup. For Bayesian analysis, best-fit substitution models were selected from jModelTest2 version 2.1.6 (Darriba et al., 2012) on XSEDE. The best-fit model for ITS1+ITS2 was SYM+I+G. Bayesian analysis was performed in

MrBayes version 3.2.7a (Ronquist et al., 2012). Two runs of four chains were run for 1,000,000 generations and sampled every 100 generations. The average standard deviation of the split frequencies was 0.008720 at the end of the runs. The burn-in phase (25%) was estimated by checking the stationarity in the plot generated by the sump command. The phylogenetic tree was checked and edited in Figtree (version 1.4.0), and then exported and further edited in Adobe Illustrator CC 2019.

Table 5.1 Names, voucher numbers, origins, and corresponding GenBank accession numbers of the taxa used in the phylogenetic analysis

Species name	Voucher	Countries	GenBank accession numbers	
			ITS	LSU
Crepidotus sp.	MT58	Thailand	OR785710	–
Cr. aff. *subverrucisporus*	G0716	Hungary	–	MK277891
Cr. affinis	PDD:72848	New Zealand	KY827291	
Cr. alabamensis	TBGT15610	India	MK459545	MK459543
Cr. albolanatus	PDD:72865	New Zealand	KY827292	–
Cr. amygdalosporus	OKM26740	the United States	–	AF205678
Cr. antillarum	OKM26827	the United States	–	AF205680
Cr. applanatus	–	the United States	MF621029	MF621023
Cr. applanatus var. *applanatus*	MCA170	the United States	–	AF205694
Cr. asiaticus	TJB9995	India	MF077337	MF077336
Cr. asiaticus	CKP9401	India	JN113586	–
Cr. aureus	OKM27300	the United States	–	AF205685
Cr. betulae	MCA384	the United States	–	AF205679
Cr. brunnescens	MCA864	the United States	–	AF367936
Cr. calolepis	ECV4050	the United States	KY777396	MF797668
Cr. caspari	FFAAS0342	China	MZ401361	MW581521
Cr. cesatii	OKM26976	the United States	–	AF205681
Cr. cf. *albissimus*	MCA697	the United States	–	AF367959
Cr. cf. *cesatii*	Montri-256	Switzerland	MK028394	–
Cr. cf. *subaffinis*	MCA604	the United States	–	AF205703
Cr. chiangraiensis	**MFLU23-0438**	**Thailand**	**OR785705**	**OR785695**
Cr. chiangraiensis	**MFLU23-0439**	**Thailand**	**OR785706**	**OR785696**
Cr. cinnabarinus	MCA387	the United States	–	AF205686
Cr. circinatus	307011	USA	MH087459	–
Cr. croceotinctus	iNat31834012	USA	MN498116	–
Cr. crocophyllus	SLO 2433	Slovakia	OM832529	OM832562

Table 5.1 (continued)

Species name	Voucher	Countries	GenBank accession numbers	
			ITS	LSU
Cr. dentatus	HMJAU 37097	China	NR-173279	–
Cr. epibryus	G0515	Hungary	–	MK277884
Cr. eucalyptorum	G1749	Hungary	–	MK277885
Cr. exilis	TBGT17157	India	–	MK878548
Cr. flavocarpus	**MFLU23-0427**	**Thailand**	**OR785699**	**OR785687**
Cr. flavocarpus	**MFLU23-0428**	**Thailand**	**OR785698**	**OR785688**
Cr. fragilis	MCA904	the United States	–	AF367931
Cr. fraxinicola	OKM26739.5	the United States	–	AF205699
Cr. globisporus	TBGT17341	India	–	MK878540
Cr. herbaceus	–	China	KX456185	–
Cr. indicus	TBGT17161	India	–	MG735357
Cr. inhonestus	420526MF0778	China	–	MG696605
Cr. iqbalii	LAH 36654	Pakistan	NR_173306	NG_079698
Cr. kangoliformis	BAP 664 (Holotype, SFSU)	Africa	KX017199	–
Cr. kauffmanii	G1956	Hungary	–	MK277887
Cr. lanuginosus	OKM27331	the United States	–	AF367940
Cr. lateralipes	**MFLU23-0440**	**Thailand**	**OR785707**	–
Cr. lateralipes	PDD 72508	New Zealand	NR_159821	–
Cr. lundellii	G0516	Hungary	–	MK277888
Cr. luteolus	16834	–	JF907963	–
Cr. lutescens	HMJAU 37002	–	NR_158400	–
Cr. macedonicus	G0394	Hungary	–	MK277889
Cr. malachioides	SLO1250	Czech Republic	NR_132047	KF154018
Cr. malachius	SLO1497	the United States	MF621032	MF621026
Cr. martinii	MCA640	the United States	–	AF367944
Cr. mollis	PBM1036	the United States	–	DQ986293
Cr. neotrichocystis	MCVE22213	Italy	MT055895	–

Table 5.1 (continued)

Species name	Voucher	Countries	GenBank accession numbers	
			ITS	LSU
Cr. nephrodes	DED 8316 (SFSU)	USA	KX017200	–
Cr. novae-zealandiae	PDD:95850	New Zealand	HQ533046	–
Cr. nyssicola	TJB8699	the United States	–	AF205690
Cr. occidentalis	MUOB:367585	USA	OK376745	–
Cr. praecipuus	PDD:72624	New Zealand	KY827312	–
Cr. pallidus	K(M):203676	United Kingdom	MZ159614	–
Cr. palodensis	TBGT 16716	India	MH844890	–
Cr. pseudomollis	HMJAU 37125	China	NR-173280	–
Cr. roseus	TBGT15507	India	MK567976	MK567977
Cr. roseocarpus	**MFLU23-0435**	**Thailand**	**OR785703**	**OR785693**
Cr. roseocarpus	**MFLU23-0437**	**Thailand**	**OR785704**	**OR785694**
Cr. rufidulus	PDD 98272	New Zealand	NR_159823	–
Cr. sinuosus	OKM26290	the United States	–	AF367945
Cr. sphaerosporus	OKM27013	the United States	–	AF205682
Cr. striatus	**MFLU23-0441**	**Thailand**	**OR785708**	–
Cr. striatus	**MFLU23-0442**	**Thailand**	**OR785709**	–
Cr. stenocystis	SLO 2557	Slovakia	OM832553	OM832581
Cr. stipitatus	NEMF 2018 iNaturalist # 14815648	USA	ON416906	–
Cr. striatus	HMJAU37087	China	MH320742	–
Cr. subfulviceps	Mushroom Observer # 316092	Mexico	MH484051	–
Cr. subverrucisporus	15720	Italy	JF907961	–
Cr. tennesseensis	TENN 029144	USA	NR_119720	–
Cr. thermophilus	Gy283	Panama	MF163183	–
Cr. tigrensis	B2200	Hungary	–	MK277892
Cr. thailandicus	**MFLU23-0424**	**Thailand**	**–**	**OR785684**
Cr. thailandicus	**MFLU23-0425**	**Thailand**	**OR785697**	**OR785685**
Cr. thailandicus	**MFLU23-0426**	**Thailand**	**–**	**OR785686**
Cr. tobolensis	LE287655	Hungary	MK522393	NG068881
Cr. tortus	TBGT17194	India	MK462161	–

Table 5.1 (continued)

Species name	Voucher	Countries	GenBank accession numbers	
			ITS	LSU
Cr. trichocraspedotus	HMJAU 37138	China	NR_173282	–
Cr. trulliformis	PDD:98274	New Zealand	KY827298	–
Cr. ungulatus	**MFLU23-0429**	**Thailand**	**OR785700**	**OR785689**
Cr. ungulatus	**MFLU23-0430**	**Thailand**	**OR785701**	**OR785690**
Cr. uber	MCA1403	the United States	–	AF367961
Cr. variabilis	SLO 2018	Slovakia	MT055890	OM832583
Cr. viscidus	**MFLU23-0432**	**Thailand**	**OR785702**	**OR785691**
Cr. viscidus	**MFLU23-0434**	**Thailand**	–	**OR785692**
Cr. versutus	PBM856	the United States	–	AY820890
Cr. volubilis	TBGT 15648	India	MH845231	MH310742
Cr. wasseri	K0696	Russia	MW722981	–
Cr. yuanchui	FFAAS0341	China	MZ401363	MW581519
N. dominicanus	F1133966	Mexico	JN033220	JN033221
N. echinospermus	MA-Fungi49404	Brazil	AJ419194	JN033222
N. plumbeus	F1068564	Puerto Rico	JN033223	NG_060271

Note the newly generated sequences in this study are shown in bold and "–" refers to the inaccessibility of the sequences.

5.3 Results

5.3.1 Phylogenetic Analysis

The combined dataset consisted of 96 *Crepidotus* and three *Neopaxillus* (Table 3.3). The final alignment, including the gaps, was 1,778 characters long and was deposited in TreeBASE (submission ID 30916). Bayesian and ML analyses resulted in similar tree topologies; thus, only the ML tree is shown with maximum likelihood bootstrap (BS) values and Bayesian posterior probabilities (PP). In the phylogram

(Figure 5.1), the new species *Cr. flavocarpus* (MFLU23-0427, MFLU23-0428) was closely related to *Cr. alabamensis* Murrill (TBGT15610) from India and *Cr. albolanatus* E. Horak (PDD:72865) from New Zealand with 86% BS, low supported for Bayesian analysis. *Crepidotus lateralipes* E. Horak (MFLU23-0440) was grouped with *Cr. lateralipes* (PDD 72508) from New Zealand with high support (100% BS, with low support Bayesian analysis) and closely related to *Cr. dentatus* T. Bau & Y.P. Ge (HMJAU 37097) from China. *Crepidotus roseocarpus* (MFLU23-0435, MFLU23-0437) was closely related to *Cr. thailandicus* (MFLU23-0425) with high support (98% BS, 1.00% PP). *Crepidotus striatus* T. Bau & Y.P. Ge (MFLU23-0441, MFLU23-0442) grouped with *Cr. striatus* (HMJAU37087) from China with high support (99% BS, 0.90% PP). *Crepidotus ungulatus* (MFLU23-0429, MFLU23-0430) was closely related to *Cr. striatus* from China and Thailand with low support (99% BS, 0.99% PP). *Crepidotus chiangraiensis* (MFLU23-0438, MFLU23-0439) was closely related to *Cr. praecipuus* E. Horak (PDD:72624) from New Zealand (with low support for BS, 0.94% PP). *Crepidotus viscidus* (MFLU23-0432, MFLU23-0434) was closely related to a seven-clade and separated from the *Cr. flavocarpus* subclade to *Cr. roseus* Singer (TBGT15507).

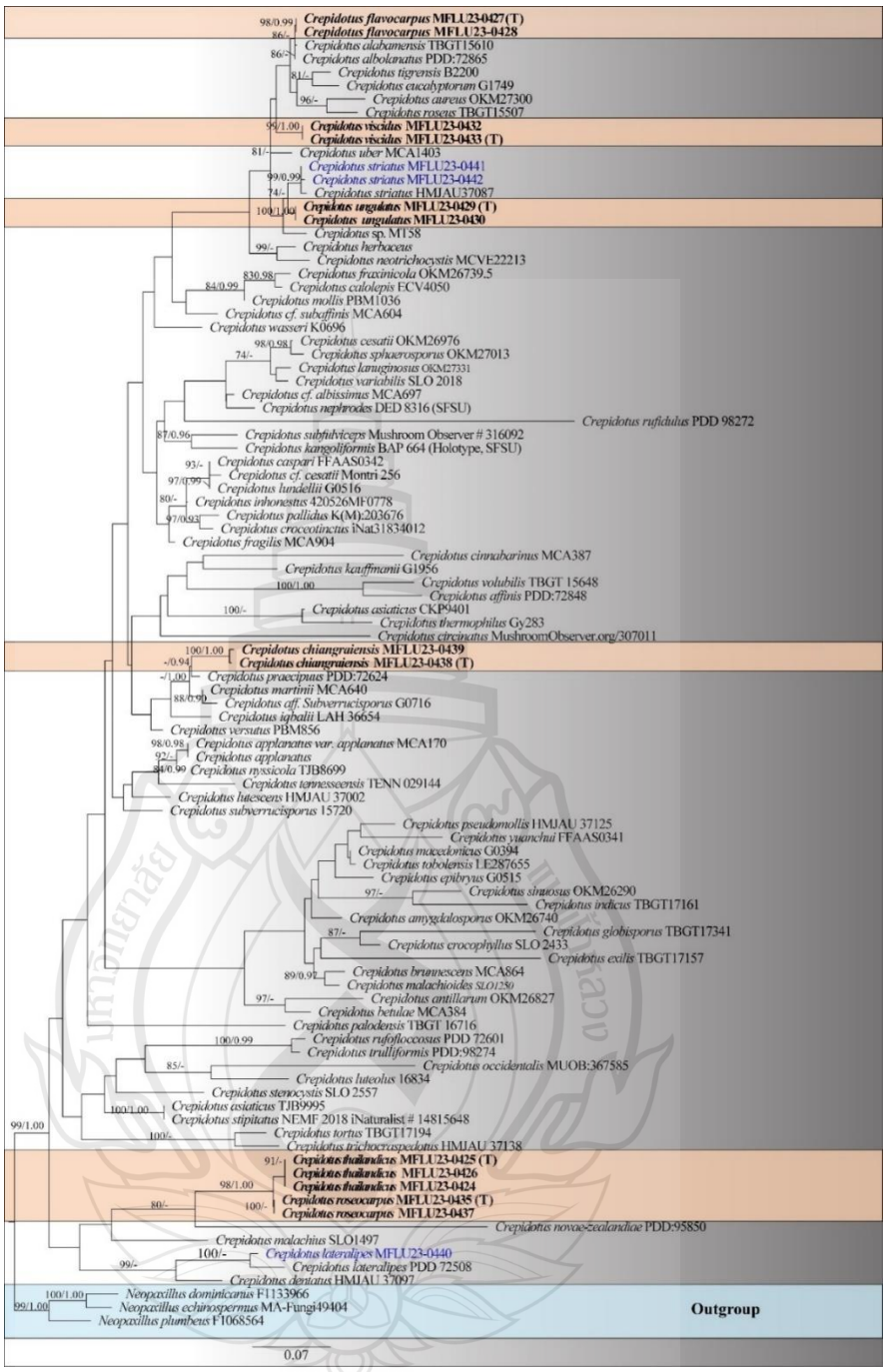


Figure 5.1 Phylogeny of selected *Crepidotus* sequences based on a maximum likelihood analysis of two nuclear gene regions (ITS and nrLSU). The bootstrap values of maximum likelihood (BS ≥ 70%) and the posterior probabilities values of Bayesian (PP ≥ 0.90) are shown on the branches. Newly sequenced collections are in bold black, and new records are in blue. Three species of *Neopaxillus* were used as outgroups. (T) designates holotypes

5.3.2 Taxonomy

5.3.2.1 *Crepidotus chiangraiensis* Phonemany, Thongkl. & Sysouph., *sp. nov*. (Figures 5.2 & 5.3)

Faces of fungi number: FoF 15096, MycoBank: MB 850908.

Etymology. *chiangraiensis* refers to the "Chiang Rai Province" where the holotype was collected.

Holotype. THAILAND, Chiang Rai Province, Mae Fah Luang District, Mae Sa Long Nok Subdistrict, 20°10'10.9"N, 99°37'21.5"E, 14 June 2019, Monthien Phonemany (MFLU23-0438).

Diagnosis. This species is recognised by small basidiomata velutinous pileus with white hairs, short stipe, subglobose to oblong obovoid basidiospores, utriform to sublecythiform with subcapitate at the apex, without pleurocytidia, regular hymenophoral trama, trichodermal pileipellis, and presence of clamp-connections.

Description: Basidiomata pleurotoid, small. *Pileus* 35–45 × 20–40 mm; orbicular, rounded flabelliform, with an involute margin when young and becoming straight when mature; white, becoming yellowish white (4A2) to pale yellow (4A3); densely velutinous with white hairs longer near the attachment zone, not hygrophanous, covered with white woolly hyphae at the attachment zone; margin wavy, split when mature. *Lamellae* 1 mm wide, adnexed, white when young, then pale yellow (4A3) to yellowish white (4A2) near the point of attachment, with dark brown spots on the surface of the lamellae, becoming brown (6D6) when fully mature, with 4 tiers of lamellulae, with smooth edge, moderately crowded. *Stipe* 1 mm wide, present in primordial stages, equal, white, rudimentary when mature, knob-liked. *Context* very thin, white. *Odour* mild. *Taste* none. *Spore print* brown (6D6).

Basidiospores [150/3/2] (6.1–)6.6–8.1–9.6(–9.6) × (4.8–)5.3–6.2–7.5(–7.7) µm, *Q* = (1.08–)1.13–1.3–1.59(–1.85), subglobose to obovoid, buff yellow (5A3) to light yellowish brown (8D5) in KOH and water, smooth, thick-walled. *Basidia* (14.1–)14.2–16–18.4(–18.4) × (5.2–)5.2–6.5–7.4(–7.4) µm, clavate to subcylindrical, 4-spored, hyaline, smooth, thin-walled. *Cheilocystidia* (22.5–)22.5–36.4–61.3(–61.3) × (6.2–)6.2–11.8–25(–25) µm, various in shape, narrowly utriform to utriform, fusiform, lecythiform to sublecythiform, with subcapitate at apex, hyaline, smooth, thin-walled. *Pleurocystidia* absent. *Hymenophoral* trama regular, hyphae 2–5 µm wide. *Pileipellis*,

a trichoderm composed of parallel hyphae with 2–5 µm wide, branched, hyaline, with cylindrical terminal elements 45.6–90.54 × 5.15–7.08 µm. *Oleiferous* hyphae 2–4 µm present in hymenophoral trama. *Clamp connections* present in all the structures.

Habitat and distribution: — Saprobic on a dead branch of an unidentified tree, scattered or gregarious with at least 10 basidiomata. So far, it has only been found in Chiang Rai Province, Mae Fah Luang District, Mae Sa Long Nok Subdistrict, northern Thailand.

Additional specimens examined: — THAILAND. Chiang Rai Province, Mae Fah Luang District, Mae Sa long Nok Subdistrict, Mae Toe village, 20°10'27.7"N, 99°36'51.9"E, 14 June 2019, Monthien Phonemany (MFLU23-0439).

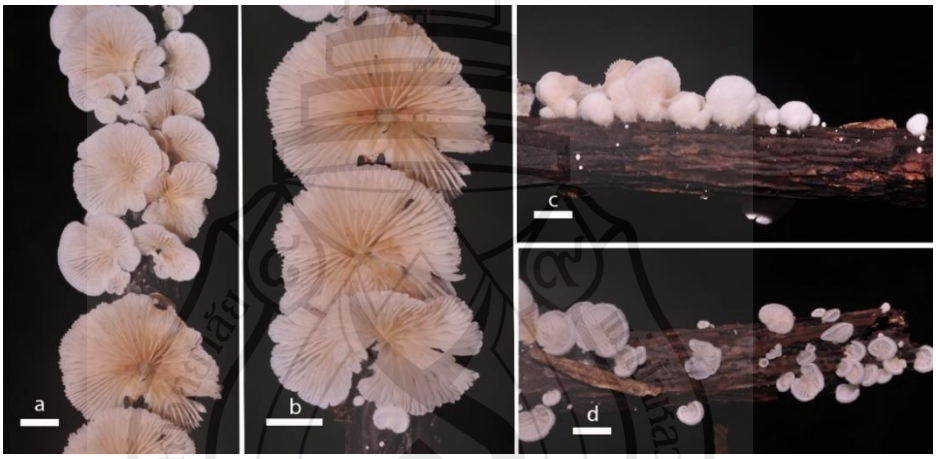


Figure 5.2 Basidiomata of *Crepidotus chiangraiensis* in the field. **a–b** MFLU230437, **c–d** MFLU23-0438. Scale bar **a–d** = 10 mm

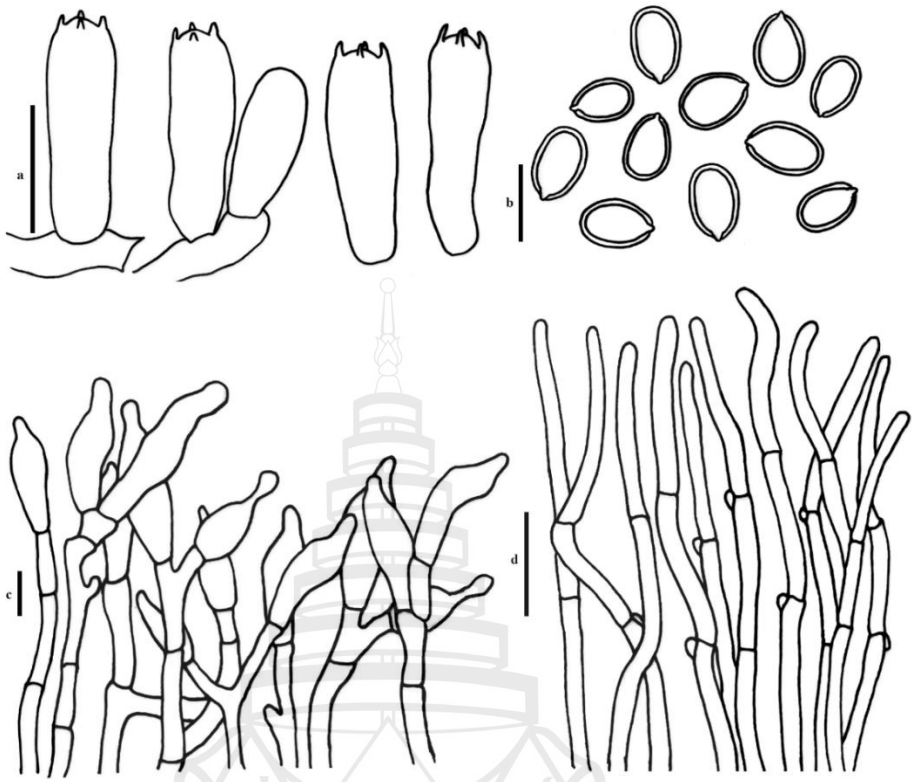


Figure 5.3 Micromorphology of *Crepidotus chiangraiensis*. **a** Basidia, **b** Basidiospores, **c** Cheilocystidia, **d** Pileipellis. Scale bar **a–b** = 10 µm, **c** = 20 µm, **d** = 50 µm

5.3.2.2 *Crepidotus flavocarpus* Phonemany, Thongkl. & Sysouph., *sp. nov.* (Figures 5.4 & 5.5)

Faces of fungi number: FoF 15097, MycoBank: MB 850911

Etymology: *flavus* refers to *yellow*, and *carpus* refers to *the body*; *flavocarpus* refers to the yellow basidiomata of the holotype.

Holotype: THAILAND. Chiang Mai Province, Mae On District, the mountain behind Thangthong lodges, 18°52'04.5"N, 99°18'26.5"E, 25 June 2020, Monthien Phonemany (MFLU23-0427).

Diagnosis: This species is characterised by small yellow basidiomata, velutinous pileus, densely white near the attachment zone and sparse toward the margin, brown basidiospores, various shape of cheilocystidia with utriform to cylindrical with sub-capitate at apex, inverse hymenophoral trama, and cutis pileipellis.

Description: Basidiomata pleurotoid, small. *Pileus* 10–30 × 9–25 mm; ungulate to spathuliform when primordial stages, becoming petaloid to rounded

flabelliform expanding to dimidiate, sometimes convex, plano-convex when mature, with an incurved margin when young and becoming straight when mature; yellow to yellowish, light yellow, pastel yellow, pale yellow becoming yellowish white then brownish grey to greyish brown when old (3B7–8, 3A2–5, 5D2–3); covered with densely velutinous at the point of attachment, with white hairs in the attachment zone and sparse toward the margin, disappearing when old, hygrophanous; margin with short striate or sulcate, concolorous with surface to paler yellow at first, then white to brownish when old. *Lamellae* 2–3 mm wide, adnexed to sinuate, pale yellow, pastel yellow to white (3A2–3, 3A1), becoming dark brown spots on the surface of the lamellae when mature, distant, with 3–4 tiers of lamellulae, with smooth edge. *Stipe* absent. *Context* very thin, white to translucent when old. *Odour* and *taste* indistinct, mild. *Spores* print brown (6D6, 6E6).

Basidiospores [150/3/3] (5.5–)5.6–6.8–7.6(–7.8) × (4.1–)4.3–5.5–6.5(–6.6) µm, Q = (1.01–)1.03–1.25–1.47(–1.53), globose to subglobose, yellowish brown (5D5–6), smooth, thick-walled. *Basidia* (14.8)14.8–18.7–21.2(–21.2) × (5–)5–6.9–8.1(–8.2) µm, subclavate, 4-spored, hyaline, smooth, thin-walled. *Cheilocystidia* (18.3)18.9–34.2–52.1(–55.9) × (4.1–)4.1–5.2–7.4(–7.8) µm, subcylindrical, narrowly utriform to sublecythiform, with subcapitate at apex, hyaline, smooth, thin-walled. *Pleurocystidia* absent. *Hymenophoral trama* inverse, hyphae 3–5 µm, with cylindrical terminal elements (17.1)18.8–25.1–30.6(–31.1) × (4.3–)4.4–5.5–6.7(–6.8) µm, hyaline, thin-walled. *Pileipellis* a cutis, hyaline to pale yellow, with cylindrical terminal elements 35–80 × 4–8 µm. *Clamp connections* present in pileipellis and hymenophoral trama.

Habitat and distribution: Saprobic on dead wood, scattered by 2–3 basidiomata. So far, only found in tropical forests in northern Thailand.

Additional specimens examined: THAILAND, Chiang Mai Province, Mae on District, Huai Kaeo Subdistrict, 18°52'04.0"N, 99°18'27.8"E, 25 June 2020, Monthien Phonemany (MFLU23-0428).

Figure 5.4 Basidiomata of *Crepidotus flavocarpus* in the field. **a–d** MFLU23-0427, **e–f** MFLU23-0428. Scale bars **a–f** =1 cm

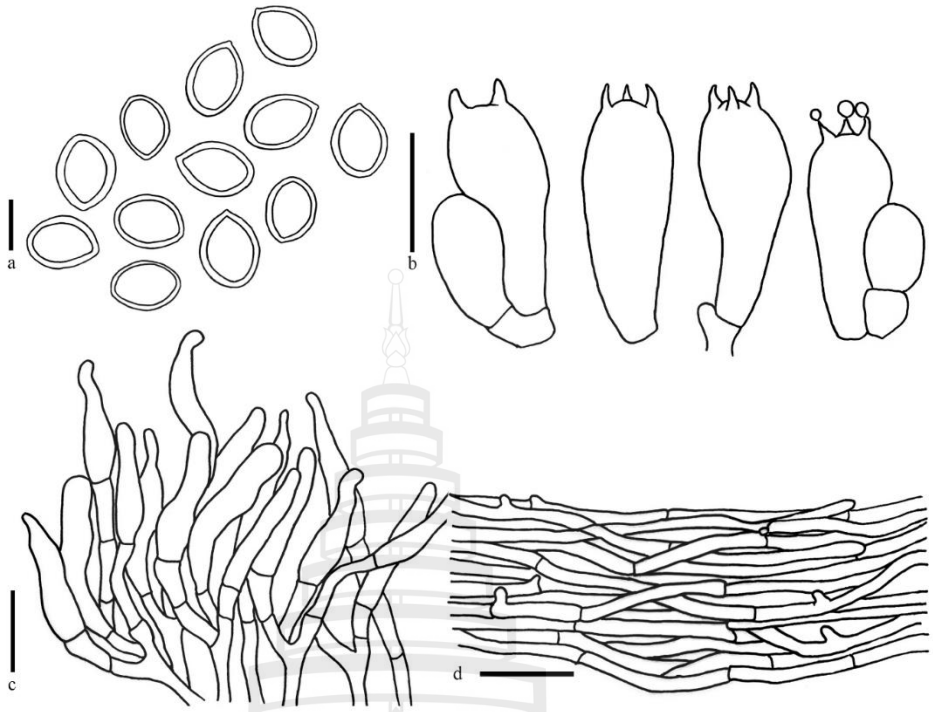


Figure 5.5 Micromorphology of *Crepidotus flavocarpus*, **a** Basidiospores, **b** Basidia and basidoles, **c** Cheilocystidia, **d** Pileipellis. Scale bars: **a** = 5 µm, **b** = 10 µm, **c** = 20 µm, **d** = 50 µm

5.3.2.3 *Crepidotus lateralipes* E. Horak, CBS Biodiversity Series 16: 32 (2018). (Figures 5.6 & 5.7)

Faces of fungi number: FoF 15098, Mycobank number: 820577.

Description: *Basidiomata* pleurotoid, small to medium. *Pileus* 20–48 × 20–30 mm; flesh, hygrophanous, dimidate, flabelliform to sub- flabelliform, with incurved margin and straight when mature; white in the primordia stage, becoming yellowish white (4A2), pale yellow (4A3), yellowish grey (4B2), finally turning orange-white (5A2) when mature; surface smooth, with exceeding margin. *Lamellae* 2 to 4 mm wide, adnexed, white when young, light brown to brown when old (5D4–5, 5E6), crowded, with more than five tiers of lamellulae, smooth edge. *Stipe* 2 × 1 mm present when young, cylindrical, white, smooth and not present when mature. *Context* 2–4 mm wide, white, yellowish white (4A2) to pale yellow (4A3). *Odour* mild. *Taste* none. *Spore print* brown (6E5) to cocoa brown (6E6).

Basidiospores [150/3/3] (4.9–)5.5–6.4–7.5(–8.0) × (4.3–)4.5–5.7–6.6(–6.9) µm, *Q* = (1.02–)1.04–1.13–1.28(–1.3), globose, rough or punctate, thick-walled, light brown (5D4). *Basidia* (16.7–)16.7–20.8–25.2(–25.3) × (7.1–)7.1–8.3–10(–10) µm, subclavate to clavate, with 2- to 4-spored, hyaline, smooth, thin-walled. *Cheilocystidia* absent. *Pleurocystidia* absent. *Hymenophoral trama* irregular, with cylindrical hyphae, 7–15 µm wide, hyaline, thin-walled. *Pileipellis* a cutis composed of cylindrical, and branched hyphae, terminal elements 60–90 × 9–13 µm. *Clamp connections* present in pileipellis and hymenophoral trama.

Habitat and distribution: Saprobic on dead wood, scattered or fasciculate by 2–4 basidiomata. It has been found in New Zealand and Thailand (this study).

Additional specimens examined: THAILAND. Chiang Mai Province, Pha Deng Village, Pa Pae Sub-district, Mare Taeng District, N19°07'14", E98°43'53", 27 May 2019, Monthien Phonemany (MFLU23-0440).

Figure 5.6 Basidiomata of *Crepidotus lateralipes*. **a–c** MFLU23-0440. Scale bar **a–c** = 10 mm

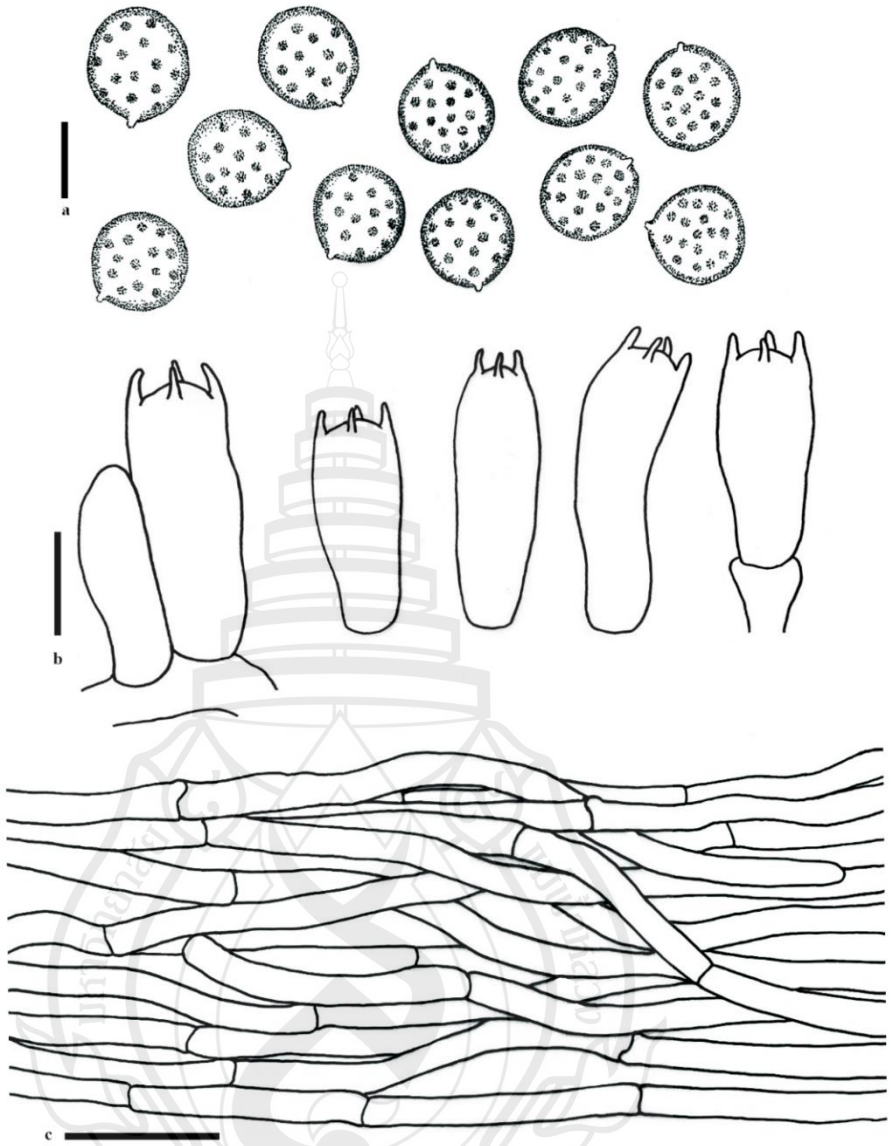


Figure 5.7 Micromorphology of *Crepidotus lateralipes*, **a** Basidiospores, **b** Basidia, **c** Pileipellis, Scale bar **a–d** = 10 µm, **c** = 50 µm

5.3.2.4 *Crepidotus roseocarpus* Phonemany, Thongkl. & Sysouph., *sp. nov*. (Figures 5.8 & 5.9)

Faces of fungi number: FoF 15099, MycoBank number: MB 850912

Etymology: *roseo* refers to pink, *carpus* refers to body or basidiomata.

Holotype: THAILAND. Chiang Rai Province, Mae Sai District, Huai Khrai Village, 20°16'05.2"N, 99°51'21.0"E, 3 October 2020, Yuwei Hu (MFLU23-0435).

Diagnosis: This species is characterised by having large pastel pink to pinkish all parts of basidiomata, ungulate to flabelliform pileus, absence of stipe, globose to subglobose punctate basidiospores, absence of cheilo- and pleurocystidia convergent hymenophoral trama with cylindrical terminal elements, a cutis pileipellis, and presence of clamp-connections.

Description: *Basidiomata* pleurotoid, small to medium. *Pileus* 15–30 × 10–45 mm; ungulate to spathuliform, flabelliform, with an inflexed margin when young and turning straight when mature; rose or pastel pink (11A5), pale red (10B6), pinkish (10A3–2); smooth or slightly fibrillose; with a split or sulcate-striate margin when mature, concolorous with surface or paler with pinkish white (11A2) when mature. *Lamellae* 2–4 mm wide, adnexed to sinuate, rose or pastel pink (11A5), subdistant to moderately crowded, with 4–5 tiers of lamellulae, smooth edge. *Stipe* 2 mm, present when primordial stages, cylindrical, pale red (10A4), smooth, not present in mature stages. *Context* 1–3 mm thick, thicker near the point of attachment then thinner toward the margin, pinkish to white (10A2–1). *Odour* and *taste* indistinct, mild. *Spores* print brown (6D6, 6E6).

