Current **A**pplied **S**cience and **T**echnology e-ISSN: 2586-9396

Curr. Appl. Sci. Technol. 2025, Vol. 25 (No. 4), e0263544

Research article

Diversity of Macrofungi in the Nature Trail of Namtok Phlio National Park, Chanthaburi Province, Thailand

Saowapha Surawut[1,2], Sorasak Nak-eiam[1,2], Chutapa Kunsook[1,2], Attakorn Khamchatra[3], Namonrug Khamchatra[1,2], Tawat Jenkarn[4], Kanokwan Chanapon[5], Onanong Boonmee[1,2], Chatmongkol Seeprasong[1,2] and Pornpimon Kanjanavas[6]*

[1]Department of Biology, Faculty of Science and Technology, Rambhai Barni Rajabhat University, Chanthaburi 22000, Thailand
[2]Research Unit of Excellence in Biodiversity, Plant Genetic Conservation Project, Rambhai Barni Rajabhat University, Chanthaburi 22000, Thailand
[3]Department of Environmental Science, Faculty of Science and Technology, Rambhai Barni Rajabhat University, Chanthaburi 22000, Thailand
[4]Namtok Phlio National Park, Chanthaburi 22190, Thailand
[5]Microbiology Program, Department of Biology, Faculty of Science and Technology, Rambhai Barni Rajabhat University, Chanthaburi 22000, Thailand
[6]Faculty of Nursing, Kasem Bundit University, Bangkok 10510, Thailand

Received: 30 May 2024, Revised: 19 November 2024, Accepted: 22 November 2024, Published: 12 March 2025

Abstract

The aim of this study was to explore the diversity of macrofungi in the nature trail of Namtok Phlio National Park, Chanthaburi Province, Thailand, the role in the ecology, and their edibility. Fifty-eight macrofungi samples were collected from the nature trail in June 2023. Twenty-eight macrofungi samples with different fruiting bodies (a similar morphology was excluded) were selected and classified according to their morphology. The results indicated that the gilled fungi were the most diverse species (42.9%). Subsequently, they were identified by sequence analysis of the internal transcribed spacer (ITS). They were classified into 2 phyla, 3 classes, 9 orders, 18 families, and 23 genera, and most commonly in the phylum Basidiomycota (89.3%), the family Polyporaceae (21.4%), and played a major role as saprotrophs (85.7%). Additionally, most of them did not have any information for the edibility data (82.1%), however, edible (10.7%) and poisonous (7.1%) macrofungi were reported. Moreover, some macrofungi samples need further investigation for molecular identification based on analysis of additional genes. These results could be used as a database of the macrofungi diversity in the nature trail of Namtok Phlio National Park.

Keywords: macrofungi; identification; ITS; Namtok Phlio National Park

*Corresponding author: E-mail: pornpimon.kan@kbu.ac.th
https://doi.org/10.55003/cast.2025.263544

1. Introduction

Namtok Phlio National Park, located in eastern Thailand, includes the areas of Mueang Chantaburi, Laem Sing, Khlung, and Makham District of Chantaburi province (33,235.67 acres). The middle of this area contains rainforest with high mountains. The waterfall called Namtok Phlio is an outstanding visiting point for travelers. The biodiversity of animals that has been explored included mammals (12 species), birds (149 species), reptiles (59 species), amphibians (19 species), and fish (9 species). Fifty families with more than 165 species of plants have been reported including *Gironniera nervosa, Heritiera javanica,* and *Scaphium scaphigerum* (Department of National Park, 2024).

Macrofungi are classified in the Kingdom of Fungi with several patterns of fruiting bodies that can be seen with the naked eyes. They play a major role in the ecological systems as decomposers. Some of them have been reported to be edible or poisonous mushrooms. Some edible macrofungi were studied to isolate the pure culture, which was spawned to grow in substrate to develop fruiting bodies (Kumla et al., 2020; Thongklang et al., 2020). The cultivation of edible macrofungi can result in valuable nutritious food and income, especially when cultivation is done at the commercial level.

Several studies on the biodiversity of macrofungi in eastern Thailand have been reported (Surawut et al., 2021; Surawut et al., 2023). The para rubber plantations in Trat province were found to be habitats of a number of ascomycetes macrofungi, a fungus that produces ascospore, which included *Cookeina garethjonesii*, *C. tricholoma*, *C. sulcipes*, *Daldinia eschscholtzii*, *Trichoderma* sp., and *Xylaria* sp. (Surawut et al., 2021). In addition, the diversity of macrofungi in the Plant Genetic Conservation Area, Chanthaburi province was reported. Fourty-one taxa of macrofungi were classified into 2 phyla, 5 classes, 11 orders, 21 families, and 34 genera with *Microporus xanthopus* being the most frequently found species. Moreover, edible macrofungi including *Amauroderma rugosum*, *Auricularia cornea*, *C. sulcipes*, *C. tricoloma*, *Dacryopinex spathularia*, *Termitomyces* sp., *Tremella fuciformis*, and *Schizophylum commune* were previously reported (Surawut et al., 2023). However, the evidence of biodiversity of macrofungi in Namtok Phlio National Park has not been discovered yet.

Generally, macrofungi are identified by morphological and nucleotide sequence analysis. Some closely related macrofungi are difficult to differentiate by only morphological properties. Consequently, the molecular biology approach consisting of DNA extraction, polymerase chain reaction (PCR), gel electrophoresis, nucleotide BLAST (Basic Local Alignment Search Tool), and phylogenetic tree were used to identify the macrofungi at the species level (Raja et al., 2017). The conserved regions such as internal transcribed spacer (ITS), beta-tubulin (*tub*), actin (*act*), and cyclooxygenase gene (*cox*) were used to identify the fungus (Raja et al., 2017). In this study, ITS was used as a target to amplify and perform