Basidiospores [150/3/3] (5.6–)5.7–6.3–6.9(–7.5) × (5–)5.0–5.4–6.2(–6.6) µm, *Q* = (1.05–)1.08–1.16– 1.31(–1.34), globose to subglobose, rough or punctate, thick-walled, light brown. *Basidia* (13.4–)13.9–18.4–21.6(–21.6) × (5–)5.1–6.1–7.4(–7.5) µm, clavate, hyaline, smooth, thin-walled, 4-spored, with short sterigma. *Cheilocystidia* absent. *Pleurocystidia* absent. *Hymenophoral* trama convergent, with terminal elements (45.5–)45.5–75–93.5(–93.5) × (12.1–)12.1–17.6–20.4(–20.4) µm, hyaline, smooth, thin-walled. *Pileipellis* a cutis composed of cylindrical hyphae with sometimes swollen or interwoven, terminal cells cylindrical, (52.6–)52.6–76.2–111.7(–111.7) × (7.6–)7.6–10.8–13.5(–13.5) µm, hyaline, interwoven hyphae, smooth hyaline, 10–25 µm wide. *Clamp connections* present in pileipellis, pileus trama, and hymenophoral trama.

Habitat and distribution: Saprobic on the bark of a living tree, scattered or fasciculate by 3–15 basidiomata. So far, this has only been found in tropical forests in northern Thailand.

Additional specimens examined: THAILAND. Chiang Rai Province, Mae Sai District, Sala Choeng Doi, 20°16'35.4"N, 99°50'34.5"E, 3 October 2020, Monthien phonemany (MFLU23-0436), (MFLU23-0437).

Figure 5.8 Basidiomata of *Crepidotus roseocarpus* in the field. **a** and **b** MFLU23-0435, **c–f** MFLU23-0437. Scale bar **a–f** = 10mm

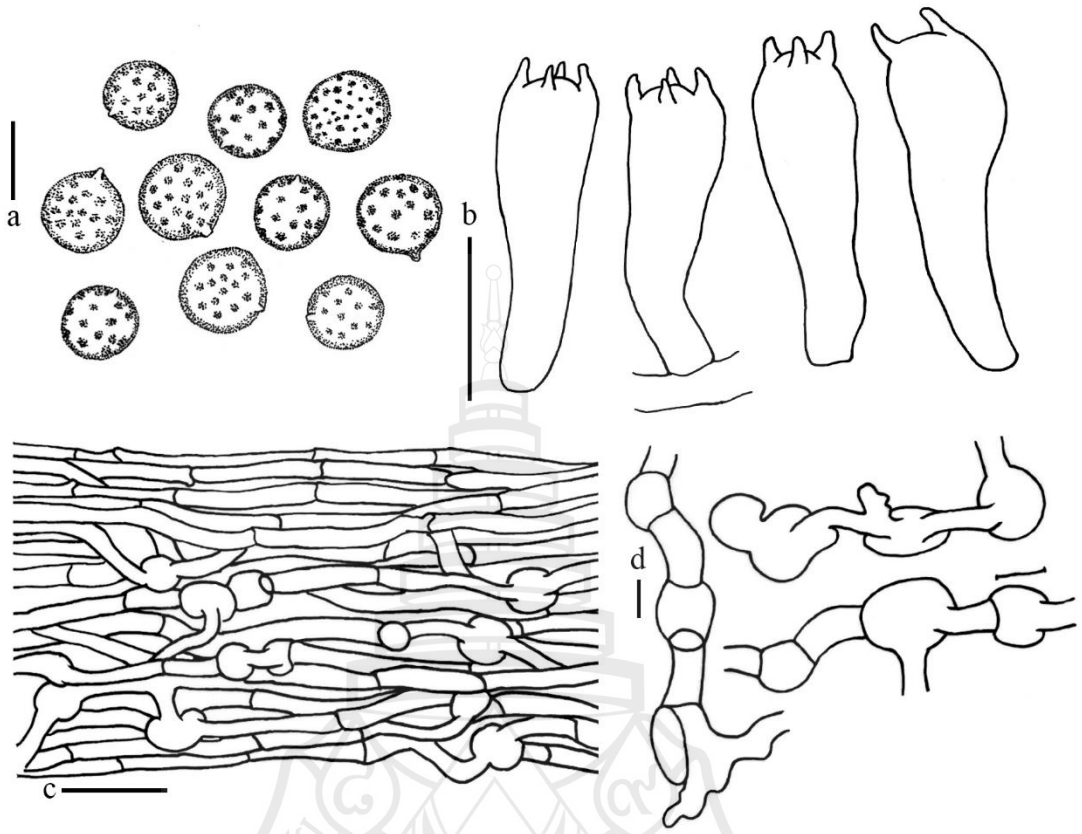


Figure 5.9 Micromorphology of *Crepidotus roseocarpus*, **a** Basidiospores, **b** Basidia, **c** Pileipellis, **d** swollen hyphae in pileipellis. Scale bar **a–d** = 10 µm

5.3.2.5 *Crepidotus striatus* T. Bau & Y.P. Ge, in Ge & Bau, Mycosystema 39(2): 251 (2020), (Figures 5.10 & 5.11).

Faces of fungi number: FoF 150100, Mycobank number: 830020.

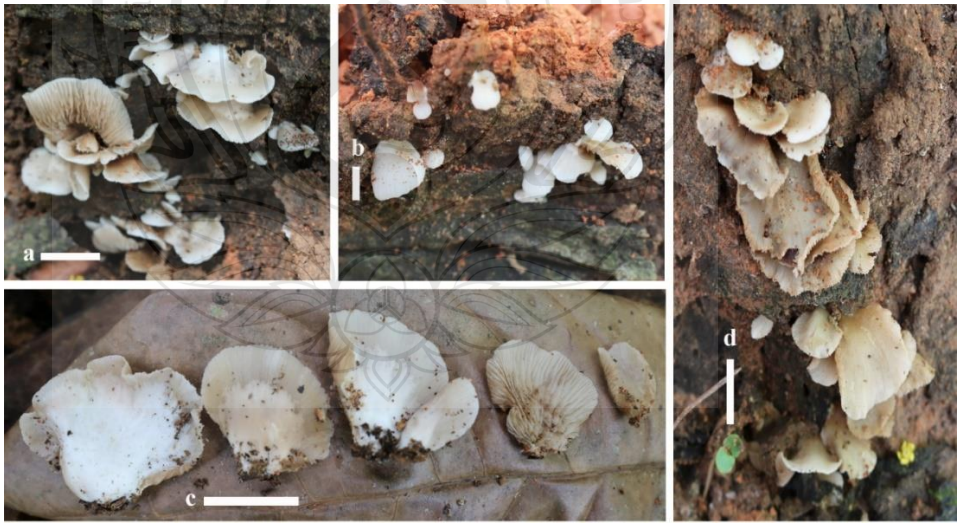
Description: *Basidiomata* pleurotoid, small. *Pileus* 5–15 × 8–20 mm; labelliform, sapathuliform, rounded flabelliform to sub-rounded flabelliform, white to whitish when young then becoming light yellow (4A4) to brownish orange (5C3); surface smooth, moist, with slightly viscid; the point of attachment covered by white woolly hyphae; margin deflexed to inflexed, becoming straight to reflexed with age, inconspicuous striae, crenate. *Lamellae* 1–2 mm, decurrent, white to whitish, becoming slightly brownish orange to brown with age (5C3, 5E4), moderately crowded, in 4–5 tiers. *Stipe* 1 mm long, or sometimes pseudostipe, whitish, translucent, cylindrical, with

tomentose. *Context* thin, translucent, *Odour* mild. *Taste* none. *Spore prints* brown (6E6).

Basidiospores [150/3/2] (6.8–)7.3–8.0(–8.6) × (4.1–)5.2–5.4– 6.2(–6.7) µm, Q = (1.3–)1.36–1.67–2.01(–2.03), ellipsoid to elongate (oblong) in side view, light brown, smooth, thin-walled, inamyloid. *Basidia* (21–)21–25.8–35(–37) × (5–)5.3–7.3–10.5(–11) µm, subclavate to clavate, with 2 to 4 sterigmata, hyaline, smooth, thin-walled. *Cheilocystidia* (24.5–)24.7–35–43.6(–44.1) × (7.6–)7.6–11.5–14.5(–14.5) µm, lageniform. *Pleurocystidia* absent. *Hymenophoral* trama irregular, hyphae 5–8 µm wide. *Pileipellis* a cutis, parallel hyphae 4–8 µm, branched hyphae up to 30 µm, with pileocystidia-liked terminal elements (41.1–)41.1–65.6–85.4(–85.4) × (4.1–)4.1–6.2–8(–8) µm. *Clamp* connections present in pileipellis, and hymenophoral trama.

Habitat and distribution: Saprobic on a dead branch, scattered by 3–8 basidiomata. This has been found in Tha Pha Village, Mae Taeng District. Chiang Mai Province, Thailand.

Additional specimens examined: THAILAND. Chiang Mai Province, Mae Taeng District, Tha Pha Village, Highway 1095 at the 22 km marker of Mae Ma Lai-Pai Road, 19°07'36"N, 98°45'48"E, 27 May July 2019, Monthien Phonemany (MFLU23-0441, MFLU23-0442).



Figures 5.10 Basidiomata of *Crepidotus striatus* in the field. **a–c** MFLU23-0441, **d** MFLU23-0442. Scale bar **a–f** = 10 mm

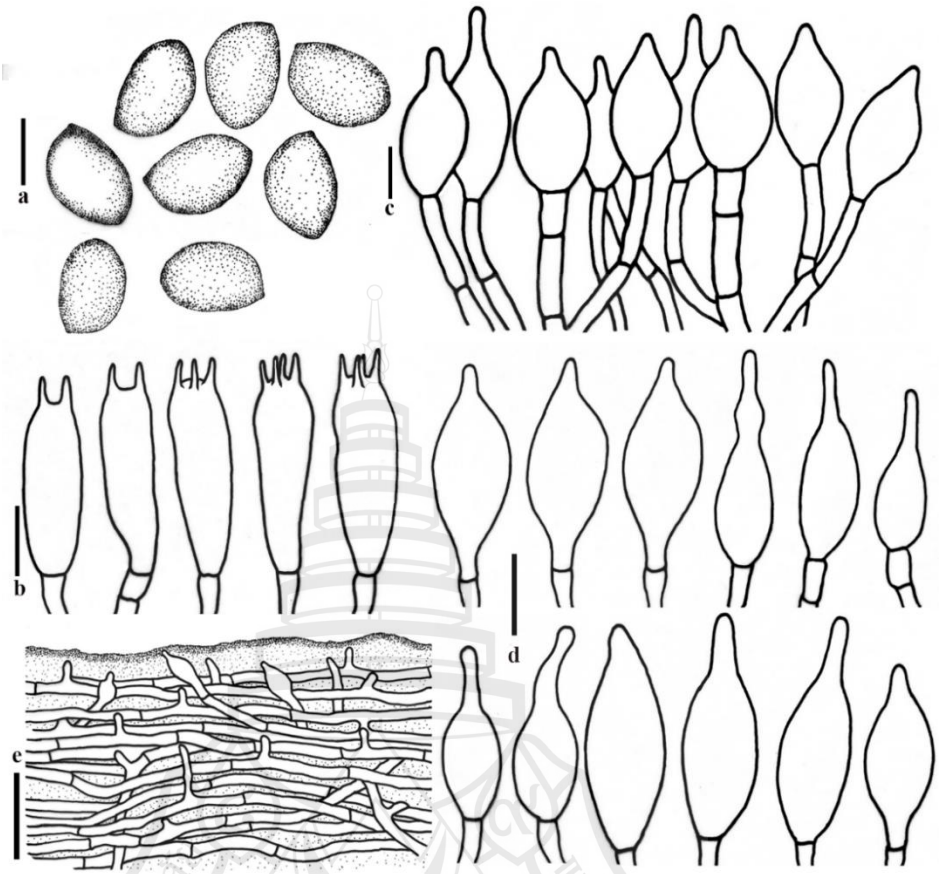


Figure 5.11 Micromorphology of *Crepidotus striatus*. **a** Basidiospores, **b** Basidia, **c** group of cheilocystidia, **d** cheilocystidia, **e** Clamp connections present in pileipellis. Scale bar **a** = 5 µm, **b** = 10 µm, **c**–**d** = 20 µm, **e** = 50 µm

5.3.2.6 *Crepidotus thailandicus* Phonemany, Thongkl. & Sysouph., *sp. nov.* (Figures 5.12 & 5.13)

Faces of fungi number: FoF 150101, MycoBank: MB 850914

Etymology: *thailandicus* refers to the country "Thailand" where the holotype was found.

Holotype: Holotype. THAILAND, Chiang Mai Province, Mae On District, Pox Kaew Village, 18°52'1"N, 99°19'29"E, 24 June 2019, Monthien Phonemany (MFLU23-0425).

Diagnosis: This species is characterised by small to medium basidiomata, tomentose pileus covering with white hair in primordial to young stages and disappeared when mature, with long transtucent-striate, globose to subglobose punctate

basidiospores, absence of cheilocystidia and pleurocystidia, a cutis pileipellis and presence of clamp-connections.

Description: Basidiomata pleurotoid, small to medium. *Pileus* 25–50 × 15–60 mm; rounded flabelliform to reniform, convex in the attachment zone, with an incurved margin when young and going straight to reflexed when mature; white when young, turning yellowish white (4A1) to yellowish grey (4B2), brown (6D6) to light brown (6D7); surface tomentose with white hair in the primordial to young stages, disappearing when mature, the attachment zone covered with white woolly hyphae; margin white, with long translucent-striate reward to the attachment zone. *Lamellar* 1–2mm wide, adnate, white when young, becoming whitish to pale yellow (4A2) to brown (5D6) when fully mature, crowded, with 4–5 tiers of lamellulae. *Stipe* 1–2 × 1–1.5 mm, pseudostipe, eccentric to lateral, whitish, translucent, cylindrical. *Context* 1 mm wide, translucent. *Odour* mild. *Taste* none. *Spore print* brown (5D6).

Basidiospores [150/3/3] (4.9–)5.2–5.9–6.7(–6.9) × (4.7–)4.8–5.3–5.8(–6.2) µm, *Q* = (1.0–)1.05–1.11–1.22(–1.29), globose to subglobose, punctate to verrucose, buff yellow to light orange-yellow in KOH, brownish in water. *Basidia* (15.3–)15.3–20.7–24.9(–24.9) × (6–)6.0–7.5–8.9(–8.9) µm, clavate to subcylindric, hyaline, smooth, thin-walled 4-spored, sterigma long 2–2.5 µm long. *Cheilocystidia* absent. *Pleurocystidia* absent. *Hymenophoral trama* subirregular to subinverse, hyphae 4–8 µm wide, with subcylindrical to sunclavate terminal elements 48.18–104.58 × 12.45–28.72 µm, hyaline. *Pileipellis* a cutis, composed of parallel hyphae 4–8 µm wide, branched, hyaline, with cylindrical terminal elements 54.6–91.34 × 9.15–14.08 µm. *Oleiferous* hyphae 2–4 µm present in the hymenophoral trama and pileipellis. *Clamp connections* are present in all structures.

Habitat and distribution: Saprobic on dead branches, scattered, or gregarious with at least 3–8 basidiomata. This has only been found in Chiang Rai province and Chiang Mai province in northern Thailand.

Additional specimens examined: THAILAND, Chiang Rai province, Mae Fah Luang District, Mae Sa Long Nok Subdistrict, 20°10'10.9"N, 99°37'21.5"E, 10 June 2019, Monthien Phonemany (MFLU23-0424), Chiang Mai province, Mae on District, Pox Kaew Village, 18°52'1"N, 99°19'29"E 24 June 2019, Monthien Phonemany (MFLU23-0426).

Figure 5.12 Basidiomata of *Crepidotus thailandicus* in the field. **a** = MFLU23-0424, **c–d** = MFLU23-0425, **d–e** = MFLU23-0426. Scale bar **a–e** = 10 mm

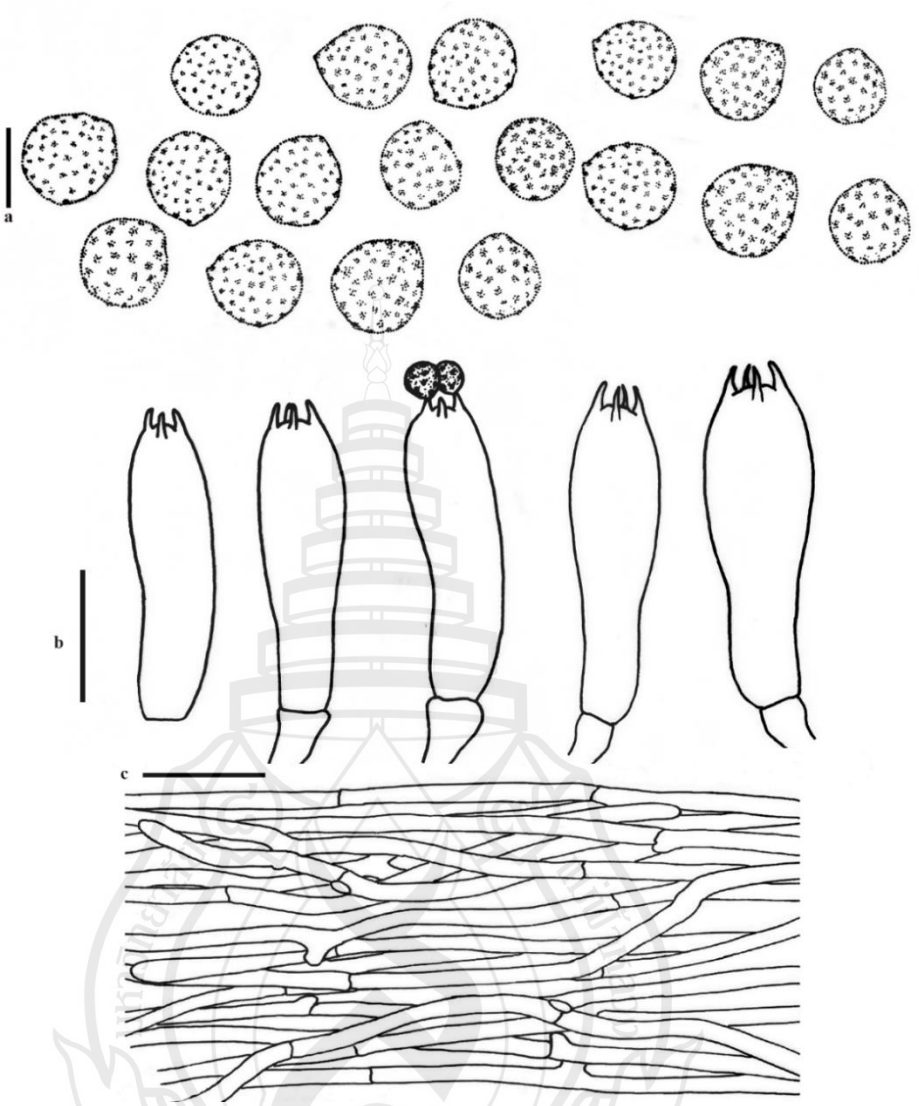


Figure 5.13 Micromorphology of *Crepidotus thailandicus*, **a** Basidiospores, **b** Basidia show clamp connections, **c** Pileipellis. Scale bar **a** = 5 µm, **b** = 10 µm, **c** = 50 µm, **d** = 100 µm

5.3.2.7 *Crepidotus ungulatus* Phonemany, Thongkl. & Sysouph., *sp. nov*. (Figures 5.14 & 5.15)

Faces of fungi number: FoF 150102, MycoBank: MB 850910

Etymology: *ungulatus* refers to the ungulate shape of basidiomata.

Holotype: THAILAND. Chiang Mai Province, Mae Taeng District, Tha Pha Village, Highway 1095 at 22 km marker of Mae Ma Lai-Pai Road, 19°07'36"N, 98°45'48"E, 15 July 2020, Monthien Phonemany (MFLU23-0429).

Diagnosis: This species is recognised by a small ungulate to spathulate basidiomata, white to light brown pileus, the absence of pseudostipe, broadly ellipsoid to subcylindrical basidiospores, lecythiform to sublecythiform cheilocystidia with subcapitate apex, absent pleurocystidia, a cutis pileipellis, the absence of oleiferous hyphae and clamp connections present.

Description: Basidiomata pleurotoid, small. *Pileus* 10–15 × 10–20 mm; ungulate to spathulate, with inflexed margin to involute margin; white when young, brownish orange (5C4–5, 5D5) near point of attachment, becoming light brown when mature (5D4–5); smooth, hygrophanous, covered with white woolly hyphae in the attachment zone; margin sulcate-striate, concolorous to the pileus surface. *Lamellae* up to 1 mm wide, decurrent, white when young, becoming pale yellow to yellowish (4A3–4A2), finally light brown (5D4–5), moderately crowded, with 4–5 tiers of lamellulae, with smooth edge. *Stipe* absent or pseudostipe, very short. *Context* is very thin, soft, and white. *Odour* none, *taste* none. *Spore prints* brown (6D6, 6E6).

Basidiospores [150/3/2] (5.5–)5.5–6–10.7(–11.2) × (4–)4–4.5–5.2(–5.4) µm, *Q* = (1.19–)1.25–1.51–2.56(–2.64), broadly ellipsoid to elongate, buff yellow to light orange-yellow in KOH and water, smooth, thick-walled. *Basidia* (12.1–)12.1–16.0–20.1(–20.6) × (4.8–)4.9–6.0–7.8(–7.8) µm, subclavate to clavate, 4-spored, sterigma 5–7 µm long, hyaline, smooth, thin-walled. *Cheilocystidia* (15.6–)15.6–28.3–75.3(–75.8) × (5–)5.2–9.5–23.7(–23.7) µm, narrowly lecythiform, lecythiform to sublecythiform with subcapitate at apex, hyaline, smooth, thin-walled. *Pleurocystidia* absent. *Hymenophoral* trama irregular, cylindrical hyphae 3–9 µm wide. *Pileipellis* a cutis composed of parallel hyphae 3–8 µm wide, hyaline, with cylindrical terminal elements 45.0–66.8 × 8.4–11.2 µm. *Oleiferous* hyphae absent. *Clamp connections* are present in all structures.

Habitat and distribution: Saprobic on the dead branch, scattered with at least 10 basidiomata. This has only been found in Chiang Mai Province, Mae Taeng District, Pang Hang Village in northern Thailand.

Additional specimens examined: THAILAND. Chiang Mai Province, Mae Taeng District, Pang Hang Village, 19°07'44.8"N, 98°45'47.8"E, 15 July 2020, Monthien Phonemany (MFLU23-0430).

Figure 5.14 Basidiomata of *Crepidotus ungulatus* in the field. **a** and **b** MFLU23-0429, **c** MFLU23-0430. Scale bar **a**–**e** = 10 mm

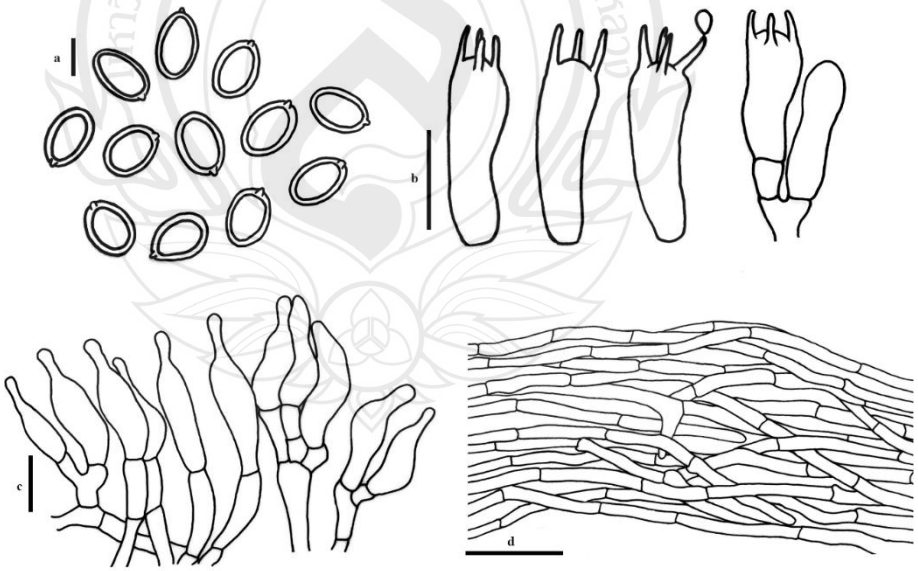


Figure 5.15 Micromorphology of *Crepidotus ungulatus*, **a** Basidiospores, **b** Basidia, **c** Cheilocystidia, **d** Pileipellis. Scale bar **a**–**b** = 10 µm, **c** = 20 µm, **d** = 50 µm

5.3.2.8 *Crepidotus viscidus* Phonemany, Thongkl. & Sysouph., *sp. nov*. (Figures 5.16 & 5.17)

Faces of fungi number: FoF 150103, MycoBank: MB 850915

Etymology: *viscidus* refers to viscid or striky basidiomata.

Holotype: THAILAND. Roi Et Province, Mayyadee District, Tambol Somsaart, Pa Phu Khao Thong Wild Temple, 16°25'55"N, 103°51'18"E, 18 August 2020, Monthien Phonemany (MFLU23-0432).

Diagnosis: This species is distinguished from other *Crepidotus* species by having a white semicircular pileus, translucent-striate with grey to dark grey margin, pale orange to orange-white lamellae, glutinous context, smooth basidiospore and hymenophoral trama present regular form.

Description: Basidiomata pleurotoid, small. *Pileus* 10–18 × 5–12 mm; semicircular, spathuliform, with incurved to straight margin; white (3A1) to pastel yellow (3A4); smooth, slightly viscid, not hygrophanus, the attachment zone covered by white woolly hyphae; margin zone, grey (1E1) to dark grey (1F1), with translucent-striate. *Lamellae* 1 mm wide, when young, white, becoming pale orange (5A2) to orange white (5A1), finally light brown with age (5D8). *Stipe* absent or pseudostipe. *Context* 1 mm wide, white, glutinous. *Odour* and taste none. *Spore print* brownish (6D8) to brown (6E8).

Basidiospores [150/3/3] (5–)5.4–6.3–7.4(–7.8) × (3.9–)4.2–4.8–5.4(–5.9) µm, *Q* = (1.04–)1.11–1.32–1.51(–1.54), globose to subglobose, ellipsoid in side view, smooth, yellow to light orange-yellow in KOH, brown in water, thin, thick-walled. *Basidia* (15.5)15.5–19.8–25.3(–25.3) × (5.8–)5.8–6.7–7.7(–7.7) µm, subclavate, 4-spored, sterigmata 3–5 µm long, hyaline, smooth, thin-walled. *Cheilocystidia* (13.5–)13.5–22.1–32.7(–32.7) × (5.6–)5.6–8.4–11.2(–11.6) µm, subcylindrical, narrowly utriform to utriform, fusiform to broadly fusiform, lecythiform to sublecythiform, with subcapitate at apex, hyaline, smooth, thin-walled. *Pleurocystidia* absent. *Hymenophoral trama* regular to subregular, hyphae 2–4 µm wide, hyaline. *Pileipellis* a cutis, composed of parallel hyphae 4–8 µm wide, branched, hyaline, with cylindrical terminal elements 56–90 × 5–7 µm. *Oleiferous* hyphae 2–4 µm present in hymenophoral trama. *Clamp connections* are present in all structures.

Habitat and distribution: Saprobic on dead wood, scattered or fasciculate by 4–10 basidiomata. This has only been found in Chiang Mai and Roi Et provinces in northern Thailand.

Additional specimens examined: THAILAND, Chiang Mai Province, Mae on District, Pox Kaew Village, 18°52'1"N, 99°19'29" E, 25 June 2019, Monthien phonemany (MFLU23-0431). Roi Et Province, Mayyadee District, Somsaart Subdistrict, 16°25'49.7"N, 103°51'26.2"E, 18 August 2020, Monthien Phonemany (MFLU23-0433), (MFLU23-0434).

Figure 5.16 Basidiomata of *Crepidotus viscidus* in the field. **a–c** = MFLU23-0432, **d** = MFLU23-0434. Scale bar **a–d** = 10 mm

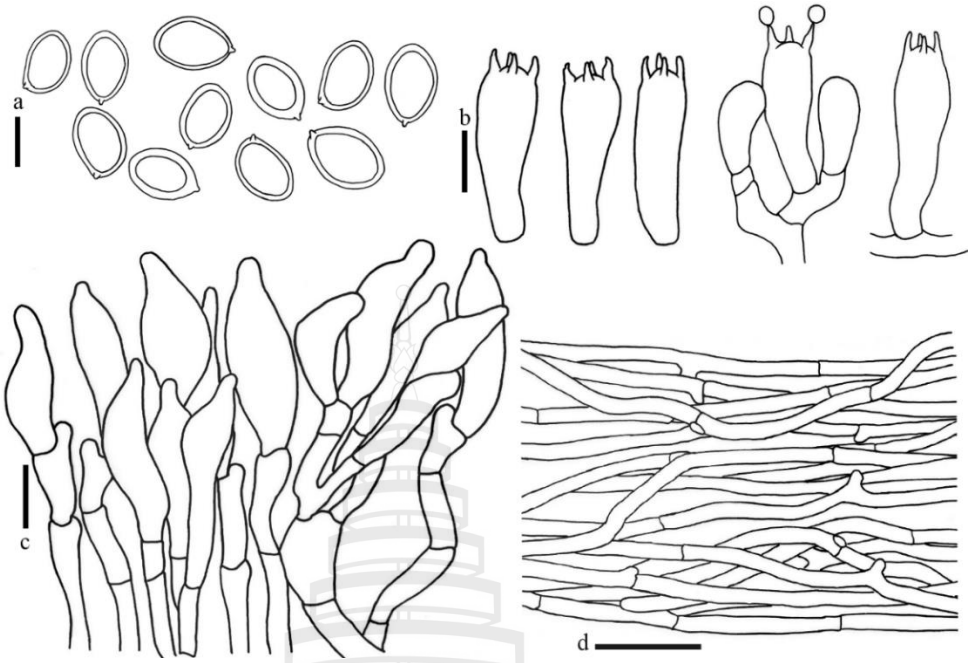


Figure 5.17 Micromorphology of *Crepidotus viscidus*. **a** Basidiospores, **b** Basidia, **c** Cheilocystidia, **d** Pileipellis, scale bar **a** = 5 µm, **b–c** = 10 µm, **d** = 50 µm

4.4 Discussion

Crepidotus chiangraiensis has the main characteristics of having small orbicular basidiomata, densely velutinous with white hairs longer near the point of attachment, not striate with wavy and split with age, and smooth globose basidiospore. According to morphology, related species include *Cr. epibryus* (Fr.) Quél., *Cr. martini* Singer, *Cr. palodensis* C.K. Pradeep & A.M. Kumar, *Cr. variabilis* (Pers.) P. Kumm. However, the species different from *Cr. chiangraiensis* are the following: *Cr. epibryus* was described as *Agaricus epibryus* Fr. differs by having smaller basidiomata (2–3 mm), wrinkled verruculose basidiospores, and lack of clamp connections (Quélet, 1888). *Crepidotus martini* described from Panama differs by having smaller (2–9 mm), punctate basidiospores, cylindric to ventricose cheilocystidia, and a pileipellis as an epicutis (Singer, 1955). *Crepidotus palodensis* described from India differs by having broadly elongate to cylindrical, finely verrucose basidiospores and cheilocystidia that present clavate to cylindro-clavate with irregular excrescences (Kumar et al., 2018). *Crepidotus variabilis* described from Norway differs in that it has verrucose, cylindric

to elliptic basidiospores, and pileipellis as a cutis (Nordstein, 1990). According to the phylogenic tree, *Cr. chiangraiensis* was related to *Cr. praecipuus* (PDD:72624) with high support (Figure 5.1). However, the comparison of morphology of both species was different, *Cr. praecipuus* has tomentose-scaly and yellowish basidiomata, yellowish-brown to brown fibrillose scales, and ellipsoid basidiospores (Kim et al., 2021). Another related species was *Cr. martini* (MCA640), and the morphology comparison found that both species were different, *Cr. martini* has brownish white and convex pileus, smaller cheilocystidia (19–30 × 7.8–13.5 µm) (Singer, 1955).

The basidiomata colour of *Cr. flavocarpus* is similar to that of *Cr. croceotinctus* Peck, *Cr. cristatus* Senn-Irlet & Immerzeel, and *Cr. parietalis* E. Horak, *Cr. praecipuus* E.Horak, *Cr. tobolensis* Kapitonov, Biketova & Zmitr., *Cr. yuanchui* Q. Na, Z.W. Liu & Y.P. Ge. These species have a yellowish, pale yellow, deep orange, and brownish-orange pileus colour pattern. However, *Cr. croceotinctus* is different from *Cr. flavocarpus* by having margin non-striate, larger basidiospores (7–8 × 5.5–6.5), vesiculose clavate cheilocystidia (Kumar et al., 2020). *Crepidotus cristatus* described in the Netherlands differs in having smaller basidiomata (2–10 mm), not hygrophanus, punctate warty, verruculose basidiospore (Senn-Irlet & Immerzeel, 2003). The description of *Cr. parietalis* from New Zealand differs by having a longer striate margin, smaller basidiospores (5–6.5 µm long), larger cheilocystidia (20–40 × 5–8 µm), with pileipellis trichoderm (Horak, 1977). *Crepidotus praecipuus* described from Korea differs by having minutely tomentose-scaly, yellowish-brown to brown fibrillose scales, and ellipsoid basidiospores (Kim et al., 2021). *Crepidotus yuanchui* described in China differs in having a wavy margin without sulcate, villose with white hair, and smaller basidiospores (5–6 × 4.2–5.2 µm) (Na et al., 2022). *Crepidotus tobolensis* described from Siberia, differs in that it has a matt surface, tomentose, non-squamulose, and broadly-lacrymoid basidiospores (Malysheva et al., 2022). Phylogenetically, *Cr. flavocarpus* (MFLU23-0427) was closely related to *Cr. alabamensis* (TBGT15610) and *Cr. albolanatus* (PDD:72865) with (86% BS, and low supported for Bayesian analysis). The genetic distance between the ITS sequence of *Cr. flavocarpus* and *Cr. alabamensis* was 0.15% (1/657) with four substitution heteromorphisms. The *Cr. flavocarpus* and *Cr. albolanatus* were 9.24% (59/638). In addition, the genetic distance between the nrLSU sequence of *Cr. flavocarpus* and *Cr. alabamensis* was 0.33% (3/904), and the

nrLSU of *Cr. albolanatus* was not available. However, the morphologies of both species are completely different, with *Cr. alabamensis* having smaller basidiomata (3–20 mm), lamellae radiating from a lateral point, and smaller cheilocystidia (17.5–40 × 5–10.5 µm) (Kumar et al., 2022). *Crepidotus albolanatus* has a pure white pileus, with larger basidiomata of 10 to 35 mm (Horak, 2018).

Crepidotus lateralipes described from New Zealand is characterized by having flesh, dimidate basidiomata, cream to golden yellow pileus, straight margin without striate, thick context, absent cheilocystidia, hymenophoral trama large hyphae 7–15 µm wide, and cutis pileipellis (Horak, 2018). The Thai specimens were similar to specimens from New Zealand-type species, but the Thai specimens were larger basidiomata. However, *Cr. lateralipes* (MFLU23-0440) were closely related to *Cr. lateralipes* (PDD 72508) with high support (100%). Therefore, both morphology and phylogenetic analysis were used for species confirmation. This study confirmed that *Cr. lateralipes* (MFLU23-0440) is new to the literature in northern Thailand.

Crepidotus roseocarpus has large pastel pink basidiomata. This species is easily confused with *Cr. roseus* Singer recorded from Florida due to pink basidiomata but smaller basidiomata (4–6 mm broad), its micromorphology presents cheilocystidia and larger basidia (22–27 × 6–7 µm) (Hesler & Smith, 1965). Another similar species is *Pleurotus djamor* (Rumph. ex Fr.) Boedijn but *P. djamor* has larger basidiomata (30–70 mm diam.), thicker context (4–8 mm), margin inflexed, and wavy when mature (Phonemany et al., 2021). *Crepidotus cinnabarinus* F.H. Møller & Westergaard recorded from Turkey was similar in colour to *Cr. roseocarpus* but differs in having smaller basidiomata (5–15 mm), pileus velvety-tomentose, present fusoid-ventricose cheilocystidia, and clamp connections absent (Güngör et al., 2014). In phylogenetic analyses, *Cr. roseocarpus* (MFLU23-0435, MFLU23-0437) was closely related to *Cr. thailandicus* (MFLU23-0425) with strong support. However, the comparison of morphology showed a difference between both species since *Cr. thailandicus* has smaller basidiomata with white pilei (this study).

Crepidotus striatus described in China has labelliform to semi-circular pileus, white, with a distinctly striate margin, lamellae edge gelatinous, cheilocystidia lageniform, pileipellis a cutis with pileocystidia-like terminal elements (Ge & Bau, 2020). The description of the morphology of *Cr. striatus* collected from northern

Thailand fit well with the holotype, and together, molecular analysis confirms that this is a new record, as in the phylogenetic *Cr. striatus* (HMJAU37087) was grouped with *Cr. striatus* (MFLU23-0441, MFLU23-0442).

Crepidotus thailandicus is characterized by a rounded flabelliform, tomentose surface with white hair when primordial to early stages, then disappearing when old; when mature, it presents long translucent-striate reward to the point of attachment that fades away at the central, punctate to verrucose basidiospores, with absent cheilocystidia and absent pleurocystidia. According to morphology comparisons do not detect a species description for *Cr. thailandicus* that exactly fits any of the literature currently available (Hesler & Smith, 1965; Dennis, 1974; Senn-Irlet ,1995; Bandala et al., 2008; Hausknecht & Krisai-Greilhuber, 2010; Kumar et al., 2018; Ge & Bau, 2020; Jančovičová et al., 2022; Kim et al., 2021; Kumar et al., 2020; Kumar et al., 2022). The most similar species were *Cr. globisporus* A.M.Kumar & C.K.Pradeep, but smaller basidomata (3–35 mm), surface fibrillose, vesiculose clavate cheilocystidia are present for *Cr. globisporus* (Kumar et al., 2020). The phylogenic tree showed that *Cr. roseocarpus* (MFLU23-0435, MFLU23-0437) was closely related to *Cr. thailandicus* (MFLU23-0425) with high bootstrap support (Figure 5.1). However, they are different in their morphological characteristics; *Cr. roseocarpus* has larger basidiomata and pastel pink, larger basidiospores (5.3–11.3 × 4.48–6.63 µm), larger basidia (13.9–21.6 × 5.1–7.4 µm), and the presence of hymenophoral trama in convergent form (this study).

Crepidotus ungulatus has the main characteristic of ungulate basidiomata, brownish orange near the point of attachment; the basidiomata colour changed to light brown in three hours, and the margin concolorous to the surface of the pileus. The available description of *Crepidotus* species, most similar to *Cr. ungulatus* was *Cr. applanatus* (Pers.) P. Kumm. But the morphology comparison found that *Cr. applanatus* had planoconvex to applanate, flat, and larger basidiomata (5–40 × 5–50 mm), stipe cylindrical whitish, with pubescent, larger basidia (13–19.4 × 5–6.6 µm), and smaller cheilocystidia (28.8–40.9 × 8.1–11.9 µm) (Jančovičová et al., 2022). In the phylogenetic tree of the species, the most closely related to *Cr. ungulatus* (MFLU23-0429) was *Cr. striatus* (HMJAU37087). However, total morphological differences such

as *Cr. striatus* have basidiomata, margin reflexed when old, lamellae edge gelatinous, pileipellis a cutis with pileocystidia-like terminal elements (Ge & Bau, 2020).

Crepidotus viscidus is characterised by a semicircular white pileus, margin zone grey to dark grey, lamellae pale orange to orange white, smooth basidiospore, narrowly utriform to utriform, fusiform to broadly fusiform, lecythiform to sublecythiform shape of cheilocystidia. In the phylogenetic study, the clustering of *Cr. viscidus* (MFLU23-0432) observed within the clade seven included closely related species are *Cr. alabamensis* (TBGT15610), *Cr. albolanatus* (PDD 72865), *Cr. aureus* (OKM27300), *Cr. eucalyptorum* (G1749), *Cr. flavocarpus*, *Cr. roseus* (TBGT15507), *Cr. tigrensis* (B2200). However, the species mentioned have different morphological characteristics; *Cr. alabamensis* described in India differs by having larger basidiomata (2–3 mm), pastel yellow pilei, smaller basidia (19–22 × 6.5 µm), and pileipellis an ixocutis (Kumar et al., 2022). *Crepidotus albolanatus* differs in having a pure white pileus, plano-convex to applanate shape of the basidiomata, with larger basidiomata (5–40 × 5–50 mm), stipe cylindrical whitish, with pubescent, larger basidia (13–19.4 × 5–6.6 µm), and larger cheilocystidia (28.8–40.9 × 8.1–11.9 µm) (Horak, 2018; Jančovičová et al., 2022). *Crepidotus eucalyptorum* described from Australia differs in having matt to fibro-villous or fibrillose scaly basidiospores (7.2–10 × 5–6.6 µm). *Crepidotus flavocarpus* described in Thailand differs in that it has yellow to yellowish pileus basidiomata, and the margin is discolorous and pale yellow (this study). *Crepidotus roseus* described in Turkey differs in that it has scarlet to cinnabar red, velvety-tomentose, verruculose basidiospores (7–8 × 5–6 µm), with thin walls (Güngör et al., 2014).

Most macromorphological characteristics of *Crepidotus* species are so confusing that it is difficult to distinguish them in the field, e.g., some *Crepidotus* species are commonly found white or whitish. To identify these species, their micromorphological characteristics should be observed in detail. Some species have differences in morphologies, but phylogenetic analyses appear to be conspecific, e.g., between *Cr. flavocarpus*, *Cr. alabamensis*, and *Cr. albolanatus*. However, the nucleotide differences between *Cr. flavocarpus* and *Cr. alabamensis* were observed as, ITS was 0.15% with four substitution heteromorphisms, and nrLSU was 0.33%. In addition, the nucleotide differences between *Cr. flavocarpus* and *Cr. albolanatus* were observed as ITS was 9.24%, and LSU had no available sequence data. Although the

percentage of genetic distance was low, the morphological differences mentioned above are well supported for *Cr. flavocarpus* to be a distinct new species. This study provides data on the ITS and LSU gene sequences and comprehensive details supporting the introduction of six *Crepidotus* species and two new records of *Cr. lateralipes* and *Cr. striatus*. Nine *Crepidotus* species have been reported from Thailand, including the eight species in this study and *Cr. asiaticus* that was previously reported by Guzmán-Dávalos et al. (2017). Further research is required for a more complete comprehension of *Crepidotus* taxonomy, and more new species may be found. A few studies have been performed on the secondary metabolites of *Crepidotus* species, and the value of this genus in this country can be increased by additional scientific research.

CHAPTER 6

TAXONOMY AND PHYLOGENY OF *HOHENBUEHELIA* COLLECTED FROM THAILAND

6.1 Introduction to *Hohenbuehelia*

Hohenbuehelia Schulzer (1866) belongs to the family Pleurotaceae Kühner in the order Agaricales Underw. In former studies, the asexual stages of *Hohenbuehelia* species were separately placed in the genus *Nematoctonus* Drechsler (Drechsler, 1941; Thorn & Barron, 1986). Following the One Fungus = One Name nomenclatural rule, both the asexual and sexual stages were placed under *Hohenbuehelia* (Taylor, 2011; McNeill et al., 2012; Thorn, 2013), with *H. petaloides* (Bull.) Schulzer as the type species. The infrasubgeneric classification of *Hohenbuehelia* genus is not as extensively detailed as some other genera, several taxonomic studies have proposed subdivisions based on morphological and molecular data. Currently, 126 taxon names are listed under *Hohenbuehelia* in Index Fungorum (http://www.indexfungorum.org/), for 50 accepted species (He et al., 2019; Wijayawardene et al., 2022), The typical characteristics of this genus are pleurotoid, gelatinous basidiomata, white basidiospores with a germ pore, lecythiform cheilocystidia (if present), and thick-walled metuloid pleurocystidia (Stevenson, 1964; Thorn & Barron, 1986; Corner, 1994; Silva-Filho & Cortez, 2017; Holec & Zehnálek, 2020). *Hohenbuehelia* and *Pleurotus* (Fr.) P. Kumm. have some similar morphological characteristics, but *Hohenbuehelia* is distinguished by the synapomorphic gelatinous layer in the context under the pileipellis, which is absent in *Pleurotus* (Mentrida, 2016). Most *Hohenbuehelia* species are decomposers and widely distributed in temperate and tropical areas (Laessoe & Petersen, 2019). *Hohenbuehelia* species have been found growing on dead branches, decayed wood, logs, and sometimes on the bark of living trees or on herbaceous stems (Holec & Zehnálek, 2020). A few *Hohenbuehelia* species have been reported as edible. However, they have very low culinary value (Cruz & Gándara, 2005). This genus is a good source of polyphenols and polysaccharides (Li et al., 2012; Li et al., 2017; Wang et al., 2018;

Wang et al., 2019). Bioactive compounds extracted from some *Hohenbuehelia* species were found to have antioxidant properties as well as antitumor and antiviral activities (Ji et al., 2012; Sandargo et al., 2018a). Furthermore, the new derivatives thiopleurotinic acid A and thiopleurotinic acid B from extracts of *H. grisea* (Peck) Singer (strain MFLUCC 12-0451) were found to exhibit cytotoxicity towards a mouse fibroblast cell line as well as antimicrobial activities (Sandargo et al., 2018a). Another compound, 4-hydroxypleurogrisein, was shown to inhibit hepatitis C virus infectivity in mammalian liver cells (Sandargo et al., 2018b). *Hohenbuehelia* sp. ZW-16 has been used for bioethanol production (Liang et al., 2013). Thorn et al. (2000) also found that the mycelia of the asexual stage of some *Hohenbuehelia* species are able to produce adhesive knobs that can capture nematodes. The diversity of bioactive compounds from *Hohenbuehelia* species and their potential applications underline the importance of detailed taxonomic study of this genus (Bohni et al., 2012; Shipley et al., 2006; Sandargo et al., 2018a; Sandargo et al., 2018b). Thailand has a high mushroom diversity with many new species yet to be discovered (Thongbai et al., 2018; Vadthanarat et al., 2021). Only four *Hohenbuehelia* species have been recorded from Thailand, namely *H. grisea*, *H. panelloides* Høiland, *H. petaloides*, and *H. reniformis* (G. Mever & Fr.) Sing. (Chandrasrikul et al., 2011; Sandargo et al., 2018a). However, most of those reports did not provide detailed morphological descriptions nor molecular data in order to confirm those identifications (*H. grisea* was identified based on ITS sequences only, without morphological data). Currently, a new species has been records is *H. nakhonphanomensis* (Somrithipol et al., 2025). In this study, during the survey of pleurotoid mushrooms in northern Thailand, several collections of *Hohenbuehelia* were obtained and studied. On the basis of morphological and phylogenetic results, two new species (*H. flabelliformis* and *H. lageniformis*) and *H. tristis* were recognised and described herein.

6.2 Material and Methods

6.2.1 Sample Collection and Morphological Study

All mushroom samples used in this study were collected from selected sites of Thailand (Table 3.1). The specimens were morphologically characterised as described in the section 2.1 of chapter 2.

6.2.2 DNA Extraction and Sequencing

DNA extraction, primers used for PCR conditions and sequencing were same in section 3.2 (Table 3.2) of chapter 3.

6.2.3 Sequence Alignment and Phylogenetic Analyses of *Hohenbuehelia*

Sequences checking, blasted sequences, and deposit new sequences same in section 3.3. All sequences are showed in Table 6.1 including the outgroup were retrieved and aligned using MAFFT v.7 (Katoh et al., 2017) on the online server (http://mafft.cbrc.jp/alignment/server/). For *tef*1, introns were delimited by comparing with the amino acid sequence of a reference sequence and locating the GT/AG motifs of the splicing sites and removed for further analyses. The ITS and LSU alignments were trimmed separately using TrimAl to eliminate ambiguously aligned positions (Capella-Gutiérrez et al., 2009). After checking for supported conflicts (BS ≥ 70%) between single-gene maximum likelihood (ML) phylogenies, a concatenated three-gene dataset was assembled.

All phylogenetic analyses were done on CIPRES science Gateway version 3.3 web server (Miller et al., 2010), accessed at https://www.phylo.org/. For both maximum likelihood and Bayesian analyses, a mixed-model (partitioned) scheme was used, with the alignment divided in the following three-character sets: ITS1+ITS2, LSU+5.8S, *tef*1. Maximum likelihood phylogenetic inference was performed using RAxML-HPC2 version 8.2.12 (Stamatakis, 2006) on XSEDE. Five *Pleurotus* species were used as outgroup. For Bayesian analysis, the best-fit substitution models were selected from jModelTest2 version 2.1.6 (Darriba et al., 2012) on XSEDE. The best-fit models were HKY+G for ITS1+ITS2, GTR+I+G for nrLSU+5.8S, and SYM+G for *tef*1. Bayesian analysis was performed in MrBayes version 3.2.7a (Ronquist et al., 2012). Two runs of five chains each were run for 500,000 generations and sampled every 200 generations.

The average standard deviation of split frequencies was 0.008720 at the end of the runs. The burn-in phase (25%) was estimated by checking the stationarity in the generation-likelihood plot in Tracer ver. 1.7.1 (Rambaut et al., 2018). The phylogenetic tree was visualised and edited in FigTree version 1.4.4 (Rambaut, 2018), and then exported and further edited in Adobe Illustrator CS3.

Table 6.1 GenBank accession numbers and geographical origins of taxa used in the phylogenetic analysis

Species	**Specimen/culture**	**Country**	**GenBank Accession Numbers**		
			ITS	**nLSU**	***tef*1**
Hohenbuehelia algonquinensis	RGT 870601/12 UWO (culture T-434)	Canada	KU355341	AF139950	KU355456
H. angustata	CBS 856.85	Canada	MH861919	MH873608	–
H. atrocoerulea	AMB 18080	Hungary	KU355304	KU355389	KU355439
H. auriscalpium	PRMJH1372007	Czech Republic	MT525862	MT534054	–
H. bonii	K(M):165700	England	KX064444	–	–
H. boullardii	JCM06005	Spain	MG553637	MG553644	–
H. canadensis, as *H. atrocoerulea* var. *grisea*	DAOM 158848	Canada	KU355356	–	–
H. carlothornii	AMB 18106	Costa Rica	KY698012	KY698013	–
H. chevallieri	WU:6528	Austria	KT388040	–	–
H. culmicola	Roux 3488	Italy	KU355323	–	–
H. cyphelliformis	Z+ZT 994	Switzerland	KU355325	KU355393	KU355445
H. faerberioides	Mertens	France	MG553638	MG553645	MW240984
H. flabelliformis	**MFLU22-0008**	**Thailand**	**OP236779**	**OM521957**	**OM714821**
H. flabelliformis	**MFLU22-0009**	**Thailand**	**OP236780**	–	–

Table 6.1 (continued)

Species	Specimen/culture	Country	GenBank Accession Numbers		
			ITS	nLSU	*tef*1
H. fluxilis	WU 29608	Austria	KU355326	–	–
H. grisea	MFLUCC 12-0451	Thailand	MF150036	–	–
H. grisea	VPI-F-0001921	USA	KY679143	KY679143	–
H. grisea	MCVE 27293	Italy	KU355329	KU355394	KU355447
H. grisea	HFJAU0029	China	MN258645	–	–
H. ilerdensis	Roux 3924	Spain	MG553639	MG553646	
H. josserandii	P.K. EG10-812-T-F	Germany	KU355353	KU355403	KU355463
H. lageniformis	**MFLU22-0010**	**Thailand**	**OP236781**	**OM521958**	**OM763737**
H. lageniformis	**MFLU22-0012**	**Thailand**	**OP236783**	**OM521959**	–
H. leightonii	WU 5846	Spain	MG553640	MG553647	–
H. ligulata	PDD:80775	New Zealand	KM975439	–	–
H. longipes	LIP 0400317	Italy	KU355333	KU355396	KU355449
H. mastrucata	TRTC 152314	Italy	KU355336	KU355397	KU355451
H. mustialensis	DAOM 46374	Canada	KY124252	–	–
H. nimueae	RGT 871128/01 UWO (culture T-489 = CBS 212.91), as *H. nigra*	Canada	KY679144	KY679144	–
H. odorata	TBGT17443	India	MN059651	–	–
H. petaloides	AMB 18088	Italy	KU355346	KU355402	KU355460
H. pinacearum	DAOM 84313	Canada	MH137814	MH137837	–
H. portegna	J.E. Wright 1136 BAFC	Argentina	AF139959	AF139959	–
H. tristis	**MFLU22-0015**	**Thailand**	**OP355451**	**OM521961**	**–**
H. tristis	**MFLU22-0016**	**Thailand**	**OP355450**	**OM521962**	**ON394004**
H. reniformis	HMJAU7091	China	GQ142024	GQ142041	–
H. robusta	CBS 130.68	_	MH859087	MH870800	–

Table 6.1 (continued)

Species	Specimen/culture	Country	GenBank accession Numbers		
			ITS	nLSU	*tef*1
H. tremula	M 0223665	Italy	KU355358	KU355406	KU355466
H. tristis	RV95/214 DUKE	Australia	–	AF042601	–
H. tristis	RV95/295 DUKE	Australia	–	AF135171	–
H. thornii	WU 21790	Portugal	KU355343	KU355401	KU355457
H. unguicularis	Z+ZT 1112	France	KU355361	KU355408	KU355467
H. valesiaca	Roux 2975	France	KU355340	KU355399	KU355455
H. wilhelmii	Z+ZT n. 1154	France	MF494947	MF494948	MF494949
Pleurotus citrinopileatus	YAASM1585	China	KX836372	–	KX840311
P. eryngii	CCMSSC00480	China	KX836350	–	KX840226
P. ostreatus	TENN 53662 (= AFTOL-ID 564)	Austria	AY854077	AY645052	AY883432
P. djamor, as *P. placentodes*	HKAS51745	China	KR827693	KR827695	KR827699
P. pulmonarius	BCRC36906	Taiwan	MH453616	MH447275	MH500353

Notes the newly generated sequences in this study are presented in bold, "–" refers to the unavailability of sequence.

6.3 Results

6.3.1 Phylogenetic Analysis

The combined dataset consisted of 39 *Hohenbuehelia* and 5 *Pleurotus* accessions (Table 6.1). The final alignment, including the gaps, was 1,878 characters long and was deposited in TreeBASE available at (https://treebase.org/treebaseweb/user/submissionList.html; submission ID 29653). The Bayesian and ML analyses resulted in similar tree topologies; thus, only the ML tree is shown with both maximum likelihood bootstrap (BS) values and Bayesian posterior probabilities (PP) (Figure 6.1). In the phylogram, the new species *H. flabelliformis* (MFLU22-0008 and MFLU22-0009) was closely related to *H. algonquinensis* (RGT 870601/12 UWO) from Canada with high support (90% BS, 1.00 PP). *Hohenbuehelia lageniformis* (MFLU22-0010 and MFLU22-0012) was closely

related to *H. odorata* (TBGT17443) from India with high statistical support (96% BS, 1.00 PP). The sequences of *H. tristis* (MFLU22-0015 and MFLU22-0016) are identical to two sequences from GenBank identified as *H. grisea* from Thailand (culture MFLUCC 12-0451) and China (HFJAU0029) with three substitution heteromorphisms (Table 6.2), and were closely related to *H. tristis* (RV95/214 DUKE and RV95/295 DUKE) from Australia with 92% BS, but low support in the BI analysis.

Table 6.2 Positions of substitution heteromorphisms in the ITS sequence of *H. tristis* MFLU22-0016 and corresponding character states in other *H. tristis* ITS sequences

Specimen name/vouchers	**Accession number**	**Position in alignment**		
		147	552	588
H. tristis MFLU22-0015	OP355451	T	C	T
H. tristis MFLU22-0016	OP355450	K	Y	Y
H. tristis (as *H. grisea* in GenBank)	MF150036	T	C	T
H. tristis (as *H. grisea* in GenBank)	MN258645	T	C	T

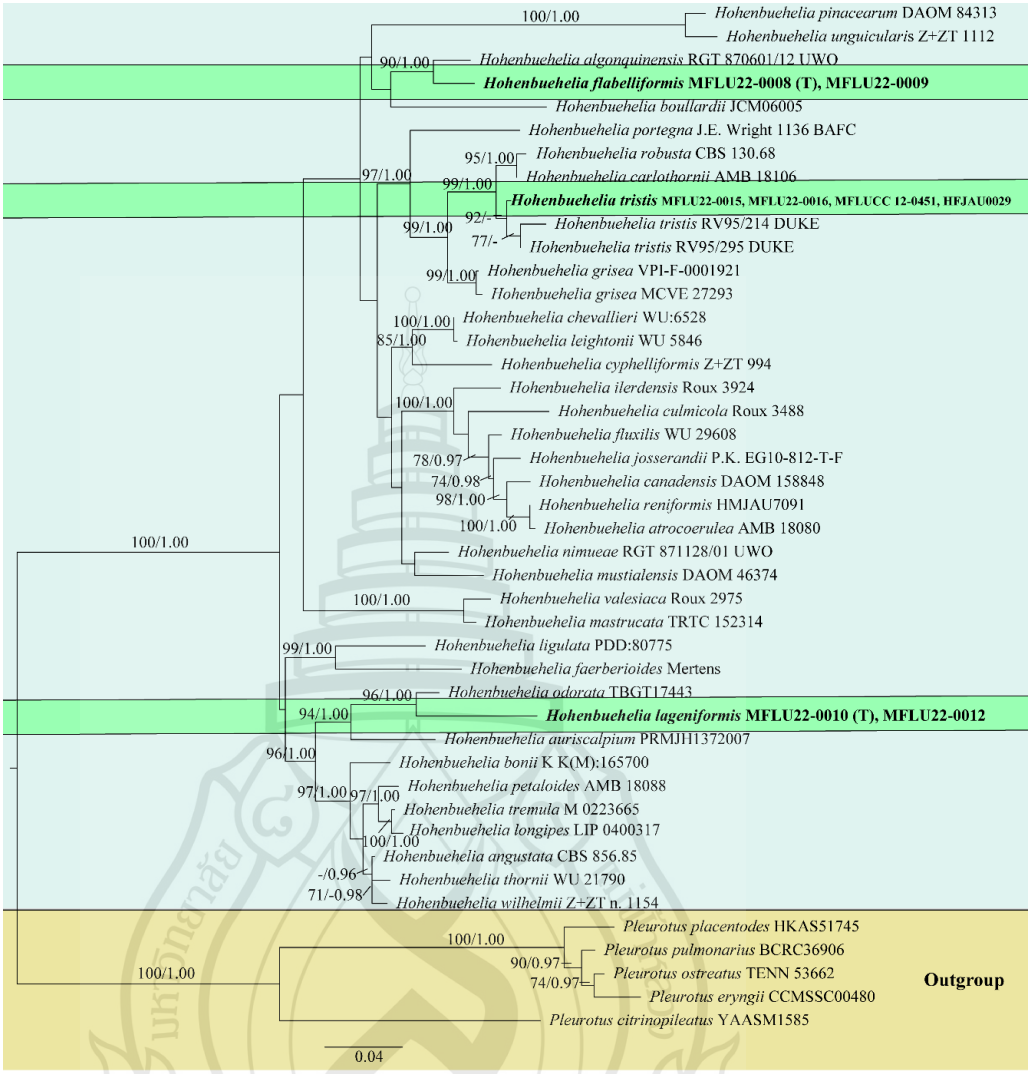


Figure 6.1 Phylogeny of selected sequences of *Hohenbuehelia* based on a maximum likelihood analysis of three nuclear gene regions (ITS, nrLSU, and *tef*1). Maximum likelihood bootstrap values (BS ≥ 70%) and Bayesian posterior probabilities values (PP ≥ 0.90) are shown on the branches. Newly sequenced collections are in bold. Five *Pleurotus* species were used as an outgroup. (T) designates holotypes. The sequence *H. flabelliformis* voucher number MFLU22-0008 was identical to MFLU22-0009 (ITS only). Sequences of *H. lageniformis* voucher number MFLU22-0010 was identical to MFLU22-0012 (ITS and LSU). Sequences of *H. tristis* voucher number MFLU22-0016 was identical to MFLU22-0015 (ITS and nrLSU) and to the two specimens MFLUCC 12-0451 and HFJAU0029 identified as *H. grisea*

(both only ITS) from GenBank, except for three substitution heteromorphisms in the ITS sequence of MFLU22-0016 (see Table 6.2)

6.3.2 Taxonomy

6.3.2.1 *Hohenbuehelia flabelliformis* Phonemany & Raspé, *sp. nov*. (Figure 6.2 & 6.3)

Faces of fungi number: FoF 10708, MycoBank: MB 843984

Diagnosis: This species is distinguished from other *Hohenbuehelia* species by large flabelliform basidiomata, yellowish white pileus that is densely villose with white hairs longer near the point of attachment and shorter towards the margin, ellipsoid basidiospores, absence of cheilocystidia, and a trichoderm pileipellis.

Holotype: THAILAND, Chiang Mai Province, Mae Taeng District, Pha Daeng village, 27 May 2019, Monthien Phonemany (MFLU22-0008).

Etymology: *flabelliformis* refers to the flabelliform shape of basidiomata.

Description: *Pileus* 35–45 × 20–40 mm, spathulate when young, expanding to spathuliform, flabelliform, rounded flabelliform, or orbicular, white when young, becoming yellowish white to pale yellow (4A2–4A3) at the centre and cream (2A3–2B3) to slightly darker elsewhere in age; surface densely villose with white hairs that are longer near the attachment and shorter towards the margin, as observed with a lens; margin white, incurved when young, becoming straight when old. *Lamellae* 1–3 mm wide, decurrent, pale yellow to yellowish white (4A3–4A2), moderately crowded when mature, with lamellulae in 1–3 tiers; edge concolorous to sides, fimbriate. *Stipe* absent or as pseudostipe 5–12 mm × 3–8 mm. *Context* consisting of two layers: (1) non-gelatinous layer, 1 mm thick, soft when young and rather leathery when old, white to dirty white (4A1–4A2); (2) gelatinous layer 0.5 mm thick, soft, sticky, colourless. *Odour* mild, pleasant. *Taste* none. *Spore print* white.

Basidiospores [150/3/2] (5.8–)6–7–8(–8.6) × (3.5–)4–4.2–5(–5.1) µm, *Q* = (1.3–)1.36–1.67–2.01(–2.03), ellipsoid to elongate (oblong) in side view, smooth, thin-walled, inamyloid. *Basidia* (21–)21–25.8–35(–37) × (5–)5.3–7.3–10.5(–11) µm, subclavate to clavate, with 4 sterigmata 4–8 µm long, hyaline, smooth, thin-walled. *Cheilocystidia* absent. *Pleurocystidia* metuloidal, present on both sides of lamellae and visible with a lens, (34–)34–42–54(–55) × (8.5–)8.5–11–14(–14.5) µm, scattered,

narrowly fusiform to fusiform, mucronate at apex, brownish in KOH. *Hymenophoral trama* irregular, hyphae 2–5 µm wide. *Pileipellis* a trichoderm, hyaline in KOH, brownish in water, with cylindrical terminal elements 34–86 × 4–7 µm. *Pileoleptocystidia* absent. *Pileus trama* consists of two different layers: (1) upper layer gelatinous, composed of horizontally arranged, smooth, colourless encrusted hyphae, 2–5 µm wide; (2) lower layer, non-gelatinous, composed of interwoven, smooth, hyaline hyphae, 2–4 µm wide. *Clamp connections* present in pileipellis, pileus trama, and hymenophoral trama.

Habitat and distribution: Saprobic on dead Dipterocarpaceae wood, scattered or fasciculate by 2–4 basidiomata. So far only found in tropical forests of northern Thailand.

Additional specimens examined: THAILAND. Chiang Rai Province, Pa Daed District, Pa Ngae village, 9 August 2019, Monthien Phonemany (MFLU22-0009).

Figure 6.2 Basidiomata of *Hohenbuehelia flabelliformis* in the field, **a, c**–**e** MFLU22-0008, **b** MFLU22-0009. Scale bars **a**–**e** = 1 cm

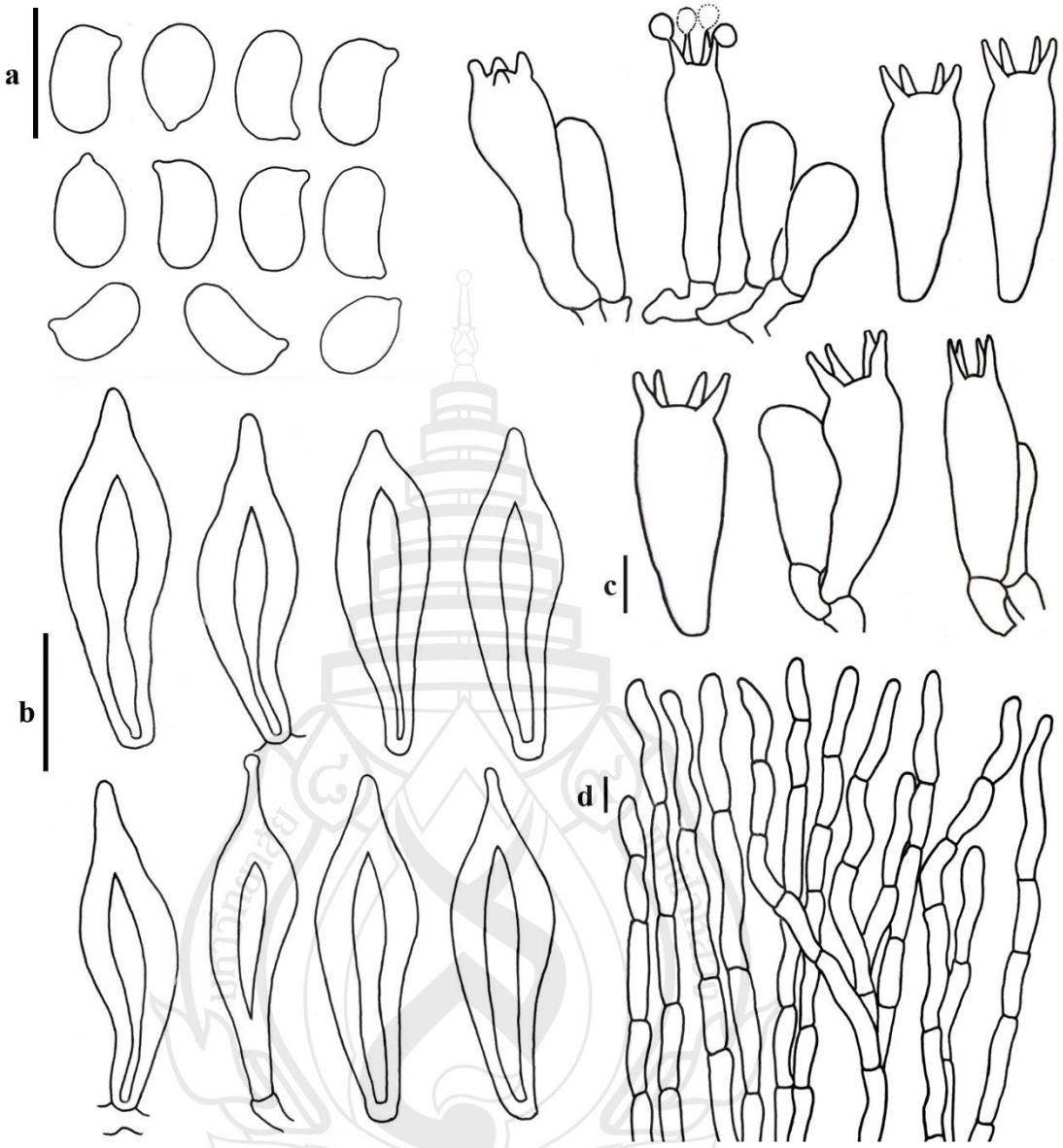


Figure 6.3 Micromorphology of *Hohenbuehelia flabelliformis*. **a** Basidiospores, **b** Pleurocystidia, **c** Basidia and basidioles, **d** Clamp connections present in pileipellis. Scale bars **a =** 10 µm, **b =** 20 µm, **c** = 10 µm, **d** = 20 µm

6.3.2.2 *Hohenbuehelia lageniformis* Phonemany & Raspé, *sp. nov*. (Figure 6.3 & 6.4)

Faces of fungi number: FoF 10709, MycoBank: MB 843985;

Diagnosis: This species is distinguished from other *Hohenbuehelia* species by having velutinous pileus with whitish hairs near the point of attachment and at the

margin, elsewhere pale greyish yellow and with only sparse white hairs, pale brown to light brown and mucilaginous context, subglobose to ellipsoid basidiospores, lageniform cheilocystidia, an ixotrichoderm pileipellis, and the absence of pileoleptocystidia.

Holotype: THAILAND, Chiang Mai Province, Mae On District, Huay Kaew Subdistrict, Pok village, 29 June 2019, Monthien Phonemany (MFLU22-0010).

Etymology: *lageniformis* refers to the lageniform shape of cheilocystidia.

Description: *Pileus* 25–30 × 15–25 mm, sapathuliform when young, rounded flabelliform to sub-rounded flabelliform when mature, light yellow (4A4), pale orange to orange white (5A2–5A3), greyish orange (5B4) becoming darker with age, sometimes sessile, or with laterally attached stipe; surface moist, shiny, velutinous with whitish (1A1) hairs near the point of attachment, elsewhere light yellow to pale greyish yellow (4A4–4B4); margin discolorous from pileus, velutinous with whitish hairs as observed under the lens, incurved even when mature, entire or sometimes undulate when old. *Lamellae* 1–2 mm broad, decurrent, white to pale orange (5A1–5A2) becoming slightly dark with age, close to moderately crowded, in 2–3 tiers. *Stipe* absent or sometimes with pseudostipe 0.5–1 mm long when young and disappearing when mature. *Context* consisting of two layers: (1) non-gelatinous layer, 1 mm thick, fleshy, white (1A1); (2) gelatinous layer, 1–2 mm thick, soft, sticky, brownish orange to light brown (5C5–5D5). *Ordour* mild. *Taste* none. *Spore print* white.

Basidiospores [150/3/3] (6.9–)7–8.8–10(–10.5) × (5.5–)6–7.0–8(–8.3) µm *Q* = (0.99–)1.11–1.26–1.49(–1.53) subglobose to ellipsoid in front view, ellipsoid to phaseoliform in side view, smooth, thin-walled, inamyloid. *Basidia* (24.3–)24–31.9–53(–58.2) × (5.4–)5.5–9.4–14(–14.6) µm, clavate, with (2)–4 sterigmata, 4–8 µm long, hyaline, smooth, thin-walled. *Cheilocystidia* (17.6–)18–21.9–26.5(–26.9) × (3.2–)3–5.0–9(–8.9) µm, lageniform with an inflated base with a thin, rostrate apex, hyaline, thin-walled. *Pleurocystidia* (31–)31–50.3–70(–71) × (9.7–)10–15.3–20(–20.3) µm, metuloidal, setiform, narrowly fusiform to fusiform, encrusted with crystals, brownish or yellowish in water, colourless but still encrusted with crystals in KOH. *Hymenophoral trama* subregular, hyphae 2–4 µm wide. *Pileipellis* an intricate trichoderm with cylindrical terminal elements 2–5 × 41–122 µm. *Pileoleptocystidia* absent. *Pileus trama* consisting of two layers: (1) upper layer gelatinous, composed

horizontally arranged, smooth, colourless, encrusted hyphae, 1–4 µm wide; (2) non-gelatinous layer, composed interwoven, smooth with hyaline hyphae, 2–5 µm wide. *Clamp connections* present in pileipellis, pileus trama, and hymenophoral trama.

Habitat and distribution: Solitary, gregarious to imbricate, on decaying branches of Dipterocarpaceae in a tropical forest in Thailand.

Specimens examined: THAILAND. Chiang Rai Province, Muang Chiang Rai District, Mae Yao subdistrict, Huai Mae Sai village, 10 July 2019, Monthien Phonemany (MFLU22-0011); ibid., 10 July 2019, Monthien Phonemany (MFLU22-0012); ibid., 10 July 2019, Monthien Phonemany (MFLU22-0013); Pa Daed District, Pa Ngae village, 7 September 2019, Monthien Phonemany (MFLU22-0014).

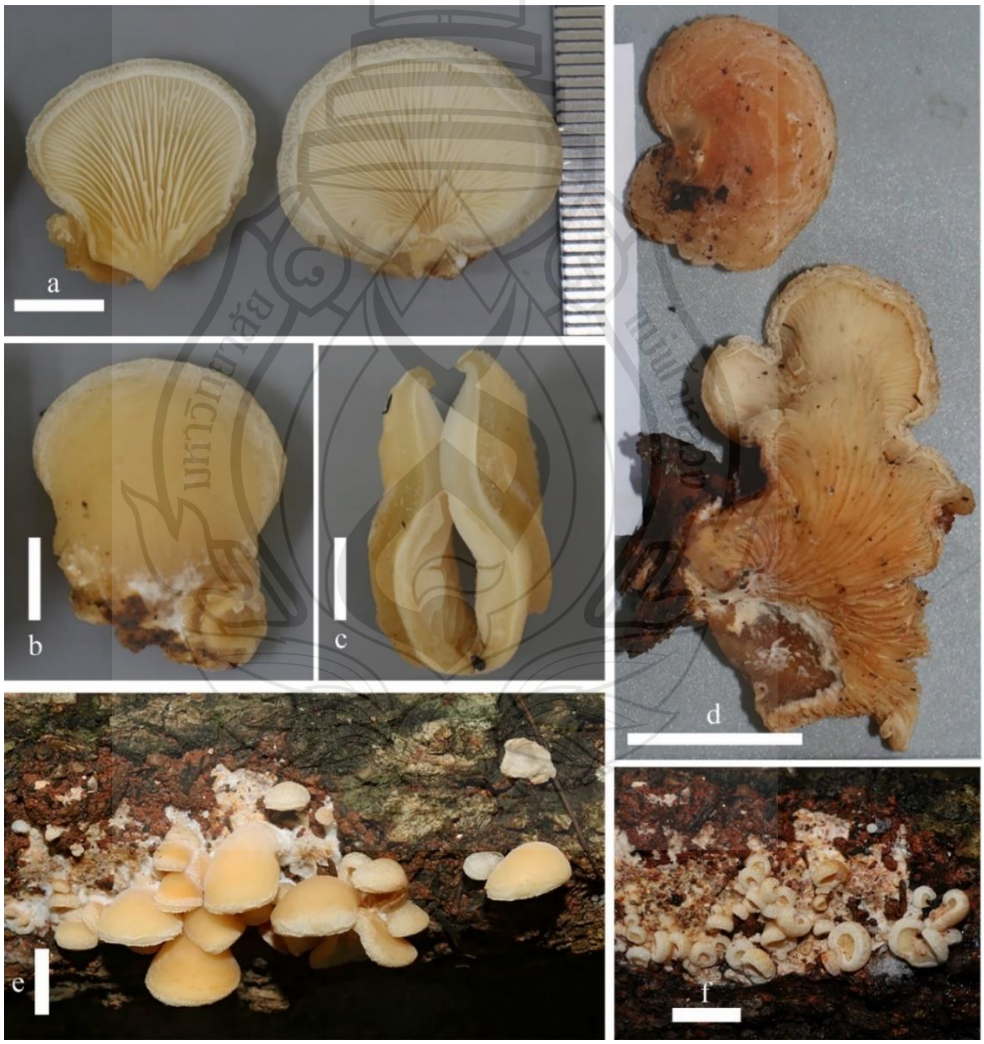


Figure 6.4 Basidiomata of *Hohenbuehelia lageniformis* on the substrate and detailed photographs in the lab, **a–c** MFLU22-0010, **d** MFLU22-0012, **e–f** MFLU22-0014. Scale bars **a–e** = 1 cm, **f** = 2 cm

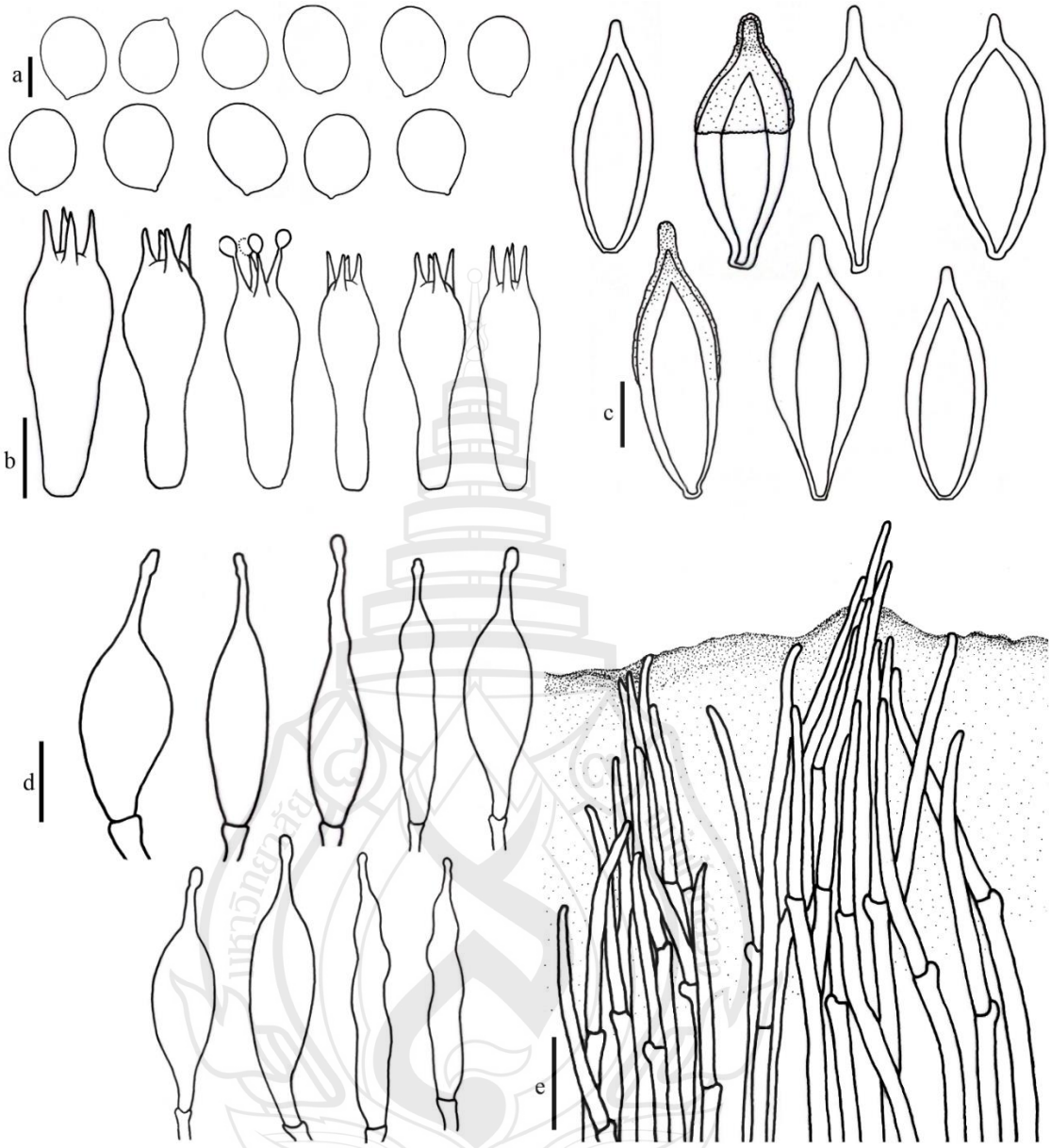


Figure 6.5 Micromorphology of *Hohenbuehelia lageniformis*, **a** Basidiospores, **b** Basidia, **c** Pleurocystidia, **d** Cheilocystidia, **e** Pileipellis. Scale bars **a =** 5 µm**, b–c** = 20 µm, **d** = 10 µm, **e** = 50 µm

6.3.2.3 *Hohenbuehelia tristis* G. Stev., Kew Bull. 19(1): 26 (1964) (Figure 6.5 & 6.6)

Faces of fungi number: FoF 10710, MycoBank: MB 844441

Description: *Pileus* 15–20 × 20–30 mm, spathuliform to reniform, dimidiate to orbicular, greyish-white (1B1) to yellowish white (1B2) when young, becoming

white (1A1) in age, glutinous, very faintly translucent, shiny when mature; upper surface minutely pubescent with greyish hairs (5A1–5B1) near the point of attachment and more sparsely so towards the margin as observed with a lens, with hairs disappearing when old; margin incurved becoming upturned in age. *Lamellae* radiating from point of attachment, 1 mm broad, very crowded, white (1A1) to pale yellow (1A3); lamellulae in (1)–4 tiers. *Stipe* absent, or pseudostipe sometimes present, laterally or dorsally attached, 1 mm long when young then disappearing when old. *Context* consisting of two layers: (1) leathery layer, 1 mm thick; (2) gelatinous layer, 1–2 mm thick, soft, sticky, colourless. *Odour* and *Test* not observed. *Spore print* white.

Basidiospores [150/3/2] (5.1–)6–6.8–8(–9) × (3.5–)3.5–4.0–5(–5.2), *Q* = (1.21–)1.38–1.70–2.08(–2.26), ellipsoid, sub-ellipsoid to elongate, smooth, thin-walled, inamyloid. *Basidia* (13–)14–21.2–24(–24.2) × (5–)5.5–6.6– 7(–7) µm, clavate to subcylindrical, mostly with 4 sometimes with 2 sterigmata, 2–4 µm long, hyaline, smooth, thin-walled. *Cheilocystidia* (10.9–)11–13.1–19(–19.8) × (4.2–)4.4–5.5–6.5(–6.5) µm, lecythiform to sublageniform, thin-walled. *Pleurocystidia* (38–)38–61.5–82(–82) × (10.7–)11–15.0–18.5(–18.6) µm, subfusiform, narrowly fusiform to fusiform, brownish, encrusted with crystals when observed in water, but crystals disappear in KOH. *Hymenophoral trama* subregular, hyphae 2–4 µm wide. *Pileipellis* as tufts of ixotrichoderm with cylindrical terminal elements 40–105 × 3–5.5 µm, with light brown intracellular pigments. *Pileoleptocystidia* absent. *Pileus trama* consisting of two layers: (1) upper layer gelatinous, composed horizontally arranged, smooth, colourless, encrusted hyphae, 1.5–2.5 µm wide; (2) lower layer non-gelatinous, composed interwoven, smooth and hyaline hyphae, with2–4 µm. *Clamp connections* present in pileipellis, pileus trama, and hymenophoral trama.

Habitat: Solitary, gregarious to imbricate, on dead small branches.

Additional specimens examined: THAILAND. Chiang Rai Province, Muang Chiang Rai District, Nang Lae Nai village, 31 July 2019, Monthien Phonemany (MFLU22-0015), (MFLU22-0016).

Figure 6.6 Basidiomata of *Hohenbuehelia tristis* in the field, **a–c** MFLU22-0015, **d** MFLU22-0016. Scale bars **a–d** = 1 cm

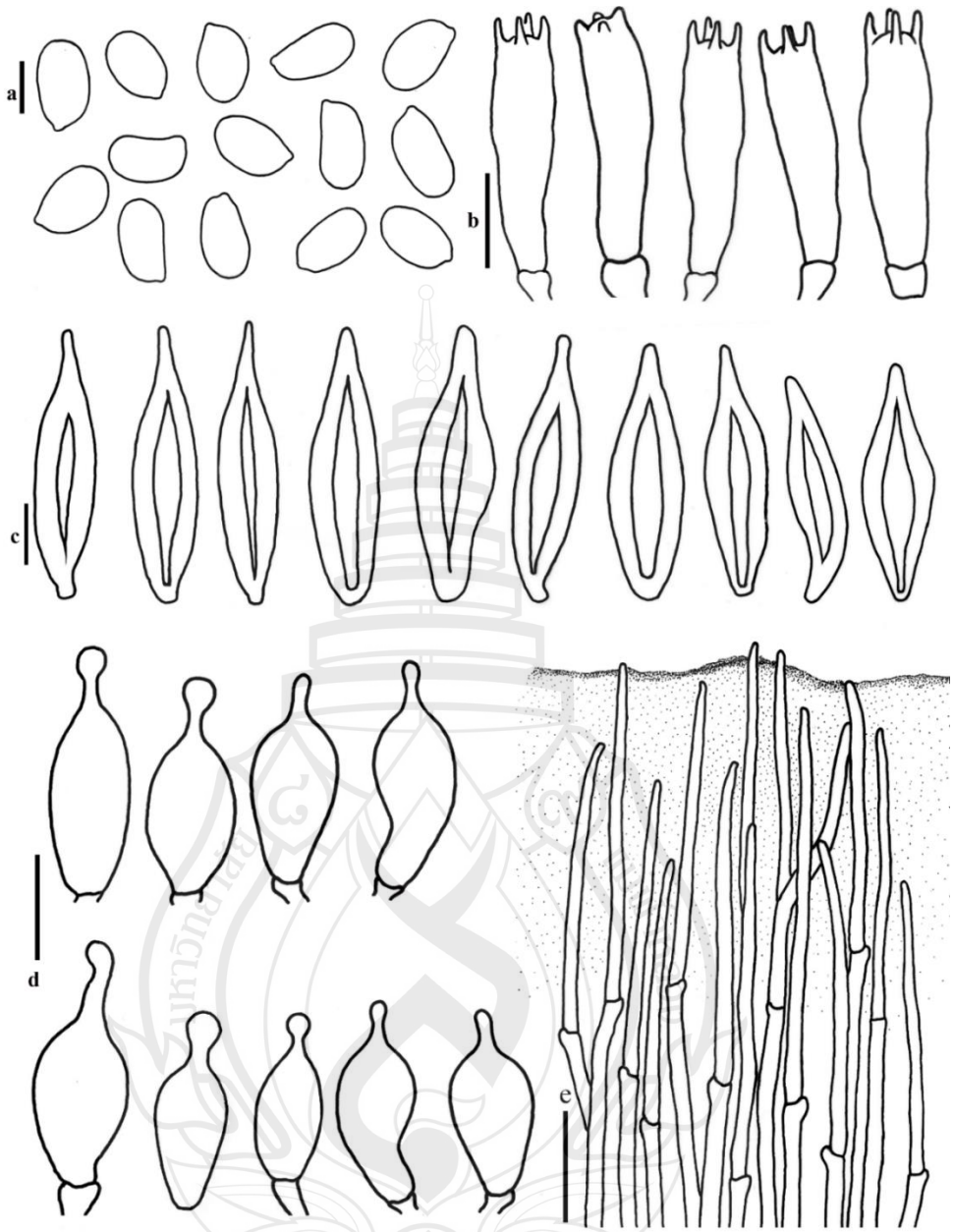


Figure 6.6 Micromorphology of *Hohenbuehelia tristis*. **a** Basidiospores, **b** Basidia, **c** Pleurocystidia, **d** Cheilocystidia, **e** Pileipellis. Scale bars **a** = 5 µm, **b** = 10 µm, **c** = 20 µm, **d** = 10 µm, **e** = 50 µm

6.4 Discussion

The Pleurotaceae belong to the Agaricales and comprise the monophyletic pleurotoid genera *Pleurotus* and *Hohenbuehelia* (Thorn et al., 2000; Koziak et al., 2007). *Hohenbuehelia* species have often been misidentified, in part because holotypes are missing or because types of species put in synonymy were not adequately studied (Consiglio et al., 2018b). In the past, most *Hohenbuehelia* species were introduced, based only on short morphological descriptions (e.g., Peck, 1891; Coker ,1944; Stevenson, 1964). Consiglio (2016, 2017a, b) and Consiglio and Setti (2017) designated lectotypes, neotypes, and epitypes to clarify older species names or names that lack modern and molecularly characterised holotypes. For example, the holotype of *H. tristis* was identified by Stevenson (1964) without any molecular evidence. Later, an nrLSU sequence of *H. tristis* was obtained for the first time by Moncalvo et al. (2000). The heteromorphisms we observed in the nrLSU and ITS sequences of one of the Thai specimens related to *H. tristis* suggested that interbreeding may occur between two divergent lineages within *H. tristis*. Although more data would be needed to confirm it, we hypothesise that those two lineages diverged in geographical isolation (between Southeast Asia and Oceania) and then came in contact in Southeast Asia. This kind of biogeographical history, revealed by DNA sequence variation, have been observed in other Agaricales, for example, *Agaricus subrufescens* Peck (Chen et al., 2016). Some of the recently described species were still introduced, based on only single-gene molecular evidence. In this study, we provide multiple-gene sequence data and detailed descriptions supporting the introduction of two new *Hohenbuehelia* species and a note on *H. tristis* from Thailand. At present, a total of six *Hohenbuehelia* species have been reported from Thailand including the ones in this study and three that were previously reported, namely *H. panelloides*, *H. petaloides*, and *H. reniformis* (Chandrasrikul et al., 2011). The report of *H. grisea* by Sandargo et al. (2018a) has to be excluded since we showed that the correct identification of their material is *H. tristis*. More studies on *Hohenbuehelia* are needed to clarify their taxonomy, and more new species might be discovered.

The basidiomata colour of *H. flabelliformis* is similar to *H. angustata* (Berk.) Singer, *H. bonii* A.M. Ainsw., *H. concentrica* Corner, *H. carlothornii* Consiglio, Setti & Thorn, *H. horrida* (Boedijn) Corner, *H. luteola* G. Stev, *H. malesiana* Corner, *H. odorata* C.K. Pradeep & Bijeesh, *H. olivacea* Yu Liu & T., and *H. testudo* (Berk.) Pegler. The basidiomata range from white, yellowish white, yellow-brown, to pinkish-orange, and form spathulate to flabelliform. *Hohenbuelia angustata*, originally described from Brazil, differs from *H. flabelliformis* by its smaller, smooth, greyish yellow basidiomata, with stipe 4.5 mm long, smaller basidiospores (3.5–5 × 2.5–3.5 µm), and smaller cheilocystidia and pileocystidia (Silva-Filho & Cortez, 2017). *Hohenbuelia horrida* and *H. odorata* differ from *H. flabelliformis* by smaller basidiospores (5.2–7.6 × 4.8–6.4 µm), lack of cheilocystidia, and presence of pileoleptocystidia (Bijeesh et al., 2019). *Hohenbuehelia testudo* differs by having larger basidiospores (9–12.5 × 3.7–4.5 µm), and pigmented metuloids (Baillière 1847). *Hohenbuehelia malesiana*, described from Brazil, is different from *H. flabelliformis* by having longer, subcylindrical basidiospores (7–9 × 3.5–4 µm), and pileipellis as an interrupted cutis, and presence of cheilocystidia (Corner, 1994). *Hohenbuehelia bonii*, from England, has larger yellow-brown basidiomata (20–75 mm diam.), smooth pileus surface, larger basidiospores (7.2–10.4 × 4.5–6.1 µm), larger pleurocystidia (56–103 × 11–19 µm), and an ixotrichoderm or ixocutis pileipellis (Ainsworth et al., 2016). *Hohenbuehelia carlothornii* described from Costa Rica, is different by having off-white basidiomata with a large pseudostipe (20–32 × 14–25 mm), presence of cheilocystidia. (Consiglio et al., 2018b). *Hohenbuehelia concentrica* from Singapore, has larger basidiomata (80 mm wide), larger basidiospores (8–8.5 × 6–6.7 µm), and absence of cheilocystidia (Corner, 1994). *Hohenbuehelia incarnata*, from the Solomon Islands, differs from *H. flabelliformis* by subglobose basidiospores, and the presence of subcylindrical to submoniliform cheilocystidia (Corner, 1994). *Hohenbuehelia olivacea*, originally described from China, has reniform basidiomata with dense and long tomentum, light brown to pallid brown in gelatinous zone, and the presence of cheilocystidia (Liu & Bau, 2009). Phylogenetically, *H. flabelliformis* was closely related to *H. algonquinensis* Consiglio, Setti & Thorn. (voucher RGT 870601/12 UWO) with 3.50 % (21/599) differences in the ITS sequence, 1.4 % (12/839) in the LSU sequence, and 6.66% (35/540) in the *tef*1 sequence. Moreover, the morphologies of both

species are completely different, with *H. algonquinensis* having a glossy black pileus, ungulate to dimidiate basidiomata and contrasting white or off-white lamellae (Consiglio et al., 2018b).

Hohenbuehelia lageniformis is characterised by velutinous pileus with whitish hairs near the point of attachment and at the margin, elsewhere pale greyish yellow and with only sparse white hairs, subglobose to ellipsoid basidiospores, and the absence of pileoleptocystidia. *Hohenbuehelia angustata, H. bonii, H. carlothornii, H. concentrica, H. flabelliformis, H. horrida, H. luteola, H. mellea* Corner, and *H. odorata* have similar pileus colour e.g. pale orange to orange white, light yellow, yellowish white, yellow-brown, honey-yellow or ochraceous brownish. However, those species have significant morphological differences. *Hohenbuehelia angustata* differs from *H. lageniformis* by having a smooth pileus surface, serrate margin, smaller basidiospores (3.5–5 × 2.5–3.5 µm), cutis pileipellis (Silva-Filho & Cortez, 2017). *Hohenbuehelia bonii* differs by having a smooth pileus surface, and larger pleurocystidia (56–103 × 11–19 µm) (Ainsworth et al., 2016). *Hohenbuehelia carlothornii* differs by having finely white-tomentose pileus, smaller basidiospores (7–8.2 × 3.1–3.7 µm), smaller basidia (19–24 × 5.2–6.8 µm), smaller cheilocystidia (13–17 × 4.9–9.4 µm), and larger pleurocystidia 55–63 × 14–18 µm) (Consiglio et al., 2018b). *Hohenbuehelia concentrica* has larger basidiomata (80 mm wide), ovoid basidiospores (8–8.5 × 6–6.7 µm), and no cheilocystidia (Corner, 1994). *Hohenbuehelia horrida* has pale grey to greyish brown lamellae, larger basidiomata (70 mm wide), smaller basidiospores (5–6 × 3–4 µm), smaller basidia (18–25 × 3–4 µm), and larger pleurocystidia (140 × 12.5 µm) (Corner, 1994). *Hohenbuehelia luteola* differs by smaller basidiospores (8–9 4.5–5 µm), and smaller pleurocystidia (45–50 ×15–20 µm) (Stevenson, 1964). *Hohenbuehelia mellea* has smaller basidiospores (5–6.5 × 2–3.5 µm), larger cheilocystidia (30–50 × 8–18 µm), and larger pleurocystidia (50–160 × 12–20 µm) (Corner, 1994). *Hohenbuehelia flabelliformis* has a thinner gelatinous layer (0.5 mm), smaller basidiospores (6–8 × 4–5 µm), smaller pleurocystidia (34–54 × 8.5–14 µm), and no cheilocystidia. *Hohenbuehelia odorata* differs by having smaller basidiospores (5.2–7.6 × 4.8–6.4 µm), smaller cheilocystidia (15–23.5 × 3–7 µm), smaller pleurocystidia (28.5–49 × 10–14.5 µm), and presence of cylindrical to flexuous pileoleptocystidia (Bijeesh et al., 2019). In the phylogenetic tree, the most closely related species to *H. lageniformis* was

H. odorata (voucher TBGT17443). However, the genetic distance between the ITS sequence of *H. lageniformis* and *H. odorata* was 4.62 % (27/584), which supports the distinction of the two species. Moreover, these two species also show morphological differences (see above).

Two accessions identified as *H. grisea*, the culture MFLUCC 12-0451 from Thailand and HFJAU0029 from China (unpublished), had the same ITS sequence than *H. tristis* (MFLU22-0015 and MFLU22-0016) except for three substitution heteromorphisms in the ITS sequence of MFLU22-0016 (see Table 6.2). The two former sequences retrieved from GenBank do not have corresponding morphological descriptions available as evidence. Therefore, these might have been wrongly identified, since ITS sequences are identical to sequences obtained from our collections of *H. tristis* (except for the heteromorphisms detailed in Table 6.2). Additional confirmation of the taxonomic identity of our specimens was obtained by comparing the morphology of our specimens with *H. grisea* which was originally described as *Pleurotus atrocoeruleus* var. *griseus* Peck from New York. The latter is distinguished by a greyish to greyish brown, sparsely tomentose pileus, the lamellae becoming cream-coloured in age (Peck, 1891). *Hohenbuehelia tristis* is characterised by reniform basidiomata, minutely pubescent pileus with greyish hairs that disappear when mature, leaving the surface gelatinous, faintly translucent and shiny, ellipsoid to sub-ellipsoid basidiospores, lecythiform to sublageniform cheilocystidia, and an ixotrichoderm pileipellis. *Hohenbuehelia tristis* described from New Zealand differs from our collections (MFLU2022-0015 and MFLU2022-0016) by having smaller basidiomata (10–20 × 10–15 mm), smaller (mostly narrower) basidiospores (7 × 3 μm), larger and pseudo-amyloid metuloids (80–90 × 15–20 μm), pileipellis as tufts of parallel larger hyphae (3–8 μm in diam.) (Stevenson, 1964). In our phylogenetic analysis (Figure 6.1), the Thai accessions of *H. tristis* formed a monophyletic group with the accessions of *H. tristis* from New Zealand. The morphological differences we observed between the Thai and New Zealand accessions suggested that they might not be conspecific. Additionally, the single synapomorphic position we observed in the nrLSU sequence (position 685 in the alignment; see Table 6.3) is not incompatible with two distinct species, since a genetic distance of only one substitution can be observed between other closely related species (e.g. *H. tremula* and *H. longipes*; Table 6.4). However, the

nrLSU sequence of the Thai specimen MFLU22-0016 showed two to three heteromorphisms corresponding to the differences between the other Thai specimens and the New Zealand specimens (Table 6.3). This suggests either incomplete lineage sorting or that the two lineages can still interbreed. Heteromorphisms were also observed in the ITS sequence of MFLU22-0016, but we were unable to compare them with the ITS sequences of materials from New Zealand, which were unavailable. In view of all the heteromorphisms, we decided not to treat the Thai materials as a new species distinct from *H. tristis*.

Table 6.3 Position of substitutions and substitution heteromorphisms in the nrLSU sequence of *H. tristis* MFLU22-0015, MFLU22-0016, and corresponding character states in *H. tristis* RV95/214 DUKE, RV95/295 DUKE

Specimen name/vouchers	**Accession number**	**Position in the alignment**			
		658	663	664	666
H. tristis MFLU22-0015	OP355451	C	G	T	G
H. tristis MFLU22-0016	OP355450	C	G	Y	R
H. tristis RV95/214 DUKE	AF042601	T	A	T	A
H. tristis RV95/295 DUKE	AF135171	T	G	C	A

Table 6.4 Genetic distance (number of substitutions, excluding heteromorphims) between LSU sequences of closely related *Hohenbuehelia* species

Species name/ specimen number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1. *H. tristis* MFLU22-0015										
2. *H. tristis* MFLU22-0016	0									
3. *H. atrocoerulea* AMB 18080	13	13								
4. *H. carlothornii* AMB 18106	3	3	14							
5. *H. reniformis* HMJAU7091	11	11	2	12						
6. *H. robusta* CBS 130.68	3	3	14	0	12					
7. *H. tristis* RV95/214 DUKE	3	2	15	5	13	5				
8. *H. tristis* RV95/295 DUKE	3	1	14	4	12	4	2			
9. *H. longipes* LIP 0400317	24	24	15	23	13	23	24	23		
10. *H. tremula* M 0223665	25	25	16	24	14	24	25	24	1	0

CHAPTER 7

TAXONOMY AND PHYLOGENY OF *PLEUROTUS* COLLECTED FROM LAOS AND THAILAND

7.1 Introduction to *Pleurotus*

Pleurotus belongs to the Pleurotaceae, *Pleurotus* is an edible mushroom that may be known as an oyster, abalone, or tree mushroom, and has been cultivated worldwide (Mahari et al., 2020). The histories of taxonomy and phylogeny of *Pleurotus* were discussed by (Sánchez, 2010; Kajendran et al., 2018). The infrasubgeneric classification of *Pleurotus* is not as strictly formalised as in some other fungal genera. The genus was identified by Paul Kummer in (1871), and its species has moved to many genera such as *Favolaschia*, *Hohenbuehelia*, *Lentinus*, *Marasmiellus*, *Omphalotus*, *Panellus*, *Pleurocybella*, and *Resupinatus* (Singer, 1986). The identification of *Pleurotus* based on the morphological characteristics of the basidiomata is complex, such as *P. djamor* (Rumph. ex Fr.) Boedijn (Zervakis et al., 2019), *P. eryngii* (DC.) Quél. (Zervakis et al., 2001), *P. ostreatus* (Jacq.ex Fr.) P. Kumm (Eger et al., 1979), *P. ostreatus-sapidus* (Anderson et al., 1973), and *P. pulmonarius* (Fr.) Quél. (Bao et al., 2005). Previously, the taxonomy of *Pleurotus* species was complicated due to their wide distribution worldwide, with one species often being referred to by multiple names (Guzmán, 2000). Later, the combination of both morphological studies and molecular phylogenetic analysis is providing greater results to understand classification of *Pleurotus* species (Zervakis et al., 2019). There are 758 taxon names listed under *Pleurotus* in the Index Fungorum (http://www.indexfungorum.org/), but only 25 species were accepted, *P. ostreatus* (Jacq) P. Kumm is the type species that is to be remembered as edible mushrooms and the most famous species for cultivation worldwide (He et al., 2019). The main morphological characteristics of *Pleurotus* are defined by pleurotoid basidiomata, decurrent lamellulae, smooth and elongated to cylindrical basidiospore shapes, dimitic hyphal system, with skeletal hyphae and generative hyphae, and present clamp connections (Corner, 1981; Seelan, 2015).

However, *P. giganteus* (Berk.) Karun. & K.D. Hyde, and *P. tuber-regium* (Fr.) Singer have omphalioid basidiomata, central attached stipe and slightly long (Karunarathna et al., 2011; Karunarathna et al., 2016). *Pleurotus* species are mostly edible and found in both tropical and temperate regions (Raman et al., 2021), and most species of *Pleurotus* cause white rot in decayed and hard deadwood (Kaur et al., 2021; Raman et al., 2021).

Pleurotus are common edible mushrooms with several important species (Miles & Chang 2004; Cohen et al., 2002). *Pleurotus ostreatus* (oyster mushroom) is the most famous species and most widely cultivated worldwide (Klomklung et al., 2012; Maftoun et al., 2015; Thongklang et al., 2016). Other edible and cultivated species are *Pleurotus columbinus* Quél., *P. eryngii* (DC.) Quél., *P. floridanus* Singer, *P. giganteus*, *P. pulmonarius* (Jacq.) P. Kumm., and *P. ostreatus* (Velázquez-Cedeño et al., 2002; Mandeel et al., 2005; Rout et al., 2005; Moonmoon et al., 2010; Kumla et al., 2013; Menolli et al., 2014). *Pleurotus* species show a number of bioactivities, such as antimicrobial, antiviral, antineoplastic, antioxidant, anti-inflammatory, anti-ageing, anti-tumour, anti-mutagenic, anti-lipidemic, hepatoprotective, hyperglycemic, hypotensive, and immunostimulant properties (Cohen et al., 2002; Patel et al, 2012; Phan et al., 2013; Suresh et al., 2017). These activities have been reported for various extracts and isolated compounds, such as polysaccharides, polysaccharide-protein complexes, proteoglycans, proteins and DNA from oyster mushroom fermentation broth, mycelia or basidiomes. In particular, polysaccharides appear to be potent anti-tumour and immunomodulating substances, besides possessing other beneficial activities (Patel et al., 2012). For instance, *P. djamor* was able to inhibit gramme-negative bacteria such as *Vibrio cholera*, *Escherichia coli* and *Pseudomonas* sp. (Zhang et al., 2016). Since most species of *Pleurotus* are fitted for human consumption, studying their distribution and cultivation is useful in order to ensure source stability for commercial use.

Many new basidiomycetes species have been studies in Thailand, and study on the pleurotoid mushrooms have been reported (Phonemany et al., 2024). There are nine *Pleurotus* have been reported (Chandrasrikul et al., 2011; Suwannarach et al., 2022). However, some species did not provide detailed morphological descriptions or molecular data to confirm their identities. Therefore, the list of species that were missing information reported should be reviewed and updated again. In previous

records of *Pleurotus* in Laos, only five species of *Pleurotus* have been reported from Laos namely, *P. djamor*, *P. eryngii*, *P. giganteus*, *P. ostreatus*, and *P. pulmonarius* (Læssøe et al., 2018; Łuczaj et al., 2021; Phonemany et al., 2021). This study aims to assign isolates to the new record of *Pleurotus* using morphology and rDNA sequences. Description, illustrations, and molecular phylogenetic analyses were provided. Based on morphological and phylogenetic results provided, three new records from Laos, including *P. djamor*, *P. giganteus*, and *P. tuber-regium*. In addition, a new record from Thailand *P. djamor* var. *fuscopruinosu*s was described here.

7.2 Material and Methods

7.2.1 Sample Collection and Morphological Study

All mushroom samples used in this study were collected from selected sites in Thailand, while dried specimens were loaned from the National Herbarium of Laos. The Lao specimens were collected from various regions, including Xiang Khouang and Oudomxay (northern Laos), Xe Kong (southern Laos), and the Houay Nhang Forest Reserve in Vientiane Capital (central Laos) (Table 3.1). The specimens were morphologically characterised as described in section 3.1 of chapter 3.

7.2.2 DNA Extraction and Sequencing

DNA extraction, primers used for PCR conditions and sequencing were same in section 3.2 (Table 3.2) of chapter 3.

7.2.3 Sequence Alignment of *Pleurotus*

DNA was extracted from dried herbarium collections using the Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou), following the manufacturer's instructions. The primers ITS1 and ITS2 were used for PCR (Gardes & Bruns, 1993). The PCR-amplified products were sequenced by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. Sequences were edited and contigs assembled using SeqMan (DNAstar, Madison, WI, USA). ITS1 and ITS2 sequences of *Pleurotus* species from this study were visually checked for quality and were blasted against the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) to check the similarity with sequences of *Pleurotus* from other countries.

7.2.4 Sequence Alignment and Phylogenetic Analysis of *Pleurotus djamor* var. *fuscopruinosus*

Sequences checking, sequences were blasted, and new sequences were deposited same as in section 3.3. The reference sequences of GenBank consisting of an outgroup are *Hohenbuehelia atrocoerulea* and *H. petaloides*. The ITS and nrLSU datasets were aligned using MAFFT v.7 (Katoh et al., 2019) in online access (http://mafft.cbrc.jp/alignment/server/), and the alignment data sets were performed in TrimAl to eliminate ambiguously aligned positions (Capella-Gutiérrez et al., 2009). The combined data set was analysed in Bayesian inference (BI) and Maximum likelihood (ML) analyses. All analyses were done on the web server CIPRES science Gateway version 3.3 (Miller et al., 2010) online (https://www.phylo.org/) Maximum likelihood (ML) phylogenetic tree inference was performed using RAxML-HPC2 on XSEDE version 8.2.12 (Stamatakis, 2006). The dataset of two gene regions was analysed in a single analysis (one for each gene) and combined. GTR+I+G for ITS. BI analyses were selected the best model from jModelTest2 in XSEDE version 2.1.6 (Darriba et al., 2012). BI analyses were performed in MrBayes on XSEDE version 3.2.7a (Ronquist et al., 2003). Two runs of five simultaneous MCMC chains will be run for 1,000,000 generations with trees and parameters sampled every 1,000th generation, for a total of 10,000 samples. The remaining samples were used to calculate posterior probabilities (PP) and the majority rule consensus tree. Trees viewed using FigTree v1.4.0.

Table 7.1 The GenBank accession numbers and geographical origins of the taxa were used in the phylogenetic analysis

Species names	Specimen/culture	Locality	GenBank Accession Numbers	
			ITS	nrLSU
Pleurotus abalonus	HKAS81197	China	MN546043	–
P. abieticola	HKAS46100	China	KP771695	KP867909
P. australis	ICMP 21585	New Zealand	MH395977	MH396002
P. calyptratus	P67	South Korea	KY962483	KY963067

Table 7.1 (continued)

Species names	Specimen/culture	Locality	GenBank Accession Numbers	
			ITS	**nrLSU**
P. calyptratus as *P. djamor* f *.calyptratus*	HMAS 63355	–	AY562495	AY562496
P. citrinopileatus	HKAS85965	China	KP867920	KP867911
P. cornucopiae	CBS 283.37	–	MH855911	MH867415
P. cystidiosus	IFO30607	Japan	AY315778	–
P. djamor	FUM-085	Iraq	KY951474	–
P. djamor	CC055	Mexico	KX573926	–
P. djamor	1092014J	Pakistan	KX056435	–
P. djamor	rxsbn-473	China	MW374226	–
P. djamor	SCK21	Thailand	MK026938	–
P. djamor	Brazil	MBsn	KF280325	–
P. djamor var *.djamor*	ABM464683	Malaysia	KC582635	–
P. djamor* var. *fuscopruinosus	**MFLU24-0015**	**Thailand**	**PP192013**	**PP192011**
P. djamor* var. *fuscopruinosus	**MFLU24-0016**	**Thailand**	**PP192014**	**PP192012**
P. djamor var. *roseus*	ABM1049204	–	KC582640	–
P. dryinus	HKAS94448	Finland	MN546046	–
P. eryngii	CCMSSC00480	China	KX836350	–
P. euosmus	CBS 307.29	China	EU424298	–
P. ferulaginis	HIK133	Italy	KF743826	HM998795
P. flabellatus	P35	South Korea	KY962451	KY963035
P. fossulatus	P146	South Korea	MG282485	MG282545

Table 7.1 (continued)

Species names	Specimen/culture	Locality	GenBank Accession Numbers	
			ITS	nrLSU
P. fuscosquamulosus	LGAMP50	Greece	AY315789	–
P. giganteus	MFLU14-0637	Thailand	KP135559	–
P. levis	DPL6135	USA	KP026244	–
P. nebrodensis	Italy UPA6	UPA6	HM998816	–
P. ostreatus	TENN 53662	Austria	AY854077	AY645052
P. opuntiae	MA-PO7	Mexico	MK757594	–
P. ostreatoroseus	P94	South Korea	MG282434	–
P. parsonsiae	ICMP 18169	New Zealand	MH395975	MH396000
P. placentodes	HKAS51745	China	KR827693	KR827695
P. populinus	P70	South Korea	KY962486	KY963070
P. pulmonarius	HKAS86009	China	KP867918	KP867906
P. purpureo-olivaceus	ICMP 20713	New Zealand	MH395976	MH396001
P. salmoneostramineus	P60	South Korea	KY962476	KY963060
P. smithii	IE74	Mexico	AY315779	–
P. tuber-regium	CBS 850.95	Nigeria	MH862563	MH874190
P. tuoliensis	CCMSSC03105	Xinjiang, China	KU612906	–
Hohenbuehelia atrocoerulea	AMB 18080	Hungary	KU355304	KU355389
H. petaloides	AMB 18088	Italy	KU355346	KU355402

Notes the newly generated sequences in this study are presented in bold, "–" refers to the unavailability of sequence.

7.2.4 Sequence Alignment and Phylogenetic Analyses of *Pleurotus tuber-regium*

Sequences checking, sequences blasted, and deposit new sequences same in section 3.3. All sequences are showed in Table 7.2 including outgroup. For both maximum likelihood and Bayesian analyses, a mixed-model (partitioned) scheme was used. Maximum likelihood phylogenetic inference was performed using RAxML-HPC2 version 8.2.12 (Stamatakis, 2006) on XSEDE. *Hohenbuehelia atrocoerulea* and *H. petaloides* species were used as an outgroup. For Bayesian analysis, the best-fit substitution models were selected from jModelTest2 version 2.1.6 (Darriba et al., 2012) on XSEDE. The best-fit model was HKY+I+G for ITS1+ITS2. Bayesian analysis was performed in MrBayes version 3.2.7a (Ronquist et al., 2012). Two runs of five chains each were run for 500,000 generations and sampled every 200 generations. The average standard deviation of split frequencies was 0.081120 at the end of the runs. The burn-in phase (25%) was estimated by checking the stationarity in generation-likelihood plot in Tracer ver. 1.7.1 (Rambaut et al., 2018). The phylogenetic tree was visualised and edited in FigTree version 1.4.4 (Rambaut, 2018), and then exported and further edited in Adobe Illustrator CS3.

Table 7.2 GenBank accession numbers and geographical origins of taxa used in the phylogenetic analysis

Species names	Specimen/culture	Locality	GenBank Accession Numbers (ITS)
Hohenbuehelia atrocoerulea	AMB 18080	Hungary	KU355304
H. petaloides	AMB 18088	Italy	KU355346
Lentinus tuber-regium	130433	China	KM405793
Pleurotus abalonus	CBS 61580	India	AY315810
P. abalonus	HKAS81197	China (Yunan)	MN546043
P. abieticola	HKAS45507	China: Sichuan	KP771697
P. abieticola	HKAS46100	China: Tibet	KP771695
P. abieticola	P97	south Koria	MG282437
P. albidus	Duke327	Brazil	AF345658
P. albidus	BAFC 50.047	Argentina	AF345660
P. australis	CBS100127	Korea	AY265812
P. australis	ICMP 21585	New Zealand	MH395977

Table 7.2 (continued)

Species names	Specimen/culture	Locality	GenBank Accession Numbers (ITS)
P. calyptratus	PC6	South Korea	MT872345
P. calyptratus	P71	South Korea	KY962487
P.citrinopileatus	HMAS63344	China: Jilin	AY696301
P.citrinopileatus	YAASM1585	China	KX836372
P. cornucopiae	TENN55191	Austria	AY450341
P. cornucopiae	CBS:283.37	–	MH855911
P. cystidiosus	IFO30607	Japan	AY315778
P. cystidiosus	AG55	USA	FJ608592
P. djamor	FUM-093	Malaysia	KY951475
P. djamor	FUM-085	Iraq	KY951474
P. dryinus	CBS 481.72	–	EU424292
P. dryinus	HKAS94448	Finland	MN546046
P. eryngii	HIK154	China	HM998841
P. eryngii	CCMSSC00480	–	KX836350
P. euosmus	CBS 307.29	–	EU424298
P. ferulaginis	HIK133	Italy	KF743826
P. ferulaginis	PN8	Italy: Remanzacco (Udine)	KF743827
P. flabellatus	P35	South Korea	KY962451
P. fossulatus	HIK127	Armenia	HM998828
P. fossulatus	P146	South Korea	MG282485
P. fuscosquamulosus	LGAMP50	Greece	AY315789
P. giganteus	PS2016-40	Laos	MT341866
P. giganteus	MFLU14-0637	Thailand	KP135559
P. levis	DPL6135	USA: Texas	KP026244
P. levis	TENN 58298	–	AF345662
P. nebrodensis	UPA6	Italy	HM998816
P. nebrodensis	CCMSSC00646	–	KU612943
P. opuntiae	CBS102543	Mexico	MN546047
P. ostreatus	HKAS53480	Germany	KP867914
P. ostreatus	TENN 53662	–	OL308083
P. placentodes	HKAS51745	China	KR827693
P. placentodes	HKAS57781	China	KR827694
P. populinus	TENN56749	USA	AY450346

Table 7.2 (continued)

Species names	Specimen/culture	Locality	GenBank accession numbers (ITS)
P. populinus	P70	South Korea	KY962486
P. pulmonarius	BCRC36906	Taiwan	MH453616
P. pulmonarius	HKAS86009	China	KP867918
P. purpureo-olivaceus	PDD:105642	New Zealand: Cragieburn Forest Park	MH395976
P. purpureo-olivaceus	GQ411523	New Zealand	PDD:91632
P. salmoneostramineus	P60	South Korea	KY962476
P. salmoneostramineus	ASI 2172	–	AY265845
P. smithii	IE74	Mexico	AY315779
P. tuber-regium	DMC 172	–	EU908193
P. tuber-regium	RV95/947.1	–	AF109966
P. tuber-regium	CBS 850.95	Nigeria	MH862563
P. tuber-regium	N.K.Zeng2244	–	MT822930
P. tuber-regium	SAM166	Thailand	MK026917
P. tuber-regium	FRI024	Malaysia	MK026919
P. tuber-regium	**HNL503400**	**Laos**	**MK894133**
P. tuber-regium	**HNL501189**	**Laos**	**MK894134**
P. tuber-regium	11812-ss1	Thailand	KP026248
P. tuoliensis	CCMSSC03105	–	KU612906

Notes the newly generated sequences in this study are presented in bold, "–" refers to the unavailability of sequence.

7.3 Results

7.3.1 Results of Sequence Alignment of *Pleurotus*

The BLAST results of ITS1 + ITS2 sequences of the studied *P. djarmor* specimens from Laos (voucher numbers HNL503398 and HNL503399) showed high similarity to *P. djarmor* from Malaysia (voucher number FUM-093) (Avin et al., 2017) with 99.84% and 99.68%, respectively. The sequence similarity between the specimen of *P. giganteus* from Laos that we studied (voucher number HNL501302) and the

sequence of *P. giganteus* from Thailand (voucher number MFLU08-1371) (Karunarathna et al., 2011) was 99.49%. All sequences generated from this study were deposited in GenBank (Table 7.3).

Table 7.3 The GenBank accession numbers and BLAST search results of ITS1 + ITS2 sequences of *Pleurotus* species from this study against the GenBank database. I = Identity and QC = Query Cover

Species	Voucher, GenBank accession No	Most similar ITS1 + ITS2 sequences in GenBank	ITS1	ITS2	ITS1+ ITS2	Species name	Voucher no.	Locality	Reference
P. djamor	HNL503398, MK894131	KY951475, I= 99.84%, QC=98%	249/249	204/204	453/453	*P. djamor*	FUM-093	Malaysia	Avin et al., (2017)
P. djamor	HNL503399, MK894132	KY951475, I= 99.68%, QC=97%	249/249	202/202	451/451	*P. djamor*	FUM-093	Malaysia	Avin et al., (2017)
P. giganteus	HNL501302, MT341866	KP120919, I= 99.49% QC=100%	289/290	196/198	485/488	*P. giganteus*	MFLU08-1371	Thailand	Karunarathna et al., (2011)

7.3.2 Phylogenetic Analysis of *P. djamor* var. *fuscopruinosus*

The phylogenetic analysis included 43 accessions, consisting of 41 representative species of *Pleurotus* and two species of *Hohenbuehelia* used as outgroups (Table 7.1). The combined dataset of ITS and nrLSU yielded a final alignment that was 1,640 characters long, including gaps. The Maximum Likelihood (RAxML) analysis is depicted in Figure 7.1. Both Bayesian and Maximum Likelihood analyses produced similar tree topologies; therefore, only the ML tree is presented, showing both maximum likelihood bootstrap (BS) values and Bayesian posterior probabilities (PP). The phylogenetic tree demonstrated that the clade of *P. djamor* varieties clustered together, including synonym species such as *P. salmoneostramineus* Lj.N. Vassiljeva, *P. flabellatus* Sacc., *P. ostreatoroseus* Singer, and *P. opuntiae* (Durieu & Lév) Sacc. The Thai specimens, *P. djamor* var. *fuscopruinosus* (MFU24-0015, MFU24-0016), were also grouped with other varieties of *P. djamor*, exhibiting high bootstrap support (BS 93%, PP 0.98%).

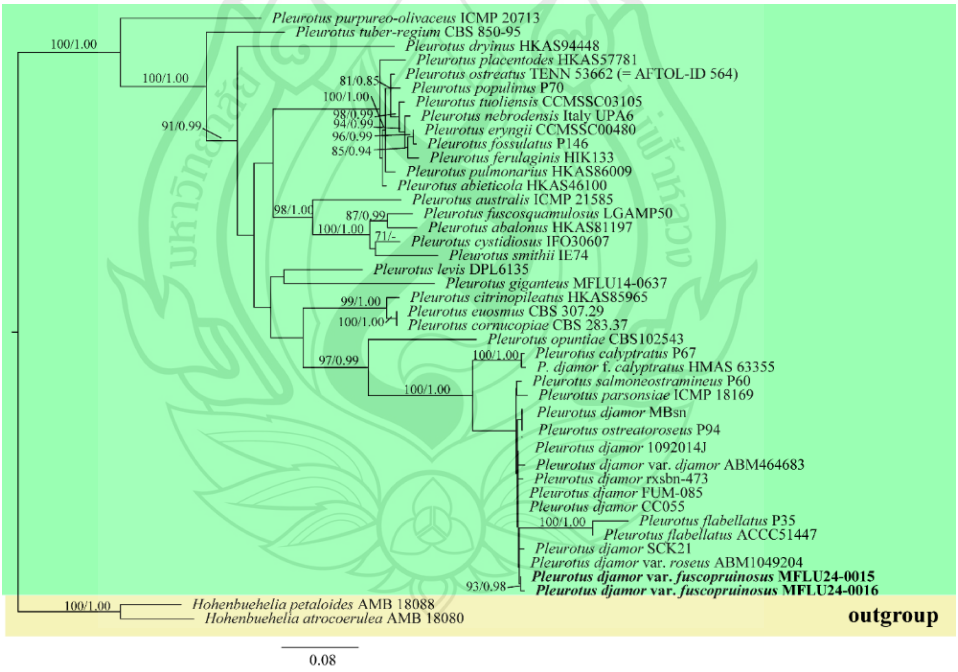


Figure 7.1 Phylogenetic analysis of *Pleurotus* species inferred from Maximum Likelihood (ML) analysis of ITS and nrLSU sequences. Bootstrap values BS ≥70% and PP ≥ 0.95% are shown above or beneath individual branches. *Hohenbuehelia atrocoerulea* (AMB 18080) and *H. petaloides* (AMB 18088) are outgroups. Sequences of our species obtained are in bold

7.3.3 Phylogenetic Analysis of *P. tuber-regium*

The combined dataset consisted of Sixty-three strains are included in the analysis, which comprises 580 characters after trimming with the trimal programme in CIPRES. The Bayesian and ML analyses resulted in similar tree topologies; thus, only the ML tree is shown with both maximum likelihood bootstrap (BS) values and Bayesian posterior probabilities (PP) (Figure 7.2). The phylogenetic analysis, *P. tuber-regium* (MK894133, MK894134) was grouped together with *P. tuber-regium* from other countries with high bootstrap support (BS 100%, PP 0.96%).

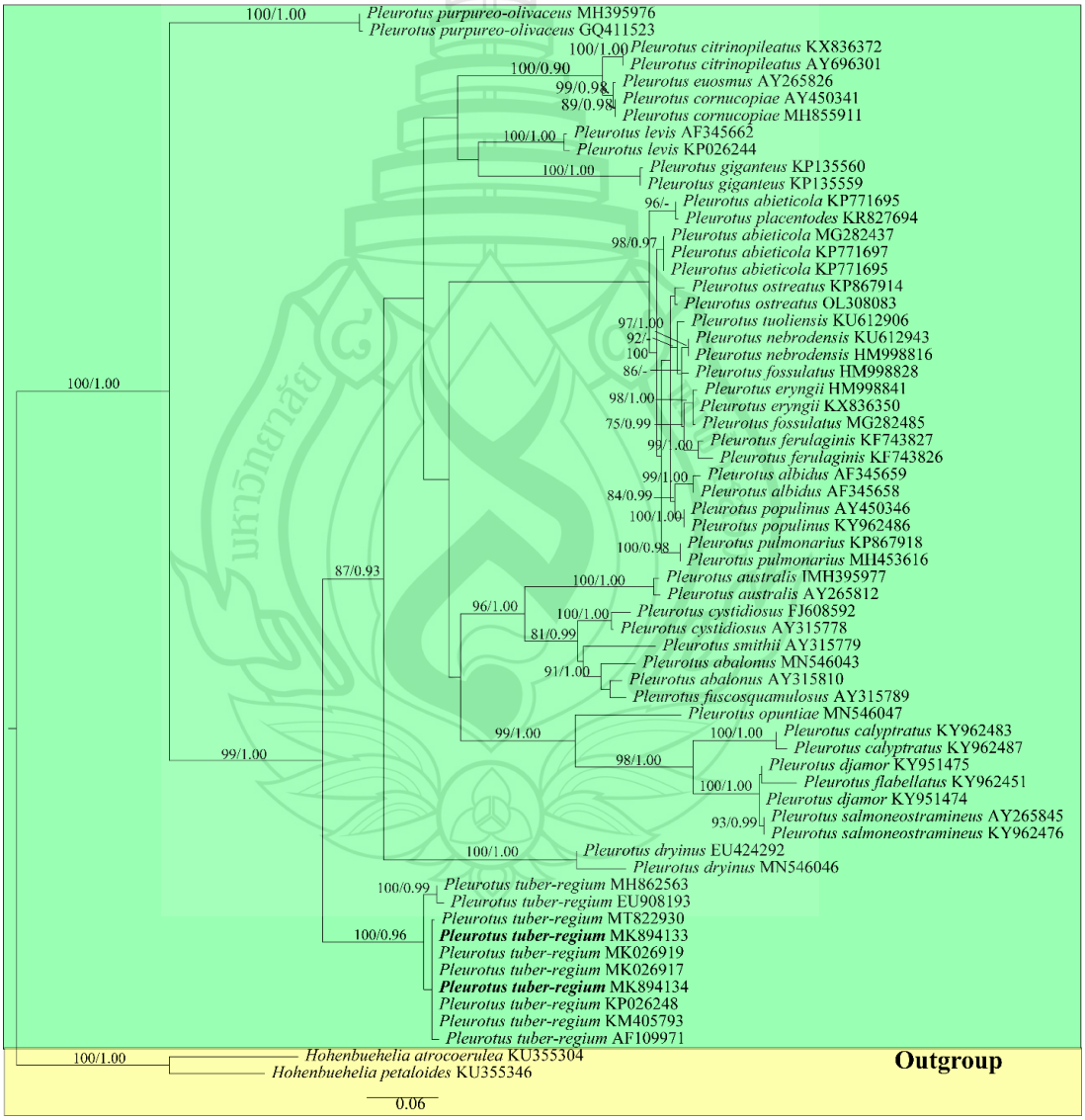


Figure 7.2 Maximum likelihood phylogenetic tree based on ITS sequences of *Pleurotus* species. Bootstrap support values BS ≥ 70% and Bootstrap values of PP ≥ 0.90 are shown on the branches. The newly generated sequences are

indicated in bold. *Hohenbuehelia atrocoerulea* (KU355304) and *H. petaloides* (KU355346) are outgroup

7.3.3 Taxonomy

7.3.3.1 *Pleurotus djamor* (Rumph. ex Fr.) Boedijn, Rumphius Memorial Volume: 292 (1959), (Figure 7.3 & 7.4)

Index Fungorum number: IF 355683

Pileus 30–70 mm diam., rounded flabelliform to reniform; surface glabrous, pinkish, white to pale red (7A3) at the centre then pastel red to reddish-orange (7A5–8) toward the margin, the entire surface turning paler to white with age; margin inflexed to straight, wavy and split when mature. *Lamellae* decurrent, narrow, 3–5 mm wide, moderately crowded, with more than 5 lamellulae lengths, pale red to pastel red (7A3–5), slightly paler near stipe, with smooth, concolorous edge. *Stipe* short or nearly absent, eccentric to lateral, up to 10 mm wide, smooth, white to pale red (7A2–3). *Context* white to pale red (7A3) and 4–8 mm thick in pileus, solid and white to pale red (7A3) in stipe. *Taste* and *odour* not recorded. *Spores print* pale pinkish to white.

Basidiospores 5.4–8.9 × 2.5–5.4 µm, avl × avw = 6.82 × 2.76 µm, *Q* = 1.12–2.82, *avQ* = 1.84, subglobose to broadly ellipsoid inside view, oblong to subcylindrical in frontal view, hyaline, smooth, thin-walled. *Basidia* 18.3–28.3 × 5.2–6.3 µm, clavate, hyaline, thin-walled, 4-spored, rarely 3 or 2-spored. *Lamellar edge* sterile. *Cheilocystidia* 12.2–25.4 × 5.5–6.2 µm, more or less convex with, subventricose to clavate and some clavate to utriform, hyaline, thin-walled. *Pleurocystidia* absent. *Hyphal system* dimitic, similar in stipe context and pileus context; skeletal hyphae 4.3–6.1 µm wide, very thick-walled, hyaline, septate, elements with 2–3 branches; generative hyphae 4.5–5.2 µm wide, cylindrical, slightly thick-walled, hyaline. *Pileipellis* a cutis composed of hyaline hyphae, 3.9–5.2 µm wide, with cylindrical terminal elements 50.6–90.8 µm in length. *Stipitipellis* not observed. *Clamp connections* present in all tissue.

Habitat and distribution: Saprotrophic on decaying wood, growing solitary or in cluster of 2–6 basidiomes. Distribution in Laos (this study), Thailand (Chandrasrikul et al. 2011), and Malaysia (Corner 1981).

Materials examined: LAOS, Vientiane Capital, Xaythany district, Houay Yang Forest Reserve, collected on 15 September 2015, P. Sysouphanthong PS172 (HNL503398, ITS = MK894131); *ibidem*, collected on 15 September 2015, P. Sysouphanthong PS173 (HNL503399, ITS = MK894132).

Figure 7.3 Fresh basidiomata of *P. djamor* on the field. **a** and **b** = HNL503398, **c** and **d** = HNL503399

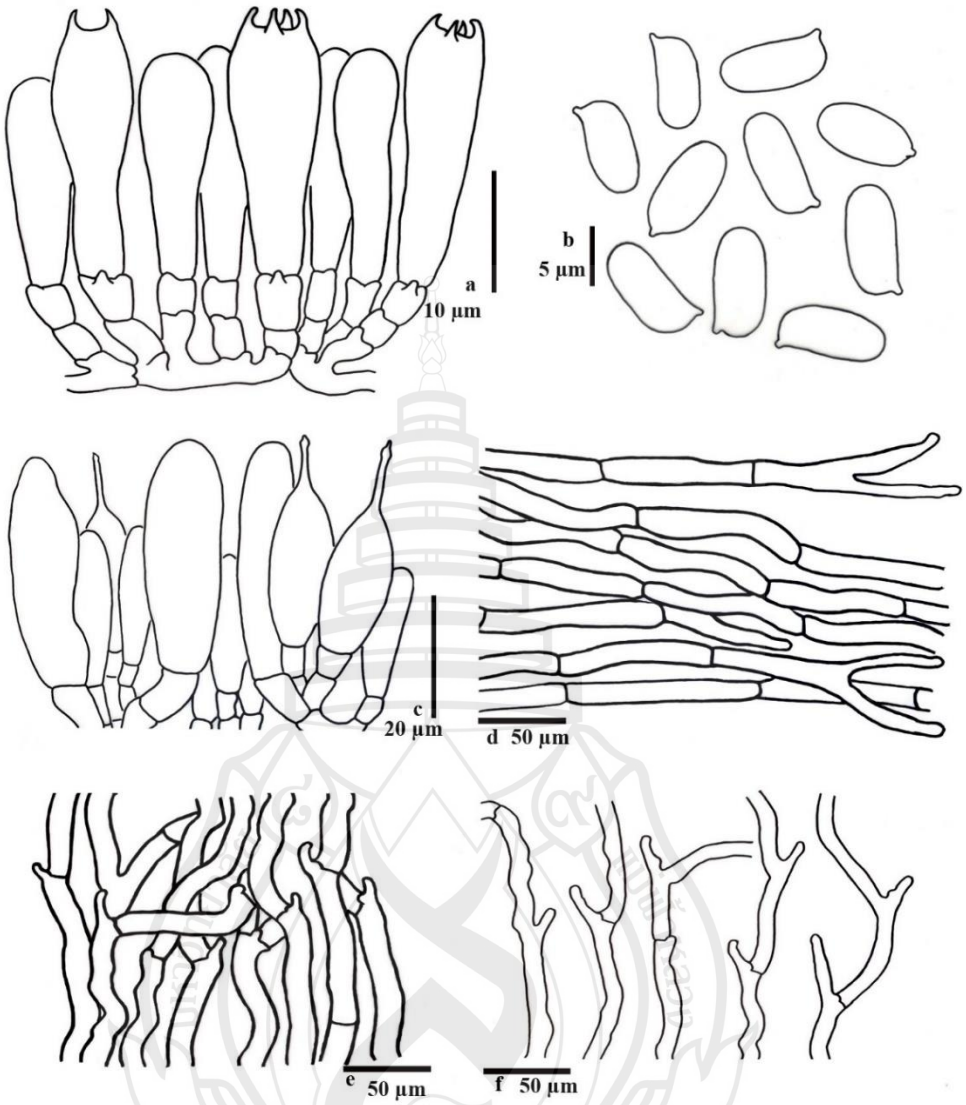


Figure 7.4 *Pleurotus djamor* (HNL503398), **a** = basidia and basidioles, **b** = basidiospores, **c** = cheilocystidia, **d** = pileipellis, **e** = skeletal hyphae from pileus context, **f** = generative hyphae from pileus context

7.3.3.2 *Pleurotus djamor* var. *fuscopruinosus* Corner. (Figures 7.5)

Faces of Fungi: FoF 15389, Index Fungorum number: IF 117633

Pileus measures 35–80 mm in diameter and has a rounded flabelliform to subreniform shape, greyish to white, transitioning to greyish-brown (5B1-5D3), and becomes pale to white or whitish with age. The base of the proximal part exhibits a greyish (5D3) fuliginous-pruinose. When young, the surface is covered with fibrillose

texture near the margin, which diminishes upon maturity. *Margin* is inflexed to straight and occasionally wavy, and it may split when fully mature. *Lamellae* are decurrent, narrow (0.5–2 mm wide), and crowded, featuring more than five lamellulae. They are white with a smooth, concolourous edge. *Stipe* measures 10–15 × 10–20 mm, is short or nearly absent, eccentric to lateral, and smooth, with colours ranging from white to pale red (7A3). *Context* is white, 1–2.5 mm thick in the pileus, and soft in texture. *Taste* and *odour* were not recorded. *Spore print* is white to pinkish (9A1, 9A2).

Basidiospores are measured as [50,3,3] (5.4–)6.0–7–8.0(–8.9) × (3.1–)3.0–4–4.5(–4.8) µm, *Q* = (1.2–)1.44–1.78–2.18(–2.52), and are broadly ellipsoid to cylindrical, hyaline, smooth, and thin-walled. *Basidia* measure (17.9–)18–21–25.2(–25.3) × (4.8–)4.8–5.7–6.7(–6.7) µm, are clavate, with four sterigmata, and are both hyaline and thin-walled. *Cheilocystidia* measure (11.5–)11.9–22.7–31.7(–33.5) × (6.1–)6.4–9.9–12.2(–12.3) µm, are short clavate to clavate with thin walls and brownish colouration. *Pleurocystidia* are absent. *Hyphal system* is dimitic, present in both the stipe and pileus contexts. Skeletal hyphae are 5–7 µm wide, very thick-walled, hyaline, and septate, with elements displaying 2 to 3 branches.

Generative hyphae measure 5–6 µm wide, are cylindrical, and slightly thick-walled, also hyaline. *Pileipellis* consists of a cutis structure with hyphae measuring 2–5 µm wide, presenting slight thickness. Some of these hyphae are encrusted with annular-like structures when treated with KOH, and they exhibit brownish walls. *Terminal elements* range from clavate to cylindrical, measuring 17–56 × 5–8 µm, and also exhibit brownish walls. Moreover, minute fascicles are present at the ends of cells, with a greater number of fascicles observed at the margins of the pileus. *Stipitipellis* was not observed, but clamp connections were found in all examined tissues.

Habitat and distribution: *P. djamor* var. *fuscopruinosus* is saprobic, growing on dead and decaying wood. It is typically found in small groups comprising two to four basidiomes.

Materials examined: THAILAND, Nakhon Si Thammarat Province, Lan Saka District, collected on 10 January 2020 by Monthien Phonemany (MFLU24-0015). The same location, Lan Saka District, was collected on 11 January 2020, Monthien Phonemany (MFLU24-0016).

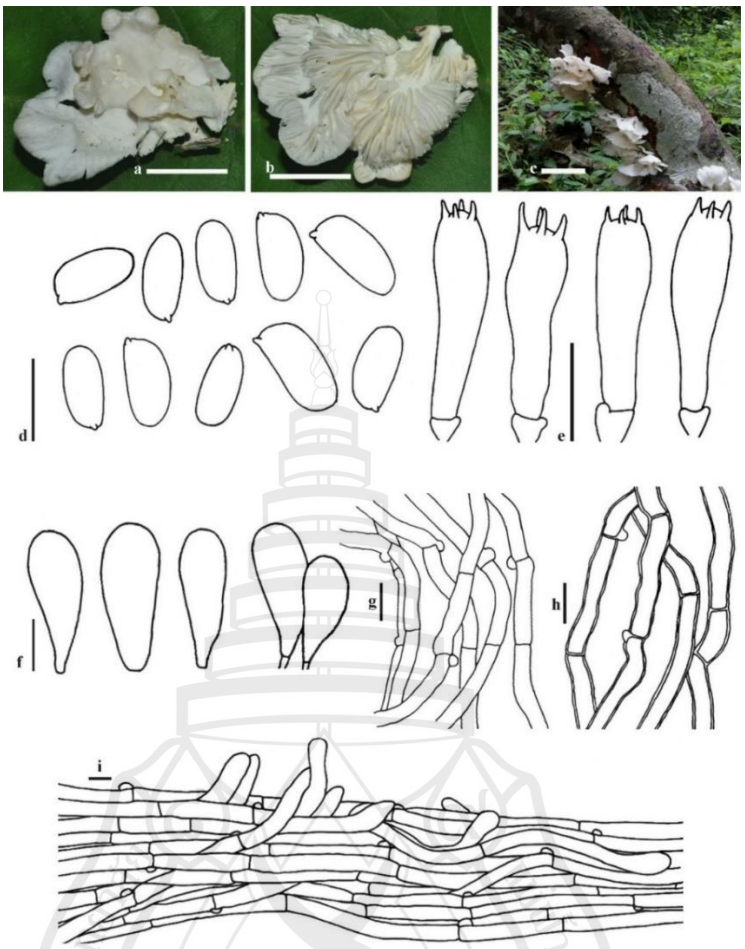


Figure 7.5 Basidiomata of *P. djamor* var. *fuscopruinosus*, **a** and **b** = MFLU24-0015, **c** =MFLU24-0016. Scale bar = 50 mm. Micromorphology of *P. djamor* var. *fuscopruinosus*; **d** Basidiospores, **e** Basidia, **f** Cheilocytidia, **g** generative hyphae of context from pileus, **h** skeletal hyphae of context, **i** Pileipellis cutis. Scale bars **d-g** =10 µm, **i** = 5 µm

7.3.3.3 *Pleurotus giganteus* (Berk.) Karun. & K.D. Hyde, in Karunarathna, Mycotaxon 118: 62 (2011) (Figure 7.6 & 7.7)

Index Fungorum number: IF 561087

Pileus 45–50 mm in diam., when mature convex to plano-concave with depressed centre, fibrillose-scaly at the centre, with brownish-orange to reddish-golden (6C6–8) uniformly dark at the surface at the centre or depress zone, with a darker centre toward the margin, on light brown (6D4–6) indefinite, radial, innate squamules, overlaid by scanty; margin brownish orange (6C5–8), straight or reflexed when young

then becoming involute with age, fringed or undulating in age. *Lamellae* decurrent, white to pale yellow (4A2) in age, narrow, up to 5 mm wide, moderately crowded, more than 5 lamellulae lengths, with concolorous entire edge. *Stipe* 50–120 × 8–20 mm, equal, with tapering pseudorrhiza, with glabrous surface or squamules concolorous with pileus. *Context* white, 5–10 mm thick in pileus, white and rather solid in stipe. *Taste and odour* not recorded. *Spore print* white to whitish.

Basidiospores 6.5–9.5 × 4.0–6.8 μm, avl × avw = 8.10 μm × 6.01 μm, *Q* = 1.05–1.82, *avQ* = 1.41, subglobose, ellipsoid to broadly ellipsoid inside view, subglobose in frontal view, hyaline, smooth and thin-walled. *Basidia* 29.9–33.1–48.2–50.3 μm, oblong to clavate, hyaline, thin-walled, 4-spored. *Cheilocystidia* 10.7–31.3 × 6.0–9.8 μm, lageniform to utriform or fusiform with long appendage and slightly swollen at the apex, hyaline, thin-walled. *Pleurocystidia* absent. *Hyphal system* dimitic, similar in stipe context and pileus context; generative hyphae 3–5μm wide, hyaline, slightly thick-walled; skeletal hyphae 4.0–5.5 μm wide, hyaline to pale brown, thick-walled. *Pileipellis* a trichoderm composed of cylindrical and hyaline to pale yellow hyphae, 3.9–5 wide, with cylindrical terminal elements, attenuate to apex, 50.4–80.3 μm in length. *Stipitipellis* not observed. *Clamp connections* present in all tissue.

Habitat and distribution in Laos: Saprotrophic on soil with a long pseudorrhiza connected with dead wood or decay wood buried in the soil, solitary or in groups of a few basidiomata in deciduous forests. Species was found in all part of Laos.

Materials examined: LAOS, Xieng Khouang province, Kham district, Nong On village, collected on 25 May 2016, P. Sysouphanthong PS2016–40 (HNL501302, ITS= MT341866); Xieng Khouang province, Phoukout district, Na Phouang village, collected on 23 May 2016, P. Sysouphanthong PS2016–22 (HNL501189); Vientiane Capital, Xaythany District, Houay Yang Forest Reserve, collected on 25 June 2014, P. Sysouphanthong PS2014–191 (HNL501862); *ibidem* collected on 15 September, P. Sysouphanthong PS158 (HNL503384); Oudomxay province, Xay district, Houay Houm village, Collected on 8 May 2014, P. Sysouphanthong PS2014–634 (HNL502305); *ibidem*, collected on 8 May 2014, P. Sysouphanthong PS2014–794 (HNL502465); Salavan Province, Salavan district, Lao Ngam village, collected on 5 October 2015, P. Sysouphanthong PS 72 (HNL503298).

Figure 7.6 Fresh basidiomata of *P. giganteus* on the field. **a** = HNL501302, **b** = HNL501862, **c** = HNL502305

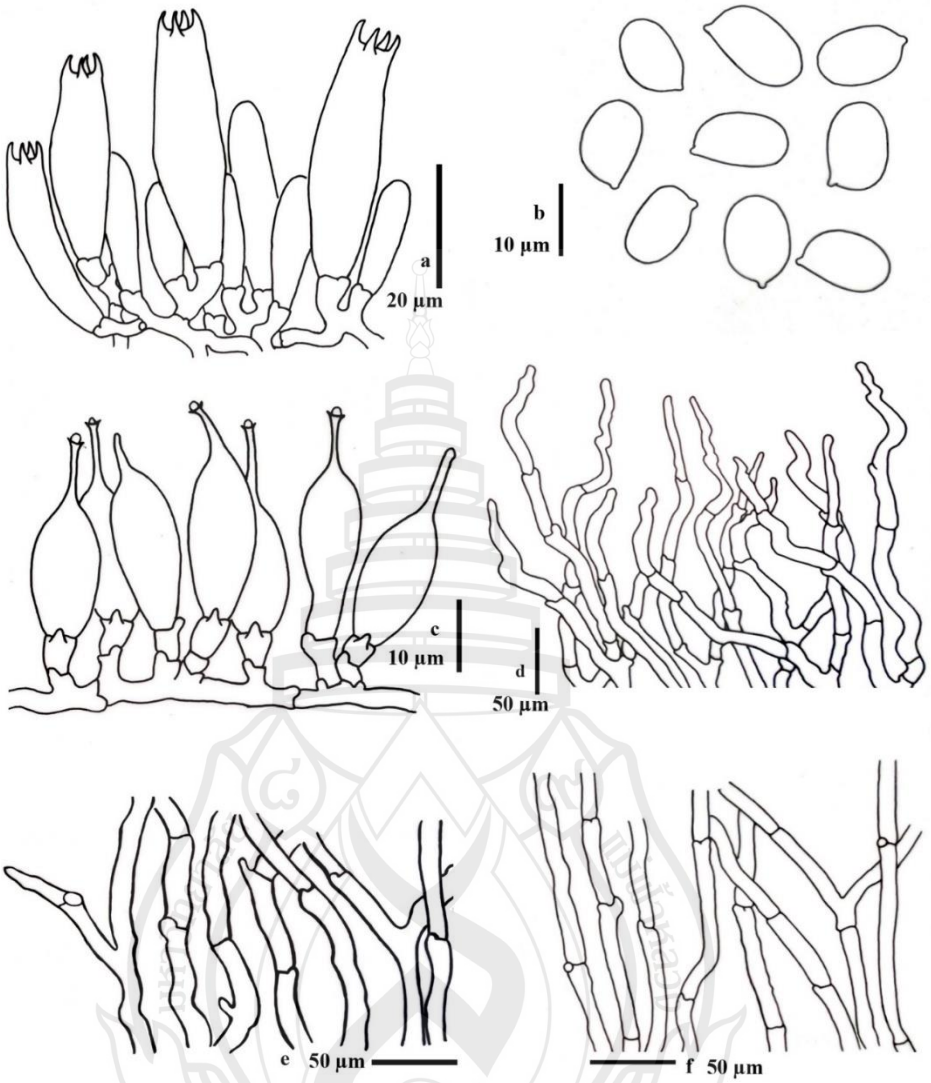


Figure 7.7 *Pleurotus giganteus* (HNL501302). **a** = basidia and basidioles, **b** = basidiospores, **c** = cheilocystidia, **d** = pileipellis, **e** = skeletal hyphae of context, **f** = generative hyphae of context from pileus

7.3.3.4 *Pleurotus tuber-regium* (Fr.) Singer, Lilloa 22: 271 (1951) [1949], (Figure 7.8)

Index Fungorum:198804, FoF number: FoF13861

Basidiomata medium to large size, tuber-liked. *Pileus* 120–150 mm diam., deeply infundibuliform, brown to light brown (6C5) to greyish yellow (4B4), fuliginous and minutely scurfy squamulose, with white in the centre, then fading with age to amber yellow (4B6), remaining dark and dry; margin incurved or involute when mature,

appendiculate and wavy. *Lamellae* decurrent, very crowded, more than 5 lamellulae of more than five, narrow, brownish orange (5C4), pale-yellow to cream-yellow (4A3, 4B3), with the entire fuscous-grey edge, very sinuous on drying. *Stipe* 60–150 × 6–10 mm, wider at base zone (15–20 mm), central, cylindrical to subcylindrical, solid, surface minutely scurfy, covered with concolorous squamulose as pileus. *Annulus* not distinct, with greyish white to greyish (1A1) subfloccose at apex, then becoming ruptured and disappearing. *Context* thick in the centre of the pileus, white, when young soft and fleshy-spongy, then becoming hard coriaceous with age; white and solid in stipe. *Odour* not severed. *Taste* not severed. *Spore print* white.

Basidiospores [150,3,3] (5.1–)5–6–7(–7.3) × (2.5–)2.6–3–4(–5) µm, *Q* = (1.41–)1.45–1.9–2.49(–2.67), cylindrical to subcylindrical, inamyloid, smooth, white, thin-walled. *Basidia* (22.2–)22–26–27.5(–27.8) × (5–)5–6–7.5(–7.9) µm, clavate, 4-spored, hyaline, thin-walled. *Cheilocystidia* (23.1–)23–34–49(–36.7) × (5.6–)6–7–10.5(–10.5) µm, submoniliform, subcylindrical, flexuose, subventricose, protruding, smooth, hyaline, thin-walled. *Pleurocystidia* absent. *The hyphal system* consists of a dimitic hyphal system with skeletal hyphae and generative hyphae, similar in the stipe context, and the pileus context; skeletal hyphae 4–8 µm thick-walled, rarely branching and narrow, with clamp connections; generative hyphae 4–6 µm, not bloat, slightly thick-walled, branched, with prominent clamp connections. *Pileipellis* a cutis composed of hyaline hyphae and cylindrical terminal elements, 3–5 × 90–100 µm, thin-walled. *Stipitipellis* same as on pileipellis. *Clamp connections* are present in all tissues.

Habitat and distribution: grow in rotten buried wood in a rain forest, solitary, distributed in many countries such as China (Karunarathna et al., 2016), Laos (this study), Madagascar (Vizzini et al., 2019), Malaysia as *Panus tuber-regium* (Fr.) (Corner, 1981), Nigeria (Oso, 1977).

Materials examined: LAOS, Xieng Khouang province, Phoukout district, Gnotphe, Na Phouang hamlet date 23 May 2016, Phongeun Sysouphanthong (HNL501189); *ibidem* collected Vientiane capital, Xaythany district, Houay Yang Preserve Forest, date 15 September 2015, Phongeun Sysouphanthong (HNL503400).

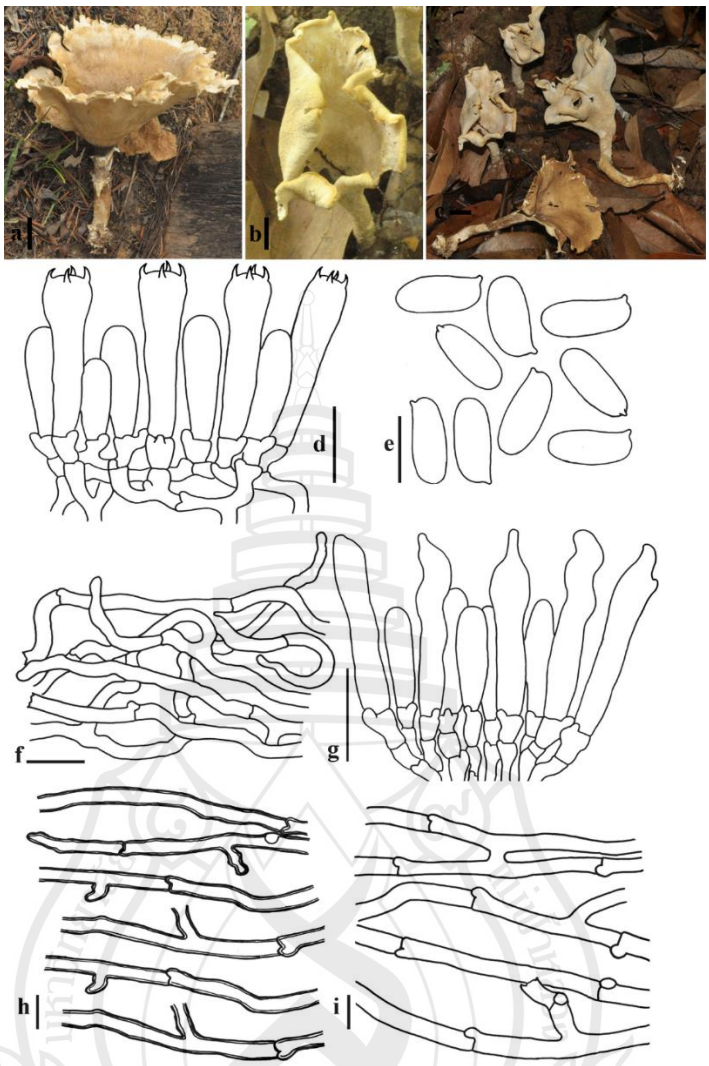


Figure 7.8 Basidiomata of *P. tuber-reguim* in the field. **a** = HNL501189, **b** and **c** = HNL503400. Micromorphology, **d** Basidia, **e** Basidiospores, **f** Irregular cutis, **g** Cheilocystidia, **h** Skeletal hyphae, **i** Generative hyphae. Scale bars **a–c** = 2 cm; **d** = 10 µm; **e** = 5 µm; **f** =50 µm; **g** = 20; **h, i** =10 µm

7.4 Discussion

Pleurotus djamor was divided into six varieties by Corner, based on differences in pileus and lamellae colour, shape of stipe, and spores. The six varieties are *P. djamor* var. *cyathiformis* Corner, *P. djamor* var. *djamor* (Rumph. ex Fr.) Boedijn, *P. djamor* var. *fuscoroseus* Corner, *P. djamor* var. *fuscopruinosus* Corner, *P. djamor* var. *roseus* Corner, and *P. djamor* var. *terricola* Corner (Corner, 1981). Our collections of *P.*

djamor from Laos were in good condition with young to mature stages. They showed pinkish, pastel red to reddish orange surface turning paler to white or whitish with age of basidiomata, pink to pale red lamellae and pale pink to white spore print. The morphology of those specimens fits well with the description of *P. djamor* var. *roseus* from Malaysia (Corner, 1981), with pinkish basidiomata, pink lamellae, oblong basidiospores 7–10 × 3–4 μm, clavate basidia 18–27 × 6–7 μm, and clavate cheilocystidia 30–45 × 8–14 μm. However, cheilocystidia of Lao specimens are smaller (12.2–25.4 × 5.5–6.2 μm) than those of the Malaysian specimens. *P. djamor* var. *cyathiformis* differ from Lao specimens by pileus white to pallid ochraceous basidiomata, *P. djamor* var. *fuscoroseus* pileus fuscous livid, *P. djamor* var. *fuscopruinosus* lamellae white to pallid ochracoeos (rarely tinged pink), *P. djamor* var. *djamor*, and *P. djamor* var. *terricola* pileus nearly or quite lateral, imbricate. Three varieties of *P. djamor* have been reported from Argentina, namely *P. djamor* var. *djamor*, *P. djamor* var. *roseus*, and *P. djamor* var. *cyathiformis* (Lechner et al., 2004). Specimens from Brazil identified as *P. djamor* (Menolli et al., 2014) have much larger basidiospores than our specimens from Laos10–11 × 3–5 μm, basidia 25–26 × 5–6 μm, and cheilocystidia 21–26 × 6– 10 μm. Menolli et al. (2014) recognised that their identification was uncertain and that their collections might belong in other varieties or even different species. ITS sequences of Laos specimens are most closely related to the specimens of *P. djamor* from Malaysia (Table 7.3), which also supports the identification of Laos specimens as *P. djamor*.

Pleurotus giganteus was described as *Lentinus giganteus* Berk (Berkeley, 1874). It was transferred to *Pleurotus* by Karunarathna et al. (2016) because some characteristics are not typical of the genus *Lentinus*. Generally, *Lentinus* species have hyphal pegs, xeromorphic basidiomes with a tough, firm texture when dry, and have a long-life. *Pleurotus* species differ from *Lentinus* by their fleshy, rather soft basidiomes and the absence of hyphal pegs (Corner, 1981; Seelan, 2015). The classification of *Pleurotus giganteus* was confirmed by phylogenetic analysis (Karunarathna et al., 2016). Lao specimens were similar to specimens from the type locality examined by Karunarathna et al. (2011b; 2016) but the pileus colour of the latter was darker. The species has been recorded from Laos by Sysouphanthong et al. (2017) and Læssøe et al. (2018) but those identifications were based on macromorphology only. In this study,

both morphology and ITS sequence data were used for species identification (Table 7.3). The morphology of *Pleurotus giganteus* collected from Laos is very similar to descriptions of the epitype described by Karunarathna et al. (2016). However, the specimens collected in this study had darker pileus than the ones described from China and Thailand. There is also a slight difference of 0.51% in the ITS sequence between the Lao (HNL501302) and Thailand (MFLU08-1371) specimens. This variation in morphology and ITS sequences can be brought about by the different environmental conditions such as the season, temperature and light conditions at the time of collections. Environmental stressors can also change the phenotype of mushrooms for their adaptive advantage (Hewitt et al., 2016).

Pleurotus tuber-regium is edible and has been cultivated in many countries (Okhuoya & Okogbo, 1990; Okjuoya, 1991; Isikhuemhen & Okhyoya, 1996). This species is not only edible, but also good in bioactivities such as antioxidant, antitumor, antihyperglycemic, and antihypertensive agents (Lin et al., 2020). *Pleurotus tuber-regium* was found in both tropical and subtropical regions that grow on decaying wood, dead wood, and was usually found in the underlying soil (Singer, 1961). Before, *P. tuber-regium* was originally described as *Agaricus tuber-regium* Fr. Later, many synonyms were reported in the Species Fungorum (http://www.speciesfungorum.org) e.g. *Lentinus tuber-regium* (Fr.) Fr., *Pachyma tuber-regium* Fr., *Panus tuber-regium* (Fr.), and *Pocillaria tuber-regium* (Fr.) Kuntze. The morphology of specimens of *P. tuber-regium* from Laos is very similar to the descriptions of the epitype described from China by Karunarathna et al. (2016), and it is consistent with those described from Malaysia as *Panus tuber-regium* by Corner (1981). Lao specimens are smaller than specimens from other areas, and the size of the species might depend on the condition of habitats (Hewitt et al., 2016). However, confirmation in the phylogenetic analysis (Figure 7.2) *P. tuber-regium* (MK894133, MK894134) from Laos was grouped together with *P. tuber-regium* from other countries with high bootstrap support (ML 100%, BI 0.96). In this report, *P. tuber-regium* is the first record in Laos.

Studies on *Pleurotus* are important because not only they are good edible mushrooms that can be cultivated, but they also produce primary and secondary metabolites (Rana & Rana, 2017; Chang & Wasser, 2018). *Pleurotus* species are also explored for their bioactivities such as hepatoprotective (Zhang et al., 2016a),

antioxidant (Zhang et al., 2016b), antimicrobial, antiviral, anti-cancer, anti-inflammatory, anti-ageing, anti-tumour, anti-mutagenic, antilipidemic, hepatoprotective, anti-hyperglycemic, hypotensive, and immunostimulant properties (Khan & Tania, 2012) with nutraceutical and pharmaceutical applications. Using both morphological and molecular evidence, this study is a first step to better document *Pleurotus* species from Laos. Most previous studies have identified taxa using morphology (Lee et al., 2017; Sysouphanthong et al., 2018; Sysouphanthong et al., 2020) and species may have been misnamed. *Pleurotus* is an important edible genus that is cultivated worldwide, and our study shows that Laos has a natural population and genetic diversity that could be used in future breeding programmes. The pink *P. djamor* var. *roseus* and the large *P. giganteus* have great industrial potential, especially in the tropics where they can be grown under natural conditions.

Five species from different geographical regions have been reported that have salmon-pinkish-coloured basidiomata, such as *P. djamor*, *P. eous* (Berk) Sacc., *P. flabellatus*, *P. ostreatoroseus*, and *P. salmoneostramineus* (Guzmán et al., 1995; Singer, 1961; Pegler, 1977; Corner, 1981; Guzmán et al., 1993; Pegler, 1997). These species are grouped in the tree topologies (Figure 7.1). *Pleurotus parsonsiae* also belongs to the same clade as *P. djamor*, but its morphology is totally different from *P. djamor* because it has the size of (pileus 7–12 cm), flesh creamy white flesh, longer stipe (8 × 8 mm), lager basidiospores (9–1 × 4–4.5) (Stevenson, 1964). *Pleurotus djamor* (Rumph. ex Fr.) Boedijn has been identified with several synonyms and was published, but there are only nine taxon names listed on the index fungroum. Corner (1981) devised *P. djamor* into six varieties by their characters such as pileus, lamellae colour, the shape of stipe, spore, and substrate. Comparison of *P. djamor* varieties by Corner (1981) found that our specimen was different from that of *P. djamor* var. *cyathiformis* Corner has a white to pallid ochraceous, lamellae edge not darker, a larger stipe (30–40 × 7–8 mm) cyathiform and smaller basidiospres; *Pleurotus djamor* var. *djamor* (Rumph. ex Fr.) Boedijn has almost lateral imbricate, terricolor, merismatiod, and smaller besidiospores (5.5–6.5 × 2.5×3 µm); *P. djamor* var. *terricolus* has nearly lateral imbricate, lignicolour, not merismatiod; *P. djamor* var. *fuscoroseus* Corner and *P. djamor* var. *roseus* Corner has pink basidiomata, gill pink. Thai specimens (MFLU24-0015 and MFLU24-0016) characteristic of flabelliform greyish to white

pileus, greyish fuliginneo-pruinosu at the base of the proximal, cheilocysidia clavate with brownish walls, pileipellis hyphae encrusted with annular-like and minute fascicles present at the end cells. Thai specimen fits well with *P. djamor* var. *fuscopruinosus* was originally described from Malaysia, but it has larger cheilocystidia 45 × 5–14 µm (Corner, 1981). Furthermore, in the phylogenetic tree, MFLU24-0015 and MFLU24-0016 were grouped in the clade of *P. djamor* with high bootstraps (MI = 93%, BI = 0.98%). The type species *P. djamor* var. *fuscopruinosus* was reported without molecular analysis, but based on its morphological characteristics, there were differences from other varieties of *P. djamor*. As mentioned above, we confirmed that the Thai specimen should be *P. djamor* var. *fuscopruinosus*. However, this variety indicates a more complicated ancestry of *P. djamor* with sterile hymenium on the upper side of the pileus, or these two varieties may represent intermediate forms of them.

CHAPTER 8

CULTIVATION, NUTRITIONAL VALUE, AND THERAPEUTIC EFFICACY OF *PLEUROTUS DJAMOR* VAR *FUSCOPRUINOSUS* AND *PLEUROTUS GIGANTEUS*

8.1 Introduction

Mushroom species have been consumed by humans for thousands of years because of their good taste and unique flavours (Valverde et al., 2015; Roncero-Ramos & Delgado-Andrade, 2017). Mushroom species have high nutritional value and medicinal properties, and can reduce risks and treat certain diseases (Oyetayo, 2011; Qin et al., 2015; Valverde et al., 2015; Lau & Abdullah, 2017; Fernandes et al., 2024). Several important elements are included in cultivations, food applications, and the value of medicine (Krakowska et al., 2020; Juárez-Hernández et al., 2023). Mushrooms have high nutrient value including protein, carbohydrate minerals, and vitamins. Their nutritional contents can be compared with foods such as eggs, milk (Akyüz & Kirbağ, 2010; Ho et al., 2020; Niego et al., 2021). The nutritional value index of mushrooms is 6 to 31 (Ferdousi et al., 2020), and it varies depending on species, their environment, condition and substrates supplied for mushroom cultivation (Carrasco et al., 2018; Chang & Wasser, 2018).

The genus *Pleurotus* belongs to the family Pleurotaceae. It was identified by Paul Kummer in 1871. Many species of *Pleurotus* have been moved to multiple genera, such as *Favolaschia*, *Hohenbuehelia*, *Lentinus*, *Marasmiellus*, *Omphalotus*, *Panellus*, *Pleurocybella*, and *Resupinatus* (Singer, 1986). The taxonomy of *Pleurotus* species has long been complicated by morphological plasticity and widespread geographic distribution. Morphological characteristics of the basidiomata often complicate identification. The key species include *P. djamor* (Zervakis et al., 2019), *P. eryngii* (Zervakis et al., 2001), *P. ostreatus* (Anderson, 1973), and *P. pulmonarius* (Bao et al., 2005). The taxonomy of the *Pleurotus* species has been challenging due to their widespread distributions. Often, one species was associated with multiple names

(Guzmán, 2000). Recent studies have combined morphological analyses with advances in molecular phylogenetics, particularly DNA sequencing of ribosomal markers, and have significantly improved classification accuracy by resolving cryptic species and clarifying evolutionary relationships. These approaches improve classification accuracy for *Pleurotus* species (Zervakis et al., 2019). The internal transcribed spacer (ITS) region is widely recognised as the primary DNA barcode for fungi due to its high variability, which allows one to distinguish closely related species (Fajarningsih, 2016). However, despite its effectiveness at the species level, ITS can sometimes fail to resolve cryptic or highly similar species because of its rapid evolution. Meanwhile, large subunit (nrLSU) ribosomal DNA is more conserved and useful for resolving higher taxonomic relationships, such as distinguishing genera (Hwang & Kim, 1999). While nrLSU provides stability in phylogenetic analyses, its lower variability makes it less effective in distinguishing closely related species. Given these limitations, an integrative approach that combines ITS and LSU sequencing offers a more robust method for accurate *Pleurotus* classification (Khatun et al., 2017). Additional markers, such as TEF1-α (translation elongation factor 1-alpha) and RPB2 (RNA polymerase II second largest subunit), may further improve species resolution, though they are less commonly used (Tekpinar & Kalmer, 2019). The Index Fungorum lists 758 taxon names under *Pleurotus* (http://www.indexfungorum.org/), but only 25 species are accepted (He et al., 2019). The type species *P. ostreatus* is well known as an edible mushroom. Morphological features of *Pleurotus* include pleurotoid basidiomata, decurrent lamellulae, smooth basidiospores, and a dimitic hyphal system with skeletal and generative hyphae (Corner, 1981; Seelan, 2015). Most species are edible and distributed in tropical and temperate regions (Raman et al., 2021). Many species of *Pleurotus* are known to cause white rot in dead hardwood (Kaur et al., 2021; Raman et al., 2021).

Pleurotus species are easy to grow and require low-cost substrates. Cultivation is economically viable due to its ability to grow on low-cost agricultural and forest residues, further enhancing its sustainability (Girmay et al., 2016; Thongklang & Luangharn, 2016; Zhai & Han, 2018). *Pleurotus* is extensively cultivated due to its ability to thrive in diverse agro-climatic conditions, rapid growth, and capacity to utilise various agricultural waste substrates (Gebru et al., 2024). Several species have been

successfully cultivated, including *Pleurotus citrinopileatus* Singer, *P. djamor*, *P. djamor* var. *roseus* Corner, *P. eryngii*, *P. flabellatus*, and *P. ostreatus* (Singh & Singh, 2011; Raman et al., 2021). Several agricultural materials can be used as substrates for *Pleurotus* cultivation, including banana leaves, peanut shells, corn leaves, wheat and rice straw, mango fruits and seeds, and sugarcane leaves (Raman et al., 2021). *Pleurotus* species can grow on both composted and non-composed substrates (Jatwa et al., 2016). Successful fruiting body formation in *Pleurotus* species depends on optimal growth conditions, including temperature, humidity, light, and aeration. Generally, mycelial colonisation is optimal at 25–28°C, while fruiting body formation occurs at 18–24°C with 85–95% relative humidity and moderate light exposure (Royse, 2004; Bellettini et al., 2019). Proper ventilation is also crucial, as high CO_2 levels can inhibit primordia formation, leading to abnormal fruiting bodies (Ade-Ogunnowo et al., 2024). Understanding these growth parameters is essential to maximise yield and quality in commercial *Pleurotus* cultivation. *Pleurotus giganteus* is an edible cultivated mushroom that can be found in the soil and buried in the woods. It is known as the giant oyster mushroom and was identified for the first time as *Lentinus giganteus* in Sri Lanka by (Berkeley, 1847), later Karunarathna et al. (2011b) transferred this species to *Pleurotus* based on morphological and phylogenetic analysis. This species was found and reported in many countries such as Malaysia (Corner, 1981), Thailand (Karunarathna et al., 2011b), and Laos (Phonemany et al., 2021). Wild *P. giganteus* has been reported to be successfully domesticated using sawdust as a substrate with a temperature for mycelia growth on soybean agar (*Glycine max* L.) at 25–30°C, and pH 5.0 to 6.5 (Klomklung et al., 2014). The best spawn was reported on soybean at 30°C (Klomklung et al., 2012). The soil casing has been shown to be important to maintain moisture and trigger the use of fruits to obtain a higher yield for *P. giganteus*, such as honeycomb cinder soil (Qin et al., 2016), and garden soil supplemented with 1% nano-KS1 (Soytong & Asue, 2014). However, this mushroom has only been investigated in terms of certain aspects of its cultivation, with its cultivation needs in terms of finding cost-effective and higher-yielding substrates, as well as the factors needed to stimulate growth-fruiting body formation of mushrooms requiring further study.

Pleurotus species are valued for their nutritional composition, including high protein, fibre, vitamins, and essential minerals (Maftoun et al., 2015; Lesa et al., 2022).

The exceptional biodiversity of *Pleurotus* species is attributed to their complex and diverse chemical composition, which includes polysaccharides, glycoproteins, and secondary metabolites such as alkaloids, flavonoids, and betalains (Sharma et al., 2021). In recent years, various *Pleurotus* species have garnered increasing interest due to their outstanding flavour and notable medicinal properties (Raman et al., 2021; Sharma et al., 2021). These mushrooms are abundant in bioactive compounds, with polysaccharides being particularly significant (Barbosa et al., 2020). These species also hold medicinal importance (Krakowska et al., 2021; Juárez-Hernández et al., 2023). *Pleurotus* species have been extensively studied for their anticancer, antioxidant, and antidiabetic properties, making them promising candidates for functional food and pharmaceutical applications (Juárez-Hernández et al., 2023). Investigations have found that *P. ostreatus* extracts induce apoptosis in human cancer cell lines, including lung, breast, and colon cancer cells (Fekry et al., 2022; Jayaprakash et al., 2024). The ethanol extracts of *P. ferulae* fruiting bodies have potent antitumor agents, especially against A549 lung cancer cells (Choi et al., 2004). Furthermore, *P. djamor* has demonstrated cytotoxic effects against colorectal and lung cancer cells, suggesting its potential as an adjunct therapy in cancer treatment (Raman et al., 2015, Juárez-Hernández et al., 2023). The anticancer mechanisms of *Pleurotus* species involve regulation of oxidative stress, cell cycle arrest, and immune modulation. For example, *P. eryngii* extracts were found to inhibit angiogenesis and metastasis in breast cancer models (Zhang et al., 2020). Additionally, lectins isolated from *P. ostreatus* have been reported to induce apoptosis via mitochondrial pathways in leukemia cells (Singh & Singh, 2011; Ebrahimi et al., 2018).

Antioxidant activity is one of the most well-documented biological properties of *Pleurotus* species. The presence of phenolic compounds, flavonoids, and polysaccharides contributes to their free radical scavenging abilities (Jayakumar et al., 2011; Elhusseiny et al., 2022). *Pleurotus ostreatus* extracts have demonstrated strong DPPH and ABTS radical scavenging activities, indicating their ability to neutralize oxidative stress and prevent cellular damage (González-Palma et al., 2016; Morris et al., 2017). Studies have shown that polysaccharides from *Pleurotus* species enhance antioxidant enzyme activities, such as superoxide dismutase and catalase, which protect cells from oxidative damage (Arunachalam et al., 2022). *Pleurotus pulmonarius*

extracts were found to reduce lipid peroxidation and increase glutathione levels, further supporting their role as natural antioxidants (Abidin et al., 2016; Etoundi et al., 2020). Some *Pleurotus* species have shown promising antidiabetic effects, mainly due to their polysaccharide content and ability to regulate glucose metabolism. Polysaccharides extracted from *Pleurotus* species have been reported to enhance insulin sensitivity, reduce blood glucose levels, and improve lipid profiles in diabetic models (Rathore et al., 2017; Liu et al., 2022). *Pleurotus ostreatus* extracts have demonstrated α-glucosidase and α-amylase inhibitory activity, which helps regulate postprandial blood glucose levels (Deveci et al., 2021; Bhardwaj et al., 2024). Additionally, *P. djamor* hot water extracts have been shown to enhance glucose uptake in 3T3-L1 adipocytes, suggesting their potential in managing insulin resistance (Chowdhury & Paul, 2020). Additionally, *P. eryngii* extracts have been found to protect pancreatic β-cells from oxidative stress-induced damage, thereby preserving insulin secretion (Arunachalam et al., 2022).

In Thailand, eight species of *Pleurotus* are documented (Rodrigues et al., 2015). Notable cultivated species include *P. citrinopileatus*, *P. djamor*, *P. eryngii*, and *P. ostreatus* (Krakowska et al., 2020; Lesa et al., 2022; Juárez-Hernández et al., 2023). The secondary metabolites of *P. ostreatus* and *P. pulmonarius* species from Thailand exhibited antibacterial activity against various pathogens, including *Bacillus cereus*, *Enterobacter aerogenes*, *Escherichia coli*, *Micrococcus luteus*, *Proteus vulgaris*, *Salmonella typhimurium*, and *Staphylococcus aureus* (Chaiharn et al., 2018). Bioactive compounds found in the extracts from both species contributed to this antibacterial effect. However, the classification of certain *Pleurotus* species remains ambiguous due to incomplete taxonomic and phylogenetic analyses. Additionally, some species lack cultivation studies, while others require optimisation to improve yield and production efficiency. Moreover, several *Pleurotus* species remain understudied in terms of their nutritional composition, and many lack comprehensive investigations into their medicinal properties and bioactive compounds. This study aims to document new *P. djamor* var. *fuscopruinosus* records in Thailand and evaluate optimal materials for *P. giganteus* cultivation, including spawn media, substrate, and casing. An integrative approach was used that combined morphological characterisation and multigene phylogenetic analysis (ITS and nrLSU). Additionally, the research investigates the

initial cultivation potential with exploration of the optimal conditions including the best media, spawn production, substrate, and environmental conditions. Evaluate the bioactive properties of *P. djamor* var. *fuscopruinosus* and *P. giganteus,* focussing on their antioxidant, anticancer, and antidiabetic potential. The findings contribute to the understanding of *Pleurotus* diversity and its potential applications in sustainable agriculture, food, and medicine.

8.2 Material and Methods

8.2.1 Cultivation of Wild *Pleurotus giganteus*

8.2.1.1 Mushroom species, and identification

Mushroom samples were collected from the Chiang Mai Province, Mae Taeng District during the rainy season. The strain of *P. giganteus* (MFULCC23-0016) was isolated from pileus tissues using sterile forceps and put into Petri-dishes with Potato dextrose agar (PDA). The mycelium culture was subcultured with PDA for the extension of mycelium and incubated at 30°C for 2 weeks. The dried samples and the culture collection were deposited in the Mae Fah Luang University herbarium and the Mae Fah Luang University culture collection. Mushroom collecting and isolation following Section 3.5.1, Figure 3.4 showed the isolation steps (General Material and Methodology). Mushroom species were used molecular analysis to confirmed.

8.2.1.2 Effect of Spawn production

The methodology of this part was following Section 3.5.2 of Chapter 3 (General Material and Methodology).

8.2.1.3 Agricultural wastes for cultivation

The methodology of this part was following section 3.5.3 of chapter 3 (General Material and Methodology).

8.2.1.4 Casing and nutrient supplementation

The methodology of this part was following section 3.5.4 of chapter 3 (General Material and Methodology).

8.2.2 Cultivation of Wild *Pleurotus djamor* var. *fuscopruinosus*

8.2.2.1 Mushroom species, and identification

Pleurotus djamor var. *fuscopruinosus* were collected from Nakhone Si Thammarat province, southern Thailand. Pure cultures were isolated from fresh basidiomata by cutting tissue context using sterile forceps placed on Petri dishes with potato dextrose agar (PDA) and incubated at room temperature for 2 weeks. Pure cultures were deposited at Mae Fah Luang University. Mushroom collection and isolation following Section 3.5.1, Figure 3.4 showed the isolation steps (General Material and Methodology). Mushroom species were used molecular analysis to confirm.

8.2.2.2 Effect of grain media for spawn production

The methodology of this part was following section 3.6.1 of chapter 3 (General Material and Methodology).

8.2.2.3 Effect of different agricultural wastes on mycelium growth

The methodology of this part was following section 3.6.2 of chapter 3 (General Material and Methodology).

8.2.2.4 Fruiting test

The methodology of this part was following section 3.6.3 of chapter 3 (General Material and Methodology).

8.2.3 Statistical Analysis for Cultivation

The analysis of cultivation test was followed by section 3.7 of chapter 3 (General Material and Methodology).

8.2.4 Determination of the Nutritional Content of Cultivated Species

The basidiomata of two *Pleurotus* species included *P. djamor* var. *fuscopruinosus* and *P. giganteus* were the proximate analysis. Fresh basidiomata were dried at 50ºC for 24 hours or until completely dried and powdered using a blender. Proximate value was optioned on the ash, carbohydrate, fat, fibre, moisture, and protein. The determination of ash, fat, fibre and moisture was followed by the AOAC (Assoc. of Analytical Chemistry) and the entire procedure for the determination of nutritional content was followed by section 3.9 of chapter 3 (General Material and Methodology). The protein content of the mushroom samples was determined using the Kjeldahl method following (Nielsen, 2017b) and the conversion factor used for nitrogen to

protein was 6.25. Carbohydrate was calculated by following equation (Raghuramulu et al., 2003):

$$\text{Carbohydrate (g/100g)} = 100 - \frac{(\text{Moisture} + \text{Fat} + \text{Protein} + \text{Ash} + \text{Crude fiber})\ \text{g}}{100\ \text{g}}$$

8.2.5 Therapeutic Efficacy of *Pleurotus djamor* var. *fuscopruinosus* and *Pleurotus giganteus*

8.2.5.1 Mushroom extract

The methodology of this part was following section 3.9.1 of chapter 3 (General Material and Methodology).

8.2.5.2 Antioxidant assay

The methodology of this part was following section 3.9.2 of chapter 3 (General Material and Methodology).

8.2.5.3 Antidiabetic assay

The methodology of this part was following section 3.9.3 of chapter 3 (General Material and Methodology).

8.2.5.4 Anticancer assay

The methodology of this part was following section 3.9.4 of chapter 3 (General Material and Methodology).

8.2.5.5 Cytotoxicity assay

The methodology of this part was following section 3.9.5 of chapter 3 (General Material and Methodology).

8.3 Results of Cultivation

8.3.1 Cultivation of *Pleurotus djamor* var. *fuscopruinosus*

8.3.1.1 Effect of grain media for spawn production

After 10 days of incubation, *P. djamor* var. *fuscopruinosus* showed the highest growth rate on sorghum (11.58 ± 0.51 cm, group a), significantly greater than paddy rice (9.16 ± 0.97 cm, group b) and barley (8.02 ± 0.92 cm, group c). Conversely, Corn, millet, and rice berry exhibited the lowest growth rates (5.96–6.96 cm, group d),

and were not significantly different from each other (Tukey's HSD, $p < 0.05$). The results are presented in Table 8.1 and Figure 8.1.

Table 8.1 Effect of cereal grains on mycelium growth of *P. djamor* var. *fuscopruinosus* MFLUCC24-0056 in 10 days

Grain Types	Mycelial growth rate (cm)	Mycelium Density
Barley rice	8.02 ± 0.92^{c}	Compact
Corn	5.96 ± 0.82^{d}	Compact
Millet	6.96 ± 0.34^{d}	Compact
Paddy rice	9.16 ± 0.97^{b}	Thin
Rice berry	6.1 ± 0.68^{d}	Lightly thin
Sorghum	11.58 ± 0.51^{a}	Compact

Notes All the Data are presented as Mean ± SD. Different letters (a-d) indicate statistically significant differences among treatments. Means with the same letter are not significantly different (Tukey's HSD, $p > 0.05$, N (independent observed data) = 30). Specifically, "a" denotes the highest growth rate group, followed by "b" and "c" for intermediate groups, and "d" for the lowest growth rate group. Mycelium density is based on visual observation.

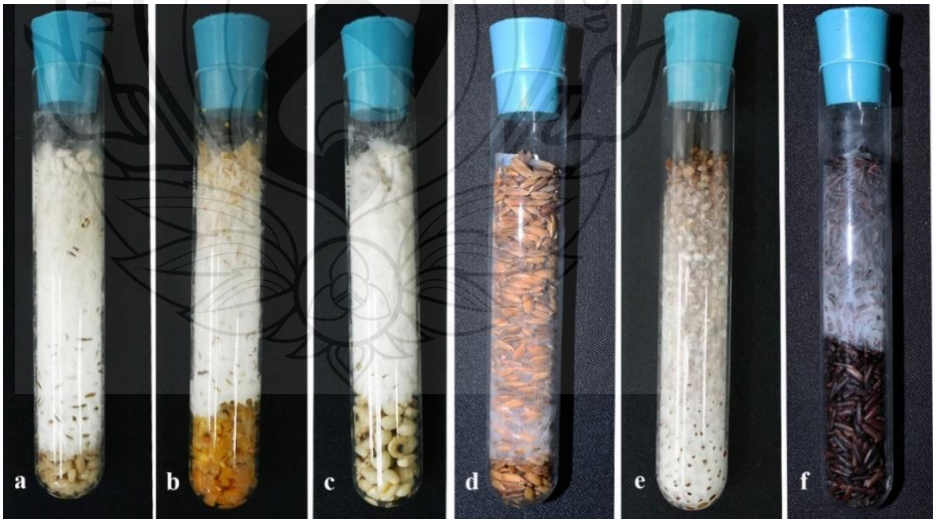


Figure 8.1 The mycelial length of *P. djamor* var. *fuscopruinosus* on different cereal grains. **a** barley, **b** corn, **c** millet, **d** paddy, **e** rice berry, **f** sorghum

8.3.1.2 Effect of different agricultural wastes on mycelium growth

The tested agricultural substrates significantly influenced the diameter of mycelial growth of *P. djamor* var. *fuscopruinosus*. After ten days of incubation, the growth of mycelium on various substrates was evaluated, with results presented in Table 8.2 and Figure 8.2. The highest diameter of the mycelial colony was observed in sawdust (4.37 ± 0.18 cm, group a) and coconut fibre (4.05 ± 0.10 cm, group a) with no significant differences, followed by rice straw (3.17 ± 0.46 cm, group b). Lower diameters were noted in rice husks, corn husks, and pineapple, which showed non-significant differences. The lowest growth diameter was recorded in banana leaves, bagasse, and corn cob, all of which did not demonstrate significant differences. These findings indicated that the choice of substrate could be crucial for optimising the growth of *P. djamor* var. *fuscopruinosus*, which has potential implications for its cultivation and commercial production. Further investigation into the nutritional composition and potential enhancements of these substrates may further improve mycelial growth and yield.

Table 8.2 Effect of different agricultural waste sources on the growth of mycelium of *P. djamor* var. *fuscopruinosus* MFLUCC24-0056 in 10 days

Substrate sources	**Mycelium colony diameter (cm)**	**Mycelium Density**
Banana leaves	$2.58 \pm 0.46^{b,c,d}$	Compacted
Bagasse	$2.58 \pm 0.11^{b,c,d}$	Compacted
Corn cob	$2.58 \pm 0.37^{c,d}$	Compacted
Corn husks	$2.74 \pm 0.53^{b,c,d}$	Lightly compacted
Coconut fibre	4.05 ± 0.10^{a}	Thin
Mix leaves	2.41 ± 0.32^{e}	Lightly thin
Pineapple	$2.57 \pm 0.25^{c,d}$	Compacted
Sawdust	4.37 ± 0.18^{a}	Compacted
Rice husks	$2.95 \pm 0.35^{b,c}$	Lightly thin
Rice straw	3.17 ± 0.46^{b}	Lightly compacted

Notes All the Data are presented as Mean ± SD. Different letters (a-d) indicate statistically significant differences among treatments. Means with the same letter are not significantly different (Tukey's HSD, $p < 0.05$, N (independent observed data) = 50). Specifically, "a" denotes the highest growth rate group, followed by "b" and "c" for intermediate groups, and "d" for the lowest growth rate group. Mycelium density is based on visual observation.

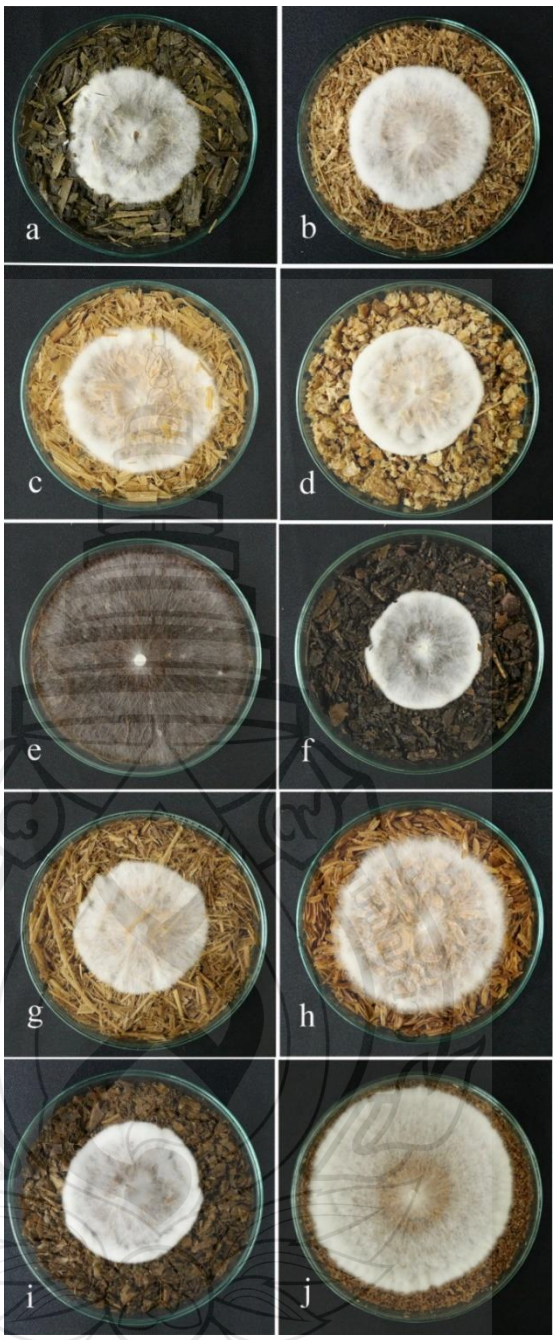


Figure 8.2 Mycelial density of *P. djamor* var. *fuscopruinosus* MFLUCC24-0056 on different substrates after ten days. **a** banana leaves, **b** sugarcane bagasse, **c** corn husks, **d** corn cob, **e** coconuts sell fibre, **f** mix leaves, **g** rice straw, **h** rice husks, **i** pineapple, and **j** sawdust

8.3.1.3 Fruiting test

The primordia of *P. djamor* var. *fuscopruinosus* were produced after the bags were opened for 5 to 10 days at temperatures between 25–28°C with humidity levels of 70–80% under illuminated conditions. The mature mushrooms developed within 4 to 5 days showed in Figure 8.3. Harvesting occurred daily, and the weight of the production was recorded. In the fruiting trials of *P. djamor* var. *fuscopruinosus*, the highest average wet weight recorded was 52.29 ± 16.61 g. The overall yield of the mushrooms was determined to be 12.19 ± 4.78 g, resulting in a biological efficiency of 13.56 ± 4.49%. A total of 116 fruiting bodies were produced during the trials, with the average pileus size measuring 30–150 × 10–80 mm. The average stipe size was 4–21 × 10–56 mm, and it was noted that the size of the mushrooms tended to decrease over time (Table 8.3).

Table 8.3 Harvesting results of *P. djamor* var. *fuscopruinosus* for 30 days

Replication	R 1	R 2	R 3	R 4	R 5	Overall/average
Average wet weight (g)	49.96 ± 0.65	37.9 ± 12.83	72.06 ± 52.54	66.51 ± 11.15	35.01 ± 17.48	52.29 ± 16.61
Yield data (g)	65.2 ± 0.6	47.3 ± 12.8	90.0 ± 52.5	83.1 ± 11.1	43.7 ± 17.4	65.3 ± 20.7
Biological efficiency (%)	12.49 ± 0.6	10.1 ± 12.8	18.96 ± 5.4	17.5 ± 11.1	8.75 ± 1.7	13.56 ± 4.49
Number of fruiting bodies	24 ± 0.44	17 ± 1.14	37 ± 3.14	24 ± 2.58	17 ± 2.21	116 ± 10.18
Pileus size (mm)	40–150 × 20–80	40–85 × 20–55	30–100 × 10–80	40–100 × 16–28	30–115 × 20–74	30–150 × 10–80
Stipe size (mm)	6–20 × 20–50	4–20 × 20–53	4–21 × 20–56	4–15 × 20–48	4–20 × 10–50	4–21 × 10–56

Notes Data are presented as mean ± standard deviation (SD) for quantitative variables. "Pileus size" and "stipe size" are presented as observed minimum–maximum ranges of width × length (w × l, in mm), based on all replications. N (independent observed data) = 50, R = Replication; w = width; l = length.

Figure 8.3 Fruiting body of *P. djamor* var. *fuscopruinosus* MFLUCC24-0056 on sawdust media. **a** primordia appeared on day 10 after open bags, **b-c** young fruiting body for three days, **d-g** mature fruiting body on day five, **h-k** old fruiting body on day seven

8.3.2 Cultivation of *Pleurotus giganteus*

8.3.2.1 Effect of grain media on spawn production

The mycelium growth rate of *P. giganteus* MFLUCC23–0016 was investigated in vitro in various grain media. After incubation at 25°C for 12 days, the millet and sorghum grains did not show a significantly higher growth rate compared to other grain media by statistical analysis. In Table 8.4, millet and sorghum grain had the highest growth rate with a compacted mycelial density (11.10 ± 0.34, 9.02 ± 0.40 mm / day, respectively); followed by rice paddy with a thin mycelial density (4.02 ± 3.14 mm/day); riceberry and wheat had the lowest growth rate with a lightly compacted mycelium density (1.21 ± 1.2 mm/day, 1.86 ± 2.21 mm/day).). The results showed that these cereal grain media could be used to promote the growth of *P. giganteus* mycelia.

Table 8.4 Effect of different types of spawn media on mycelia growth rates (mm/day) of *P. giganteus*

Spawn media	Mycelial growth rate	Mycelial density
Millet	11.10 ± 0.34^{a}	Compacted
Rice paddy	4.02 ± 3.14^{b}	Thin
Rice berry	1.21 ± 1.26^{cb}	Lightly compacted
Sorghum	9.02 ± 0.4079^{a}	Compacted
Wheat	1.86 ± 2.21^{cb}	Lightly compacted

Notes All the Data are presented as Mean ± SD. Different letters (a-d) indicate statistically significant differences among treatments. Means with the same letter are not significantly different (Tukey's HSD, $p < 0.05$, N (independent observed data) = 25). Specifically, "a" denotes the highest growth rate group, followed by "b" and "c" for intermediate groups, and "d" for the lowest growth rate group. Mycelium density is based on visual observation.

8.3.2.2 Effect of agricultural waste on fruiting production

The small primordia of *P. giganteus* were formed after four weeks of incubation at 28°C and at a humidity of 85% in darkness. Subsequently, the bags were opened and transferred to the mushroom house temperature at 30.6 ± 0.9°C, humidity 90% and illuminated conditions. The fruiting bodies of *P. giganteus* matured in 7 days. The mushroom product from sawdust, sawdust + corn cobs, sawdust + rice straw, and sawdust + sugarcane bagasse is shown in Table 8.5. After sixty days we found that there was no significant difference in the total fresh weight of the fruiting body product of sawdust and sawdust + corn cobs at 180.77 ± 44.41g, 176.60 ± 17.65g, with no significant difference in the average weight of the fruit at 51.13 ± 13.25g, 44.13 ± 4.41g, respectively. The number of fruiting bodies was 35 fruits and 29 fruits, respectively; followed by no significant total fresh weight of sawdust + rice straw and sawdust + sugarcane bagasse at 35.30 ± 14.15g, 34.52 ± 11.29g, respectively, such that the average weight of the fruit and the number of fruits of the fruiting body (21) did not show statistically significant differences between the conditions. Biochemical efficiency was measured in sawdust + corn cobs and sawdust with no significant differences at 45.45 ± 4.54%, 42.20 ± 10.36%, respectively; followed by sawdust + rice straw and sawdust + sugarcane bagasse with no significant differences at 11.51 ± 4.61%, 10.55 ± 3.45%, respectively.

Table 8.5 Effect of different types of substrates on fruiting body growth rates of *P. giganteus*

Treatment (w/w)	Total number of fruiting bodies	Total fresh weight (g)	Average fruit weight (g)	Biological efficiency (%)
Sawdust	35	180.77 ± 44.41[a]	51.13 ± 13.25[a]	42.20 ± 10.36[a]
Sawdust + corn cobs	29	176.60 ± 17.65[a]	44.13 ± 4.41[a]	45.45 ± 4.54[a]
Sawdust + rice straw	21	35.30 ± 14.15[b]	10.12 ± 2.14[b]	11.51 ± 4.61[b]
Sawdust + bagasse	21	34.52 ± 11.29[b]	14.14 ± 3.33[b]	10.55 ± 3.45[b]

Notes All the Data are presented as Mean ± SD. Different letters (a-d) indicate statistically significant differences among treatments. Means with the same letter are not significantly different (Tukey's HSD, $p < 0.05$, N (independent observed data) = 40). Specifically, "a" denotes the highest growth rate group, followed by "b" and "c" for intermediate groups, and "d" for the lowest growth rate group.

8.3.2.3 Effect of soil casing

Primordia appeared after the soil casing was applied for 14 days with a 7-day cycle at 30.8 ± 1.17°C, 95% of humidity and under illuminated conditions. The effect of soil casing with three different formulas that add various degrees of nutrient supplementation on total fresh weights and the number of fruiting bodies of *P. giganteus* is shown in Table 8.6. Each formula did not result in significant differences for the average weight of the fruits. However, the first formula (T1) produced the highest fresh fruit weights at 218.35 ± 92.53g and the number of fruiting bodies at 44 fruits; followed by the third formula (T3) which resulted in fresh fruit weights of 278.54 ± 89.04g with 48 resultant fruits; and the second formula (T2) with the lowest number of fruiting bodies at 137.28 ± 67.42g and the number of fruiting bodies at 25 fruits. Figures 8.4 to 8.6 show fruiting bodies of *P. giganteus* grow under different formula soil casings.

Table 8.6 Effect of soil casing with nutrient supplementation on the production of fruiting bodies of *P. giganteus*

Treatment	Total number of fruiting bodies	Total fresh weight (g)	Average fruit weight (g)
T1	44	278.54 ± 89.04[a]	78.13 ± 28.1
T2	25	137.28 ± 67.42 [ab]	76.11 ± 16.18
T3	48	218.35 ± 92.53[b]	49.75 ± 7.16

Notes All the Data are presented as Mean ± SD. Different letters (a-d) indicate statistically significant differences among treatments. Means with the same letter are not significantly different (Tukey's HSD, $p < 0.05$, N (independent observed data) = 15). Specifically, "a" denotes the highest growth rate group, followed by "b" and "c" for intermediate groups, and "d" for the lowest growth rate group.

Figure 8.4 Fruiting bodies of *P. giganteus* grow under soil casing formula (T1)

Figure 8.5 Fruiting bodies of *P. giganteus* grow under soil casing formula (T2)

Figure 8.6 Fruiting bodies of *P. giganteus* grow under soil casing formula (T3)

8.4 Result of Nutritional Analysis

8.4.1 Determination of Nutrient Contents of Cultivated *Pleurotus* Species

The nutritional composition of *P. djamor* var. *fuscopruinosus* (MFLUCC24-0056) and *P. giganteus* (MFLUCC23–0016) was determined including the content of ash, fibre, protein, fat, and moisture of dried mushrooms grown on a sawdust substrate. Table 8.7, shows the nutritional values in 100 g of dried *P. giganteus* which included the following: ash content was 5.61 ± 0.9%, carbohydrates content 61.32 ± 0.0%, fat content was 2.98 ± 1.5 8g, fibre content was 14.28 ± 0.3%, moisture content was 15.6 ± 0.5%, and protein content was 20.318 ± 0.8g. The proximate value obtained from *P. djamor* var. *fuscopruinosus* is shown the ash content is recorded at 7.59 ± 1.0%, the carbohydrate content is 15.99 ± 0.2%, the fat content is recorded at 2.07 ± 0.5%, the fibre content is 19.10 ± 0.9%, the moisture content is 30.25 ± 0.5%, the protein content is 25.00 ± 1.5g.

Table 8.7 Comparing the proximate nutritional values of some *Pleurotus* species

Species name	Ash (%)	carbohydrates (%)	Fat (g/100 g DW)	Fibre (%)	Moisture (%)	Protein (g/100 g DW)	References
P. cornucopiae	6.58 ± 0.0	71.05 ± 0.0	1.30 ± 0.0	N/ A	9.07 ± 0.0	11.08 ± 0.0	Landingin et al. (2021)
P. cystidiosus	6.30 ± 0.00	N/ A	2.05 ± 0.0	20.05 ± 0.0	91.13 ± 0.0	15.68 ± 0.0	Hoa et al. (2015)
P. djamor	0.87 ± 0.22	N/ A	0.17 ± 0.01	3.10 ± 0.24	90.15 ± 0.27	0.12 ± 0.0	Zurbano et al. (2017)
P. djamor* var. *fuscopruinosus	**7.59 ± 1.0**	**15.99 ± 0.2**	**2.07 ± 0.5**	**19.10 ± 0.9**	**30.25 ± 0.5**	**25.00 ± 1.5**	**This study**
P. eryngii	5.99 ± 0.08	N/ A	3.4 ± 0.1	N/ A	9.47 ± 0.08	16.2 ± 0.3	Rodrigues et al. (2015)
P. floridanus	8.69 ± 0.09	42.83 ± 2.54	2.4 ± 0.40	12.25 ± 0.30	89.23 ± 0.10	27.89 ± 0.23	Alam at al. (2008), Prasad et al. (2018)
P. giganteus	**5.61 ± 0.9**	**61.32 ± 0.0**	**2.98 ± 1.5**	**14.28 ± 0.3**	**15.6 ± 0.5**	**20.31 ± 0.8**	**This study**
P. giganteus	N/ A	64.7 ± 0.0	3.10 ± 0.0	32.45 ± 0.07	N/ A	19.2 ± 0.0	Phan et al. (2019)
P. ostreatus	10.91 ± 1.22	37.8 ± 2.5	2.18 ± 0.21	10.41 ± 1.84	8.45 ± 1.65	33.5 ± 0.22	Cohen et al. (2014), Tolera and Abera (2017)

Notes FW = fresh weight; DW = dry weight; N/A = no data available; This study is designed in bold.

8.5 Results of Therapeutic Efficacy of *Pleurotus djamor* var. *fuscopruinosus* and *Pleurotus giganteus*

8.5.1 Antioxidant activity

Three different extractions, namely ethyl acetate ($C_4H_8O_2$), methanol (MeOH) and hot water extract (H_2O) from *P. dajmor* var. *fuscopruinosus* and *P. giganteus*, were used for the antioxidant assay in terms of the DPPH and the ABTS radical scavenging activities of different extracts. The radical-scavenging activity results are shown in Table 8.8. The findings ethyl acetate extract of *P. dajmor* var. *fuscopruinosus* indicated that the DPPH radical scavenging activity measured 694.47 ± 3.92 μg/mL for the ethyl acetate extract, while the ABTS scavenging activity was 652.92 ± 2.53 μg/mL. The ethyl acetate extract of *P. giganteus* indicated that the DPPH radical scavenging activity was 743.78 ± 6.02 μg/ml, and the ABTS scavenging activity was 704.82 ± 2.28 μg/ml.

In contrast, the results for the MeOH and the H_2O extracts showed inactivity. Ascorbic acid (vitamin C) recorded an ABTS scavenging assay, yielding an activity of 8.57 ± 0.43 μg/mL, with DPPH scavenging activity measuring 13.03 ± 0.48 μg/mL. Troxol (vitamin E derivative) recorded an ABTS scavenging activity of 12.29 ± 0.37 μg/mL and a DPPH scavenging activity of 12.88 ± 0.43 μg/mL.

Table 8.8 Antioxidant activities of Ethyl acetate extracted from *Pleurotus* species in terms of the DPPH and ABTS radical scavenging activities (IC_{50} μg/ml)

Treatment	DPPH scavenging activities (IC_{50} μg/ml)	ABTS scavenging activity (IC_{50} μg/ml)
P. dajmor var. *fuscopruinosus*	694.47 ± 3.92	652.92 ± 2.53
P. giganteus	743.78 ± 6.02	704.82 ± 2.28
Ascorbic	8.57 ± 0.43	13.03 ± 0.48
Troxol	12.88 ± 0.43	12.29 ± 0.37

Notes Values represent mean ± SD, N (independent observed data) = 90, ns = no significant activity detected at the maximal tested concentration.

8.5.2 Anticancer Activity

The extract of *P. djamor* var. *fuscopruinosus* demonstrated inhibitory effects against lung cancer cells (A549) and colorectal cancer cells (SW480), as detailed in Table 8.9. The ethyl acetate exacted from *P. dajmor* var. *fuscopruinosus* showed an IC_{50} values for against of colorectal cancer cells SW480 at 382.03 ± 4.55 µg/ml, and lung cancer cells A549 at 245.73 ± 7.60 µg/ml. The ethyl acetate exacted from *P. gigantus* showed an IC_{50} values for against of colorectal cancer cells SW480 at 302.67 ± 11.71 µg/ml, and lung cancer cells A549 at 149.11 ± 5.31 µg/ml. The MeOH and hot water extract showed the results not active. Doxobrucirin showed an IC_{50} values of inhibition at 14.96 ± 1.58 µg/ml.

Table 8.9 Inhibition of cancer cells A549 and SW480 from the Ethyl acetate extracts of *Pleurotus* species

Treatment	**IC_{50} value of inhibition (µg/ml)**	
	P. dajmor* var. *fuscopruinosus	***P. gigantus***
Colorectal cancer cells (sw480)	382.03 ± 4.55	302.67 ± 11.71
Lung cancer cells (A549)	245.73 ± 7.60	149.11 ± 5.31
Doxobrucirin	14.96 ± 1.58	14.96 ± 1.58

Notes Values represent mean ± SD, N (independent observed data) = 90.

8.5.3 Cytotoxicity

The cytotoxicity of *P. djamor* var. *fuscopruinosus* and *P. gigantus* are shown in Table 8.10. The ethyl acetate exacted from *P. dajmor* var. *fuscopruinosus* showed an IC_{50} values for against of Raw cells (264.7) at 213.08 ± 4.08 µg/ml, the ethyl acetate exacted from *P. gigantus* showed an IC_{50} values for against of Raw cells (264.7) at 165.49 ± 2.92, and the standard positive control (Doxobrucirin) showed an IC_{50} values of inhibition at 14.80 ± 0.14 µg/ml. The MeOH and hot water extracts showed the results not active.

Table 8.10 Inhibition of Raw cell (264.7) from the extract of *Pleurotus* species

Treatment	IC_{50} value of inhibition (µg/ml)	
	P. dajmor* var. *fuscopruinosus	***P. gigantus***
Raw cells (264.7)	213.08 ± 4.08	165.49 ± 2.92
Doxobrucirin	14.80 ± 0.14	14.80 ± 0.14

Notes Values represent mean ± SD, N (independent observed data) = 45.

8.5.4 Antidiabetic activity

8.5.4.1 α-glucosidase inhibitory activity

Inhibition of the α-glucosidase enzyme in vitro was examined and the values obtained are shown in Table 8.11. *Pleurotus dajmor* var. *fuscopruinosus* exhibited strong inhibitory activity against alpha-glucosidase in hot water extracts (582.91 ± 3.0 µg/ml) as compared to ethyl acetate (473.87 ± 1.4 µg/ml) and methanol (357.63 ± 3.3 µg/ml). *Pleurotus giganteus* exhibited strong inhibitory activity against alpha-glucosidase in hot water extracts (583.04 ± 4.1 µg/ml) as compared to methanol (579.05 ± 0.1 µg/ml), and ethyl acetate (552.17 ± 1.0 µg/ml). Acarbose showed an IC_{50} value at 635.70 ± 4.9 µg/ml.

Table 8.11 α-glucosidase inhibitory activity of *Pleurotus* species from differences in solvents extraction

Treatment	IC_{50} inhibition value (µg/ml)/ solvents		
	Ethyl acetate	**MeOH**	**H_2O**
P. dajmor var. *fuscopruinosus*	473.87 ± 1.4	357.63 ± 3.3	582.91 ± 3.0
P. giganteus	552.17 ± 1.0	579.05 ± 0.1	583.04 ± 4.1
Acarbose	635.70 ± 4.9		

Notes Values represent mean ± SD, N (independent observed data) = 45.

8.5.4.2 Glucose consumption

In the glucose consumption assay, 3T3-L1 cells were used to evaluate the bioactivity of the formulations with three different extractions and the values obtained are shown in Table 8.12. *Pleurotus dajmor* var. *fuscopruinosus* showed an IC_{50} value of hot water extract at 582.91 ± 3.0 µg/ml, and *P. giganteus* showed an IC_{50} value at 340.78 ± 5.32 µg/ml. The ethyl acetate and MeOH are not shown active. Metformin was used as a positive control showed IC_{50} value at 99.58 ± 0.59 µg/ml.

Table 8.12 Glucose consumption activity of *Pleurotus* species from Ethyl acetate extracted

Treatment	**Solvent & IC_{50} µg/ml**
	Hot water extract
P. dajmor var *fuscopruinosus*	187.31 ± 5.79
P. giganteus	340.78 ± 5.32
Metformin	99.58 ± 0.59

Notes Values represent mean ± SD, N (independent observed data) = 45.

8.6 Discussion

8.6.1 Cultivation of *Pleurotus djamor* var. *fuscopruinosus*

Although there are 25 species of *Pleurotus*, a limited number have been studied extensively for their commercial importance, potential for cultivation, and adaptability to various substrates, particularly those derived from agroindustrial lignocellulosic waste. *Pleurotus djamor*, known as the pink oyster mushroom, is a prominent edible mushroom with various recognized varieties distributed worldwide, including Argentina, Brazil, Indonesia, Japan (as *P. salmoneostramineus* Lj.N. Vassiljeva), Laos, Malaysia, Mexico, and Thailand (Corner, 1981; Neda et al., 1988; Guzmán et al., 1993; Lechner et al., 2004; Chandrasrikul et al, 2011; Menolli et al., 2010; Menolli et al., 2014; Maftoun et al., 2015; Phonemany et al., 2021). The variety *P. djamor* var. *fuscopruinosus* was first identified by Corner in (1981), but at that time, it was noted

primarily based on morphological characteristics without molecular validation. The Thai specimens examined in this study provided critical morphological details and molecular analysis, enhancing our understanding of this species.

The identification of the Thai specimens (MFLU24-0015, MFLU24-0016) as a new recorded variety reinforced the necessity for more molecular studies in the classification of fungi. Sorghum demonstrated the fastest growth rate for *P. djamor* var. *fuscopruinosus*, indicating its suitability as a cost-effective substrate for spawning in Thailand. This aligns with numerous reports indicating that sorghum is an effective substrate for spawn production. For example, Thulasi et al. (2010) documented the optimal spawn production of *P. eous* and *P. florida*, Kumla et al. (2013) identified optimal conditions for *P. giganteus*, and Thongklang and Luangharn (2016) noted similar findings for *P. ostreatus*. Oei and van Nieuwenhuljzen (2005) further supported the concept that sorghum is widely utilized for such purposes across the globe.

The effect of agricultural waste on mycelium growth showed promising results, demonstrating that *P. djamor* var. *fuscopruinosus* could thrive on various substrates derived from agriculture. The data suggest that while coconut fiber can support mycelium growth, sawdust is the superior substrate for mycelial density and diameter. This finding may enhance the implementation of sawdust as a substrate source for mushroom production, which aligns with previous findings by Hoa and Wang (2015), who suggested that sugarcane residue, acacia sawdust, and corn cob are beneficial for mycelial growth in other *Pleurotus* species. Kuforiji and Fasidi (2007) also indicated that corn cob, rice straw, and sawdust can effectively promote growth in *P. tuber-regium*.

The results of the fruiting body tests established that *P. djamor* var. *fuscopruinosus* thrives particularly well in sawdust-based substrates for fruiting body production. This observation corresponds with earlier studies that noted the successful cultivation of *Pleurotus* species on a variety of agricultural and natural substrates, such as logs and sawdust (Kumla et al., 2013; Oei & van Nieuwenhuljzen, 2005; Thongklang & Luangharn, 2016). Shah et al. (2004) reported high yields for *P. ostreatus* cultivated in sawdust, while Jegadeesh et al. (2018) successfully cultivated *P. djamor* var. *roseus* using various agro-residues efficiently. The present study is the first to successfully cultivate *P. djamor* var. *fuscopruinosus* with sawdust as the primary substrate combined

with additives. This aligns with Mandeel et al. (2005), who reported that the nutrient content, growth, and yield of *Pleurotus* cultivated in sawdust depend on its chemical constituents.

8.6.2 Cultivation of *Pleurotus giganteus*

Comparing the morphology of *P. giganteus* (MFLUCC23-0016) with other *P. giganteus* strains reported on from different countries, such as the strains MFLU08–1371 from Sri Lanka described by Karunarathna et al. (2011b), the strains of *P. giganteus* CMU54–1 from Thailand by Kumla et al. (2013), and the strains of *P. giganteus* HNL501302 from Laos by Phonemany et al. (2021), the characterization of MFLUCC23-0016 fit well with these reports which was further confirmed by molecular analysis.

Pleurotus giganteus has been used as a medicinal substance and source of food and income for local people (Mortimer et al., 2012; Mortimer et al., 2014; Valverde et al., 2015). Therefore, studying and developing approaches to optimal cultivation is critical. Our results show that the mycelium growth rate was highest with the compacted mycelium density of *P. giganteus* in millet and sorghum. For other types of media grain, such as rice paddy, growth occurred but the mycelium density was thin. For rice berry and wheat grain the results did not suggest optimal use based on the mycelium growth of *P. giganteus*. Our results suggest that with rice berry and wheat grain the mycelium growth rate was very low with lightly compacted mycelium density, which agreed with Jayachandran et al. (2017) who reported that wheat grain was comparatively less effective for the production of *P. floridanus*. The effect of grain media for spawn production was similar to that of Klomklung et al. (2012) and Kumlar et al. (2013) who similarly reported the best substrate for spawn production of *P. giganteus*. Thulasi et al. (2010) reported that sorghum grain was the optimal substrate for spawn production of *P. eous* and *P. florida*. Agriculture wastes for fruiting bodies production, such as corn cob, rice straw, sugarcane bagasse, could be used as an alternative to mixing with sawdust for cultivated *P. giganteus*. The fruiting bodies product being dependent on the type of substrates that contained different percentages of lignin, cellulose and hemicellulose used for its cultivation, in agreement with Thongklang and Luangharn (2016) who reported using agricultural wastes for the production of *P. ostreatus*. Furthermore, according to previous studies *P. giganteus* was first grown on sawdust in

northern Thailand at the laboratory scale, by Klomklung et al. (2012, 2014), Kumla et al. (2013), and Soytong and Asue (2014). The soil casing is important for the cultivation of *P. giganteus* to obtain a higher mushroom product by applying soil on top of the substrates and our study reported that three soil casing formulas could be used for the fruiting products. However, 25% humus soil mixed with 25% calcium carbonate, 35% lime sand, 15% peat obtained a higher product compared to the total wet weight of the fruiting product from 100% humus soil, and without humus soil mixed with 50% lime sand, 25% calcium carbonate, 25% peat. Based on our results on the effect of the soil casing, we suggest that 25% humus soil + 25% calcium carbonate + 35% lime sand + 15% peat be used as the soil casing. Furthermore, based on the result of this study, it was found that 100% humus soil could be an alternative to the soil casing and 50% lime sand + 25% calcium carbonate + 25% peat could also be used due to its good air permeability, but could negatively impact the ability to retain much moisture. This study agrees with Soytong and Asue (2014) who used ordinary garden soil as the main casing material and reported garden soil supplemented with 1% nano-KS1. Additionally, reported by Qin et al. (2016) who tested seven types of soil casing used for cultivated *P. giganteus* (named as *Panus giganteus*) such as sugarcane filter mud, mushroom fermentation residue, paddy soil, yellow clay soil, honeycomb and cinder soil, spent straw mushroom substrate and water-soaked rotten straw. The using of agricultural waste has a high potential for cultivation of *P. giganteus*. Therefore, more study is necessary on how to increase fruiting production by changing the ratio and supplementing for sawdust + corn cob substrates. In addition, the formula for the soil casing changes the percentage of the nutrient supplement, as well as the types of soil.

8.6.3 Determination of Nutrient Contents of Cultivated *Pleurotus* Species

The nutritional profile of *P. djamor* var. *fuscopruinosus* and *P. giganteus* manifests high culinary value, revealing considerable levels of carbohydrates, protein, moisture, fibre, ash, and low-fat content. This nutritional content implies the mushroom's potential to match or even surpass other conventional food sources, such as eggs and milk (Ho et al., 2020; Niego et al., 2021). Generally, mushrooms are recognized for their ability to provide more protein than many other crops and most wild plants (Sande et al., 2019). In the proximate of *P. djamor* var. *fuscopruinosus* (Table 8.7), the protein content is 24.99%. However, the protein content of mushrooms

is affected by a number of factors, including the type of mushroom, the stage of development, the sampled part, the level of nitrogen available, and the location (Bellettini et al., 2019; Wang et al., 2021).

The findings from the nutritional analysis indicate that *P. djamor* var. *fuscopruinosus* possesses a high culinary and nutritional value. The mushroom exhibited a fat content of 2.07%, which is comparable to that of *P. cystidiosus* O.K. Mill. (2.05 ± 0.0%) (Hoa et al., 2015). This low-fat content is advantageous for health-conscious consumers who seek nutritious food options. In comparison with other species, *P. djamor* var. *fuscopruinosus* has lower fat levels than *P. eryngii* (DC) Quél. (3.4 ± 0.1%) and *P. giganteus* (3.10 ± 0.0%) (Phan et al., 2019). The ash content of 7.59% and the fibre content of 19.10% are noteworthy, especially when compared to previous studies that reported lower amounts of ash (0.87 ± 0.22%) and fibre (3.10 ± 0.24%) (Zurbano et al., 2017). The findings are consistent with those of Landingin et al. (2021), who provided data for *P. cornucopiae*, noting lower fat content alongside higher protein, ash, and carbohydrate content. This demonstrates that *P. djamor* var. *fuscopruinosus* is not only nutritious but also aligns with the trend of mushrooms serving as health-promoting foods.

The results of the proximate of *P. giganteus* in sawdust substrates were found to have a very low amount of fat but were rich in carbohydrates, protein, moisture, and ash content with a large amount of fibre. Our study had similar results to previous studies by Landingin et al. (2021) who reported the approximate mass of *P. cornucopiae*, which had a large amount of carbohydrates, protein, ash, and moisture content, with a lower fat content. The comparison of the proximate nutritional values of some species of *Pleurotus* is shown in (Table 8.7). Phan et al. (2019) who reported a review of the proximate of *P. giganteus* found that the amount of fibre and fat was higher (32.45 ± 0.07%, 3.10 ± 0.0%, respectively) than strains MFLUCC23-0016 (14.28 ± 0.3%, 2.98 ± 1.5%); the protein contents of strains MFLUCC23-0016 were determined to be higher (20.31 ± 0.8 g/100g) than previously reported (19.2 ± 0.0 g/100g); the carbohydrates in this study (61.32 ± 0.0%) can be compared with Phan et al. (2019) at (64.7 ± 0.0%). The ash content of our study was 5.61%, which is relatively low compared to other *Pleurotus* species in (Table 8.7), e.g., *P. ostreatus* (10.91 ± 1.22%) (Cohen et al., 2014; Tolera & Abera, 2017); *P. floridanus* (8.69 ± 0.09%) (Alam

et al., 2008, Prasad et al., 2018); and *Agaricus* sp. (10.1–10.90%) (Zakhary et al., 1983). However, nutritional status is affected by several factors, such as the type of mushrooms, the stage of development, the part sampled, the level of nitrogen available, and the location (Flegg & Maw, 1977; Motskus, 1973). Mushrooms have been consumed by humans for decades, and nutritional analyses of mushroom species reveal that they are rich in protein, carbohydrates, and dietary fibre with low fat (Cheung, 2010; Valverde et al., 2015).

The results of the nutritional analyses of *P. dajmor* var. *fuscopruinosus* and *P. giganteus* cultivated found in this study confirmed that this mushroom can be compared with other species of *Pleurotus* (see Table 8.7) and them can be an alternative food that provides health benefits.

8.6.4 Therapeutic Efficacy of *Pleurotus djamor* var. *fuscopruinosus* and *Pleurotus giganteus*

There are more than 270 types of mushrooms recognized for their potential health benefits (Panda et al., 2022). Many pleurotoid mushrooms have been investigated for their bioactive compounds, which can be therapeutic in treating various diseases (Atri et al., 2019; Phan et al., 2019; Deshmukh et al., 2022). The pharmacological safety of mushrooms is an important consideration, with treatments being viewed as safe and comparable to other natural remedies (Sisodiya et al., 2023). In this study, the bioavailability assay evaluated the antioxidant activity of mycelium extracts from *P. djamor* var. *fuscopruinosus* and *P. giganteus* using three different solvents. Notably, 50% (v/v) ethyl acetate proved to be the most effective solvent for antioxidant activity. Among them, *P. djamor* var. *fuscopruinosus* demonstrated slightly higher antioxidant activity than *P. giganteus* in both DPPH and ABTS assays, as indicated by its lower IC_{50} values. This agreed with Phan et al. (2013) who reported *P. giganteus* could potentially be used in a well-balanced diet and as a source of dietary antioxidants to promote neuronal health. The 50% (v/v) ethyl acetate was the most active for the antioxidant assay. This finding aligns with the work of Cheung et al. (2003), who reported that *Lentinula edodes* Singer, at a concentration of 6 mg/ml, demonstrated a higher DPPH radical scavenging rate of 55.4%. Meanwhile, the literature Floegel et al. (2011) suggested that the ABTS assay is superior to the DPPH assay when evaluating various plant foods with differing antioxidant compounds.

However, Gulcin and Alwasel (2023) reported *P. ostreatus* extracts have a high affinity for DPPH radicals that are comparable with the aqueous study environment, giving ascorbic acid an advantage because of their high-water solubility compared to the extracts that may be less soluble, thus limiting their overall effective concentration and scavenging action. Other species such as *P. floridanus,* and *P. ostreatus* extracts also have the greatest antioxidant activity in the ABTS and DPPH assay (Gulcin & Alwasel, 2023; Krishna et al., 2023). This study indicates that *P. dajmor* var. *fuscopruinosus* exhibited stronger antioxidant activity in ABTS assays (lower IC_{50} values) compared to DPPH assays (higher IC_{50} values), suggesting that this mushroom showed antioxidant properties, its potency is significantly lower than ascorbic acid and trolox.

The therapeutic potential of *Pleurotus* species has gained significant traction as cancer treatment research progresses. Numerous species within this genus have demonstrated anticancer properties, as extracts from their fruiting bodies or mycelium have exhibited cytotoxic effects against various cancer cell lines, including human hepatoma HepG2 cells, bladder carcinoma, and ovarian cancer (Maness et al., 2011; Ragasa, 2018; Abdullah, 2021; Nowakowski et al., 2021; Juárez-Hernández et al., 2023). In this study, *P. djamor* var. *fuscopruinosus* and *P. giganteus* verifiable anticancer effects *in vitro*, with the ethyl acetate extract effectively targeting both colon and lung cancer cells. Although its cytotoxic activity was significantly lower than that of doxorubicin, the extract still exhibited notable cancer cell elimination, highlighting its potential as a natural source of bioactive compounds for future therapeutic exploration. Our findings correlate with previous research Xu et al. (2014), which highlighted the telomerase inhibitory properties of ethyl acetate extracts from *P. ostreatus*, as well as the potent anticancer activity of water extracts from *Ustilago esculenta* Henn. against SNU-1 cells exhibited strong anticancer effects. The present investigation into cytotoxicity revealed that while the extracts of *P. djamor* var. *fuscopruinosus* and *P. giganteus* demonstrated selective cytotoxic effects against cancer cells, they also exhibited relatively low cytotoxic effects on RAW 264.7 cells as compared to doxorubicin. This observation is significant as it supports their potential for safe applications.

Diabetes is a major global health concern characterized by elevated blood sugar levels. This condition has prompted the development of numerous medications, each

with distinct mechanisms of action and potential side effects. These treatments may vary in effectiveness based on individual physiological responses and unique characteristics (Tor Sak & PatthamWan, 2011). In this study, *P. djamor* var. *fuscopruinosus* and *P. giganteus* demonstrated significant *in vitro* antidiabetic activity, particularly through its α-glucosidase inhibitory action. The MeOH extract of *P. dajmor* var. *fuscopruinosus* has the strongest α-glucosidase inhibition (lowest IC_{50}). Both mushrooms extracts show some inhibitory activity, but MeOH extracts appear more effective. Additionally, the extract is effectiveness was affirmed with statistically significant results at the dose level tested, highlighting its potential as a natural adjunct in diabetes management. Our findings align with previous research by Deveci et al. (2021), which reported that *P. ostreatus* exhibits strong α-amylase inhibitory activity along with high α-glucosidase inhibition, both of which are relevant to type 2 diabetes management.

Further investigation of glucose consumption activity revealed that the hot water extract of *P. djamor* var. *fuscopruinosus* and *P. giganteus* demonstrated significant activity. In contrast, the well-known antidiabetic drug metformin exhibited a lower IC_{50} value. In contrast, the well-known antidiabetic drug metformin exhibited a lower IC_{50} value. This indicates that while *P. djamor* var. *fuscopruinosus* and *P. giganteus* may not surpass the potency of metformin, but it still possesses meaningful antidiabetic properties that merit further exploration. These findings align with previous research that documented the antidiabetic effects of *P. djamor*, particularly based on basidiomata powder, as stated by Nayak et al. (2021). Moreover, the hot water extract of *P. pulmonarius* (Fr.) Quél has demonstrated both *in vitro* and *in vivo* antidiabetic effects (Balaji et al., 2020). Various other *Pleurotus* species have also been reported to exhibit high antidiabetic activities; for example, *P. florida* (Bello et al., 2017; Venkata Krishna et al., 2023), and *P. ostreatus* (Shamtsyan & Pogacnik, 2020). Wei et al. (2020) further corroborated these findings, asserting that medicinal mushroom extracts, including those from *Agaricus blazei* Murill, have substantial effects on glucose reduction, demonstrating activity comparable to that of metformin. The capacity of *P. djamor* var. *fuscopruinosus* to regulate blood sugar levels and inhibit carbohydrate digestion via α-glucosidase activity indicates its potential use as a functional food for managing diabetes. This mushroom could serve as a complementary option to

traditional pharmacological treatments, contributing to a holistic approach to diabetes management. Future studies should explore the bioactive compounds responsible for these antidiabetic effects and their mechanisms of action. Moreover, examining their efficacy *in vivo* models will provide valuable insights into their potential integration into dietary practices aimed at regulating blood sugar levels. This study suggests that to improve the IC_{50} concentration of the extract, efforts should focus on purification, compound identification, enhancing bioavailability, and optimizing experimental conditions. These strategies can help achieve potency levels comparable to or exceeding those of the standard reference compound.

CHAPTER 9

CONCLUSION

This study reviews information on the taxonomic classification and distribution of pleurotoid mushrooms in Laos and Thailand. Culturing some important species, including the substrate used and the biological efficiency, was also discussed. In addition, nutritional analyses of edible species and secondary metabolites of pleurotoid species with their bioactivities were also included. Laos and Thailand share borders in the Greater Mekong Subregion with the same climate and types of forest; therefore, it is expected that the density and diversity of mushrooms should be relative. However, Thailand has recorded more pleurotoid mushrooms, with eight families, 11 genera, and 30 species, while Laos only identified three families, three genera, and 12 species. Many forest areas in Laos have not been extensively investigated, so more undiscovered species may exist to explore. Further taxonomic studies in Laos may reveal a high diversity of Basidiomycota.

9.1 Species Diversity of Pleurotoid Mushrooms

Thailand has abundant types of forests; therefore, the density and diversity of mushrooms are expected to be high. However, Thailand has recorded pleurotoid mushrooms, with eight families, 11 genera, and 30 species. The current number of pleurotoid mushrooms recorded in Thailand is 100 species, including those in this study and 30 species that were previously reported. Many forest areas in Thailand have not been extensively investigated, so more undiscovered species may exist to explore. Further taxonomic studies in Thailand may reveal a high diversity of pleurotoid mushrooms. The importance of studying the diversity of pleurotoid mushroom species in community forestscan respond to many issues, such as (1) conservation of biodiversity helps in understanding and maintaining the diversity of pleurotoid mushrooms that exist in the ecosystem, which plays an important role in maintaining the balance of forest and ecosystem. (2) Economic benefits, some pleurotoid

mushrooms have a high economic value and can be used as food, herbs, or medical products. The study helps identify mushrooms with economic potential and develop cultivation practices. (3) Research and innovation; the study of the diversity of pleurotoid mushrooms can be a starting point for further research on active compounds in mushrooms that may lead to the development of new drugs or other useful products. The study in community forests also helps to raise awareness of the importance of nature conservation in the community and encourages local people to play a role in protecting natural resources. This study provided an extensive survey of pleurotoid mushrooms in Thailand during the period 2019-2023. However, it should be noted that the frequency of sample collections for each site was not consistent due to unsuitable or inaccessible conditions.

9.2 Phylogeny and Taxonomy of Pleurotoid Mushrooms

Laos and Thailand are tropical countries located in Southeast Asia that share a border of 1,845 km in length under the Greater Mekong Subregion, Northern Thailand and Laos are similar in seasonal climate and biodiversity. We proposed investigating and documenting the number of pleurotoid mushrooms in this interesting region. This study described 15 species of pleurotoid mushrooms, including six new species of *Crepidotus* and two new records, two new species of *Hohenbuehelia* and a note on *Hohenbuehelia tritis*, four new records of *Pleurotus* species, based on the details of the morphology and molecular analysis data (see Table 9.1). Furthermore, two wild *Pleurotus* species have been tested for their ability to cultivation, and some agricultural waste effects on mushroom product nutritional content screening of mushroom products in terms of their biological activities.

Table 9.1 Lists of described species, number of specimens, new species, and new records of pleurotoid mushrooms from Laos and Thailand

No.	Name	No. of investigated specimens	
		Thailand	**Laos**
1	*Crepidotus chiangraiensis*	2*	0
2	*Crepidotus flavocarpus*	2*	0
3	*Crepidotus lateralipes*	1**	0
4	*Crepidotus roseocarpus*	3*	0
5	*Crepidotus striatus*	2**	0
6	*Crepidotus thailandicus*	3*	0
7	*Crepidotus ungulatus*	2*	0
8	*Crepidotus viscidus*	4*	0
9	*Hohenbuehelia flabelliformis*	2*	0
10	*Hohenbuehelia lageniformis*	4*	0
11	*Hohenbuehelia tristis*	2**	0
12	*Pleurotus djamor*		2**
13	*Pleurotus gigateus*	1**	7**
14	*Pleurotus tuber-regium*		2**
15	*Pleurotus djamor* var. *fuscopruinosus*	2**	0
	Total	**29**	**11**

Note * = new species from this study; ** = new record from this study.

9.3 Cultivation of Two wild *Pleurotus* Species

Four species of *Pleurotus* were identified based on morphological and molecular evidence, including eleven specimens borrowed from HNL (Laos) and three specimens collected from Thailand. Most of the specimens examined were from Thailand. This study recorded two species, *P. giganteus* and *P. djamor* var. *fuscopruinosus*, which is reported for the first time in Thailand.

During pleurotoid mushroom surveys, two samples were isolated and deposited in the MFLUCC culture herbarium. *Pleurotus giganteus* was selected to investigate its

suitable conditions for cultivation, including the effect of grain medium on spawn production, the effect of agricultural waste on fruit production, and the effect of soil casing. Mycelium grew best with the compacted mycelium density in millet and sorghum; the best substrate for fruiting bodies was sawdust and sawdust + corn cobs at temperature of 30.6 ± 0.9°C, 90% and illuminated conditions. In addition, using 25% humus soil, 35% lime sand, 25% calcium carbonated and 15% peat, this formula is the best casing soil that can offer high yields, while using 100% humus soil is an alternative casing that can be used instead of the casing of the best formula, but the yield will depend on the different components of the soil, with different soil structures creating soil with different properties that affect water flow.

Pleurotus djamor var. *fuscopruinosus*, a newly identified variety in Thailand, was characterized through molecular and morphological analyses. Its mycelial growth was optimized on sorghum grain and sawdust, with successful fruiting observed in sawdust-based cultivation. Primordia formation occurred four weeks after the mycelium had fully colonised the substrate and mature fruit bodies developed within 4–5 days under illuminated conditions, at a temperature of 25–28°C and a humidity of 70–80%.

9.4 Nutritional Analysis and Biological Activity

Nutritional analysis revealed that *P. djamor* var. *fuscopruinosus* and *P. giganteus* cultivated on sawdust substrates contains very low levels of fat while being rich in essential nutrients, including protein, carbohydrates, moisture, ash, and dietary fibre. This nutrient profile highlights the culinary potential of these mushrooms, making them an attractive addition to a healthy diet. Furthermore, the bioactivity assays performed showed variable results, dependent on the polarity of the solvents used for extraction. The ethyl acetate extract displayed significant antioxidant activities in both the DPPH and ABTS assays, although these activities were found to be lower than those typically observed in fruits and vegetables.

The anticancer assay indicated notable cytotoxicity against different cancer cell lines, including lung and colon cancer cells. The ethyl acetate extract was confirmed to

be toxic to both cancerous cells, prompting considerations of its application in a therapeutic context. Such findings are significant, as they suggest that while low toxicity levels for cancer cells are desirable, treatments, such as Doxorubicin, are known for their indiscriminate destruction, affecting rapidly dividing normal cells, including those in the digestive tract, blood, and hair. In addition, the antidiabetic activity exhibited by *P. djamor* var. *fuscopruinosus* and *P. giganteus* were demonstrated through its ability to inhibit the enzyme α-glucosidase, effectively reducing postprandial blood sugar levels. This capacity to inhibit sugar absorption highlights the mushroom's potential as a functional food in diabetes management.

In conclusion, *P. djamor* var. *fuscopruinosus* and *P. giganteus* show considerable promise as an important dietary fungus, given its rich nutrient composition and potential health benefits. Future research should focus on optimising the cultivation methods for increased fruiting body production, which may involve adjusting substrate ratios and supplementing sawdust with other agricultural wastes to enhance growth and yield. Moreover, bioactivity assays demonstrated notable antioxidant activity, α-glucosidase inhibition, and selective cytotoxicity against cancer cells. These findings highlight its potential as a functional food and therapeutic agent. Further studies should focus on isolating bioactive compounds, evaluating safety, and performing *in vivo* evaluations to validate their pharmacological properties.

9.5 Research Advantages

9.5.1 This study is important for providing a comprehensive study documenting the diversity of pleurotoid mushrooms from selected communities in Thailand, an extensive survey of the diversity of their distribution and ecological significance.

9.5.2 This study provided accurate species identification diagnostics using taxonomy and phylogenetic analysis for the classification of new species and records, significantly expanding the known taxonomy of pleurotoid mushrooms and contributing to the global fungal database.

9.5.3 This study provided a review checklist of pleurotoid mushrooms, including nutritional analysis, cultivation, and the bioactive compound of pleurotoid mushrooms reported from Laos and Thailand.

9.5.4 Investigation of a wide range of applications of pleurotoid mushrooms, including their nutritional composition, cultivation potential, and bioactive properties.

9.5.5 Successful first-time cultivation of *P. djamor* var. *fuscopruinosus*, along with finding alternative substrates and soil casing options to enhance the yield of *P. giganteus* cultivation.

9.5.6 The study showcases the rich nutrient profiles of *P. djamor* var. *fuscopruinosus* and *P. giganteus*, offering potential as healthy dietary options. The findings on antioxidant, anticancer, and antidiabetic properties suggest potential applications in functional foods and pharmaceuticals.

9.6 Future Perspective

Pleurotoid mushrooms are becoming more popular due to their high nutritional content and good medical effects for humans. As a result, they may be a natural source for the production of nutraceuticals. Some pleurotoid mushrooms from Thailand have been investigated for their secondary and bioactive properties, such as *Clitopilus chalybescens*, *Hohenbuehelia grisea*, *Lentinus sajor-caju*, *L. squarrosulus*, *Pleurotus eryngii*, *P. ostreatus*, and *P. pulmonarius* (Jatuwong et al., 2016; Chaiharn et al., 2018; Sandargo et al., 2018a; Sandargo et al., 2018b; Sysouphanthong et al., 2023). However, no studies have been performed on the secondary metabolites of pleurotoid mushrooms and their bioactivities in Laos, and limited studies have been conducted in Thailand. The value of pleurotoid mushrooms in these countries can be increased by additional scientific research.

There is no scientific literature on the cultivation of pleurotoid mushrooms in Laos. Pleurotoid species are grown exclusively in the community for personal use and for sale in the neighbourhood market. Instead of being grown on arable land, cultivated mushrooms are grown in specific structures or underground facilities that may have been constructed or modified especially for that purpose. Pleurotoid mushrooms

cultivated in Thailand come in five different species, such as *Lentinus arcularius, L. sajor–caju, L. squarrosulus*, *Pleurotus giganteus*, and *P. ostreatus*. Other genera are not well documented; therefore, the cultivation of pleurotoid species needs additional research to improve agricultural waste or lignocellulosic waste as an alternative substrate, helping to break down lignin and hemicellulose. To reduce the amount of waste, the cultivation of pleurotoid species can be an alternative livelihood for locals. Moreover, since many pleurotoid species are suitable for human consumption, studying their distribution and cultivation is useful to ensure source stability for commercial use.

Some pleurotoid species have been shown to offer considerable health benefits due to their high protein and low-fat content (Singh, 2017; Hyde et al., 2019). The bioactive substances derived from mushrooms are well known for their therapeutic benefits. It is now possible to isolate and identify important substances such as dihydropleurotinic acid, 4-hydroxypleurogrisein, pleurotin, pleurothiazole, thiopleurotinic acid A, thiopleurotinic acid B, and other bioactive substances due to advances in chemical technology.

9.7 Future Work

Pleurotoid mushrooms, which are saprotrophic in nature, display notable diversity in both Laos and Thailand. Between 2019 and early 2023, a total of 250 specimens were collected. From these, 70 species were identified, with 55 species classified based on morphological characteristics. However, some of these morphologically identified species were not deposited in the herbarium due to uncertainties in their identification, and only a portion of them have undergone DNA sequencing.

Due to time constraints and the intensive nature of detailed analysis, this study focused on 29 specimens representing 15 selected species. These species were chosen based on their abundance, distinct morphological traits, and clearer taxonomic placement, allowing for a more reliable and in-depth examination within the available resources.

Future research will include additional molecular sequencing to improve the accuracy of species identification and ensure proper deposition of all specimens in the herbarium. Continued investigation is likely to reveal new species and new distribution records, contributing to a more comprehensive understanding of pleurotoid mushroom diversity in the region.

The diversity of pleurotoid mushrooms in Thailand (discussed in Chapter 4) has been fully documented and the corresponding article is currently being reviewed for publication in a selected journal. Many forests in both Laos and Thailand remain underexplored, suggesting the potential to discover numerous undiscovered species. Further taxonomic studies in Laos, in particular, could reveal a high diversity of Basidiomycota. One limitation of this study is the inconsistency in sampling frequency across the 19 community forests, which may have affected the reliability and comparability of the diversity results. This inconsistency was largely due to access-related challenges, such as remote locations, difficult terrain, and transportation constraints, as well as seasonal factors like heavy rainfall or dry periods that limited mushroom fruiting and collection opportunities. To improve the accuracy of future studies, it is recommended to implement a standardized sampling schedule that ensures equal coverage across all study sites and key seasons. Additionally, using long-term monitoring plots or rotating sampling efforts across multiple seasons could help address temporal variation and ensure a more comprehensive representation of fungal diversity.

Some pleurotoid species have often been misidentified, in part because holotypes are missing, or because types of species put in synonymy were not adequately studied. In the past, some species were introduced based only on short morphological descriptions without molecular study, therefore those species need to be checked and investigated again. The same species confused in the past, some taxa were placed in the wrong species and based only on morphology. Currently, study in fungi using modern technique taxonomy based on both morphological and molecular studies helps us to understand them more in detail. For example, two accessions identified as *H. grisea* (MFLUCC 12-0451) reported from Thailand, HFJAU0029 from China is *H. tristis* with heteromorphisms and genetic distance evidence. Future studies will incorporate multiple genetic markers, such as RPB1, RPB2, and TEF1-α, to improve phylogenetic resolution. Furthermore, advanced genomic techniques like whole-genome sequencing

or RADseq can offer deeper insights into population structure and evolutionary relationships. Integrating these molecular approaches with ecological, morphological, and physiological data would support a more comprehensive and accurate taxonomic assessment of *H. tristis* and related taxa.

One significant challenge in validating molecular data in fungal taxonomy is the frequent lack of detailed morphological information in public databases like GenBank. While GenBank provides a vast repository of genetic sequences, many submissions are incomplete, missing essential accompanying data such as macro- and micromorphological descriptions, habitat details, or geographic origin. This limitation makes it difficult for researchers to accurately interpret or compare genetic data, especially when confirming species identities or assessing genetic variation within or between species. Without corresponding morphological and ecological context, the risk of misidentification increases, potentially leading to taxonomic confusion or incorrect conclusions about species boundaries. To enhance the reliability and usefulness of molecular data, future studies will aim to submit comprehensive datasets that integrate genetic sequences with detailed morphological characteristics and ecological information. Such integrative submissions would greatly improve the quality and reproducibility of fungal taxonomy and biodiversity research, facilitating more accurate species identification and a better understanding of fungal diversity.

The discovery and cultivation of additional wild edible pleurotoid species hold significant potential. Newly identified cultivable wild species will be evaluated to determine optimal growth conditions, enabling the development of large-scale cultivation techniques and their eventual introduction as commercial species. Comprehensive laboratory testing is imperative to fully assess its toxicity, as many commonly consumed edible mushrooms possess varying levels of inherent toxicity. Current cultivation trials have varied results depending on the substrates used. To find the best cultivation methods and improve yields, I will conduct systematic tests with more substrate types, different mixes, and nutrient additions.

Further testing of extracts from the mycelium of *P. djamor* var. *fuscopruinosus* and *P. giganteus* is essential for evaluating additional bioactivities and identifying the specific bioactive compounds responsible. A comparative analysis of extracts derived from both the mycelium and fruiting bodies is also necessary to provide a

comprehensive understanding of their biological properties. However, before any therapeutic or medicinal use can be recommended, it is critical to conduct detailed safety and toxicity assessments to ensure these mushrooms are safe for human consumption. Thorough future testing is needed to evaluate potential toxic effects, especially when extracts are used at high concentrations or over long periods. Furthermore, investigating the bioactivities of additional species within the pleurotoid group may reveal promising applications in the pharmaceutical, nutraceutical, and medical fields, provided their safety and efficacy are well-established through rigorous scientific validation.

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APPENDIX A

CHEMICAL REAGENT

Chemical Reagents used for this Study

1) Potassium Hydroxide (KOH): 3-5% aqueous solution (Lagent et al., 1977)

- 3 (-5) g of potassium hydroxide
- 97 (-95) ml of water
- Place the dried specimen to be studied in a drop of potassium hydroxide on a glass slide; add Congo Red if the hyphae are hyaline
- Use for: 3-5% KOH is the reagent used to rehydrating the hyphae of dried specimens.

2) Congo Red: 1% aqueous solution (Lagent et al., 1977).

- 1 g of congo Red
- 99 ml of water
- Use for: Congo Red will stain the wall of hyphae to red, and this is used measurement micro-morphology, to observe walls hyphae and spores for drawing. Using congo Red normally combine with water for fresh material or potassium hydroxide for dried material.

3) Distilled water (H_2O) (Lagent et al., 1977).

Use: the reagent used to revive the hyphae of dried specimens of some species have thin hyphae, and to see real colour.

APPENDIX B

ABSTRACT OF PUBLICATIONS

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Additions to *Crepidotus* species (Basidiomycota): Six new species and two new records from Thailand

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Abstract

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 recognized as densely velutinous with white hairs longer near the point of attachment, not striate margin, and not hygrophanous. *Crepidotus-flavocarpus sp. nov.* is recognized by yellow to yellowish pileus basidiomata, margin discolorous paler yellow, densely velutinous with white hairs near the point of attachment, smooth globose basidiospores, and inverse hymenophoral trama. *Crepidotus thailandicus sp. nov.* is recognized by a tomentose surface with white hair when primordial to early stages and then disappearing with age when mature present long translucent-striate reward to the point of attachment becoming fade away at central. *Crepidotus roseocarpus sp. nov.* is recognized by large basidiomata, pileus, lamellae, and context are pastel pink, concolorous darker pink at the margin, hymenophoral trama convergent, with cylindrical terminal elements. *Crepidotus-ungulatus sp. nov.* is recognized by ungulate basidiomata, brownish orange near the point of attachment, the basidiomata color changed to light brown in three hours, and the margin is concolorous to the surface of the pileus. *Crepidotus viscidus sp. nov.* is recognized by white semicircular pileus, translucent-striate with gray to dark grey margin, lamellae pale orange to orange-white, glutinous context, smooth basidiospore and hymenophoral trama present regular form. *Crepidotus lateralipes* and *C. striatus* are reported as new records to Thailand.

Key words: Agaricales, molecular phylogeny, new species, pleurotoid mushrooms, taxonomy

Introduction

Crepidotaceae (S. Imai) Singer (Agaricales, Agaricomycetes) is a gill mushroom that consists of six genera, namely *Crepidotus* (Fr.) Staude, *Episphaeria* Donk, *Nanstelocephala* Oberw. & R.H. Petersen, *Pellidiscus* Donk, *Pleuroflammula* Singer, and *Simocybe* P. Karst (He *et al.* 2019). *Crepidotus* is a member of the Crepidotaceae, originally described as *Agaricus* trib. *Crepidotus* Fr., later Staude (1857), described and transferred it to *Crepidotus*. Currently, 319 taxa are listed under *Crepidotus* in Index Fungorum (2024), but 200 species are accepted, and *C. mollis* (Schaeff.) Staude is the type species (He *et al.* 2019). The main characteristics of this genus are having pleurotoid basidiomata, smooth or ornamented with brown to yellowish basidiospores, which are mostly absent stipe or pseudo-stipe, and currently some species present stipe (e.g., *C. asiaticus* Guzm.-Dáv., C.K. Pradeep & T.J.Baroni, *Crepidotus iqbalii* A. Izhar, Usman & Khalid) (Guzmán-Dávalos *et al.* 2017; Izhar *et al.* 2021). *Crepidotus* species are saprotrophic and can be found in temperate and tropical regions (Laessoe & Petersen 2019), mostly growing mainly on decaying wood; some species are found on soil, often on buried substrate, and only a species is biotrophic in basidiomata, e.g., *Craterellus lutescens* (Fr.) Fr. (Singer 1986). Bioactive compounds from some species of *Crepidotus* have been investigated, such as Strobilurin E, a new antibiotic of the (E)-β-methoxyacrylate was isolated from *C. fulvotomentosus* (Peck) Peck that can inhibit fungal respiration (Weber *et al.* 1990).

Thailand is rich in biodiversity. Many new species of fungi have been reported in Thailand, but many new species have yet to be discovered (Hyde *et al.* 2018; Vadthanarat *et al.* 2021). Nine species have been reported in

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Research Article

Additions to *Hohenbuehelia* (Basidiomycota, Pleurotaceae): two new species and notes on *H. tristis* from northern Thailand

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Abstract

Two new species and a 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 for Thailand for the first time. Based on the polymorphism observed in part of the nrLSU gene, the presence of two divergent lineages within *H. tristis* is discussed.

Key words: Agaricales, DNA sequence heteromorphisms, molecular phylogeny, pleurotoid mushrooms, Southeast Asia, taxonomy, two new species

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Introduction

Hohenbuehelia Schulzer belongs to the family Pleurotaceae Kühner in the order Agaricales Underw. In former studies, the asexual stages of *Hohenbuehelia* species were separately placed in the genus *Nematoctonus* Drechsler (Drechsler 1941; Thorn and Barron 1986). Following the One Fungus = One Name nomenclatural rule, both the asexual and sexual stages were placed under *Hohenbuehelia* (Taylor 2011; McNeill et al. 2012; Thorn 2013), with *H. petaloides* (Bull.) Schulzer as the type species. Currently, 126 taxon names are listed under *Hohenbuehelia* in Index Fungorum (http://www.indexfungorum.org/), for

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Nutritional analysis of cultivated *Pleurotus giganteus* in agricultural waste as possible alternative substrates

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Phonemany M, Thongklang N 2023 – Nutritional analysis of cultivated *Pleurotus giganteus* in agricultural waste as possible alternative substrates. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 13(1), 92–103, Doi 10.5943/cream/13/1/7

Abstract

Pleurotus giganteus is a wild edible mushroom that was successfully domesticated in Thailand. In this research, a *P. giganteus* strain (MFLUCC23–0016) was isolated into PDA agar medium with the best growing spawn detected in millet and sawdust at 11.10 ± 0.34 mm/day, 9.02 ± 0.40 mm/day respectively. Fruiting bodies occurred 14 days after unsealing the substrate bag, which produced large basidiomata and obtained a higher total yield of fresh weight from the sawdust and sawdust + corn cob substrate at 180.77 ± 44.41 g, 176.60 ± 17.65 g, respectively, compared to sawdust + rice straw and sawdust + sugarcane bagasse (35.30 ± 14.15 g, 34.52 ± 11.29 g, respectively) in 60 days with developed fruiting bodies in a 7-day cycle. Three soil casing formulas could be used for the casing of the soil for the *P. giganteus* fruiting trial, in which T1 obtained a higher total fresh weight at 278.54 ± 89.04 g followed by T2 at 218.35 ± 92.53 g and T3 obtained the lowest yield at 137.28 ± 67.42 g. Nutritional analysis of 100 g of dried *P. giganteus* showed 61.32 ± 0.0% of carbohydrates content, 20.318 ± 0.8 (g/100 g) of protein content, 15.6 ± 0.5% of moisture content, 14.28 ± 0.3% of fiber content, 5.61 ± 0.9% of ash content, and 2.98 ± 1.58 (g/100 g) of fat content.

Keywords – cultivation – edible mushroom – fiber content – nutritional analysis – *Pleurotus* – soil casing

Introduction

Pleurotus (Fr.) P. Kumm. belongs to Pleurotaceae Kühner (Agaricales). 769 taxon names for *Pleurotus* are listed in Index Fungorm (http://www.indexfungorum.org/, 2022), with a total of 25 identified species with *P. ostreatus* (Jacq.) P. Kumm. as is the type species (He et al. 2019). The main morphological characteristics of *Pleurotus* are defined by pleurotoid basidiomata, decurrent lamellulae, smooth and elongated to cylindrical basidiospores, a dimitic hyphal system with skeletal hyphae and generative hyphae, and clamp connections (Corner 1981). This genus is distributed in both tropical and temperate areas (Chang & Miles 2004). *Pleurotus* is an edible mushroom that may be known as the oyster, abalone, or tree mushroom, and has been cultivated worldwide (Mahari et al. 2020).

Pleurotus giganteus (Berk.) Karun. & K.D. Hyde is an edible cultivated mushroom that can be found in the soil often on buried wood. It is known as the giant oyster mushroom and was identified for the first time as *Lentinus giganteus* in Sri Lanka by (Berkeley 1847). Later,

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Contributed Paper

Two *Pleurotus* Species (Pleurotaceae) From Lao People's Democratic Republic

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ABSTRACT

Pleurotus is widely distributed and the most commonly cultivated edible mushroom genus worldwide. In addition to being widely consumed, some species have medical, biotechnological and environmental applications owing to their bioactivities. In this study, we report two *Pleurotus* species from Laos. *Pleurotus djamor* is reported for the first time from Laos, while *P. giganteus* a common species in Laos is also reported. Molecular phylogenetic analyses of the nuclear ribosomal internal transcribed spacer region (nrITS) were performed in addition to the morphological analysis to confirm their identification. Full descriptions, color photo plates, micrographs, and taxonomic comparisons are provided.

Keywords: basidiomycota, distribution, diversity, southeast Asia, taxonomy

1. INTRODUCTION

Pleurotaceae Kühner includes four genera, *Agaricochaete* Eichelb, *Antromycopsis* Pat. & Trap, *Hohenbuehelia* Schulzer and *Pleurotus* (Fr.) P. Kumm. The latter genus, consisting of 25 species [1], is diverse in both tropical and temperate areas [2]. *Pleurotus ostreatus* (Jacq.) P. Kumm. is the type species [1]. *Pleurotus* species are saprobic causing white rot in both decayed and hard deadwood [3]. The main morphological characteristics of *Pleurotus* are pleurotoid basidiomata with an eccentric to laterally attached stipe or lacking a stipe and decurrent lamellae, smooth and elongated to cylindrical basidiospores, a dimitic hyphal system, and presence of clamp connections [4, 5]. *Pleurotus giganteus* (Berk.) Karun. & K.D. Hyde and *P. tuber-regium* (Fr.) Singer however, produce omphaloid basidiomata, with a centrally attached stipe and with slightly long pseudorrhiza [6, 7]. A number of *Pleurotus* species are commonly known as oyster mushrooms. The taxonomic history and phylogeny of *Pleurotus* were discussed by Menolli et al. [8] and Shnyreva and Shnyreva [9].

Pleurotus are common edible mushrooms with several important species [2, 3]. *Pleurotus ostreatus*

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Research Article

https://gmsmushrooms.org/: A Comprehensive Online Database of Mushrooms in the Greater Mekong Sub Region

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ABSTRACT

The Greater Mekong Sub-region (GMS) is a global biodiversity hotspot and habitat for many ecologically and economically important mushrooms species. The diversity of mushrooms in the GMS has been studied and published in different journals, and many have been documented from different countries in the GMS region but data remain mostly fragmented. A comprehensive website, https://gmsmushrooms.org/, was established to update the taxonomy, illustrations, descriptions and other useful information for mushroom species reported from the GMS into a single platform. The website is managed by expert mycologists who regularly update information as new macrofungi are introduced from the GMS.

Keywords: basidiomycetes, Laos, macrofungi, Thailand, Vietnam, website, Yunnan province

1. INTRODUCTION

The GMS represents a land area across Mekong River, comprising Cambodia, Yunnan Province and the Guangxi Zhuang Autonomous Region of the People's Republic of China, Lao People's Democratic Republic (Laos), Myanmar, Thailand, and Vietnam [1]. This region has an area

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Pleurotoid (Basidiomycetes) from Laos and Thailand: A Review of their Taxonomy, Distribution, Cultivation, Nutrition and Bioactivities

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Abstract

Pleurotoid mushrooms are characterised by their fan-shape basidiomata with a stipe that can be present or absent. They exhibit 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 current article, a comprehensive exploration of the distribution of pleurotoid mushrooms is carried out in Laos and Thailand, offering 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. This article reviews the importance of pleurotoid mushrooms by integrating knowedge on their distribution, cultivation, nutrition, and biological activity.

Keywords – basidiomycota – edible mushrooms – health benefits – medicinal properties.

Introduction

The majority of pleurotoid mushrooms are decomposers that can be found in decaying wood, stump trees, the bark of living trees, or herbaceous stems (Laessoe & Petersen 2019). A few species are ectomycorrhizal fungi, e.g., members of *Lactifluus* (Pers.) Roussel (Wang et al. 2012, Morozova et al. 2013, De Crop et al. 2018). Pleurotoid mushrooms include various genera such as *Campanella* Henn., *Clitopilus* (Fr. ex Rabenh.) P. Kumm., *Crepidotus* (Fr.) Staude, *Deconica* (W.G. Sm.) P. Karst., *Entoloma* (Fr.) P. Kumm., *Gloiocephala* Massee, *Hohenbuehelia* Schulzer, and *Lentinellus* P. Karst. are decomposers (Laessoe & Petersen 2019). Some pleurotoid mushrooms have been widely consumed, especially members of *Pleurotus* (Fr.) P. Kumm. *Pleurotus ostreatus* (Jacq.) p. Kumm. (oyster mushroom) is the most famous species cultivated worldwide (Khan & Tania 2012, Lesa et al. 2022). Additionally, other commercially cultivated *Pleurotus* species are *Pl. citrinopileatus* Singer (yellow oyster mushroom or golden oyster mushroom), *Pl. cornucopiae*

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A mini review on the potential pharmacological properties, cultivation, and market value of edible *Lentinus* mushrooms (*Polyporaceae*)

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Abstract

Lentinus is a saprobic genus consisting of many edible species. Some species are widely consumed and important for the commercial market. *Lentinus* species are mostly distributed in tropical and subtropical regions, and few species like growing in temperate regions. According to our literature reviews, some *Lentinus* species have high nutrient value, medicinal properties, biotechnological, and environmental applications due to their reported bioactivities. *Lentinus* can also be considered as an important part for the economy as they are edible. In this short review, potential pharmacological properties, cultivation methods and economical value of *Lentinus* species are provided.

Keywords – economic – edible mushrooms – fungus – lentoid – medicinal mushrooms.

Introduction

Lentinus Fr. is a genus of *Polyporaceae*, which was established with *L. crinitus* (L.) Fr. as the type species. (Corner 1981, Grand et al. 2011, He et al. 2019). There are 635 taxon names listed under *Lentinus* in the Index Fungorum (http://www.indexfungorum.org/). For a total of 55 species including asexual morphs (He et al. 2019). This genus is widely distributed, and many phylogenetic studies have been done to resolve the relationships among *Lentinus* species (Corner 1981, Grand 2004, Grand et al. 2011, Seelan 2015, Seelan et al. 2015, Senthilarasu 2015). Corner (1981) defined the genus *Lentinus* different from other related genera by having skeleton-binding hyphae, and the genus mostly relates to *Polyporus*. Later Pegler (1983) published an extensive monograph of *Lentinus* as a polyphyletic and there are two subgenera of *Lentinus* sensu are *Lentinus* subg. *Lentinus* and *Lentinus* subg. *Panus*, which they separate is based on different hyphal systems and hymenophoral trama (Pegler 1983). However, *Lentinus* has widely document taxonomically controversial (Hibbett & Vilgalys 1991, Pegler 1983, Binder et al. (2005, 2013), Seelan et al. 2015).

Lentinus was confused in previous taxonomic classification in groups of lentinoid and pleurotoid, which were *Heliocybe* Redhead & Ginns, *Lentinus* Fr., *Lentinula* Earle, *Lentinellus* P. Karst., *Neolentinus* Redhead & Ginns, *Panellus* P. Karst., *Panus* Fr., *Pleurotus* (Fr.) P. Kumm. (Donk 1964,

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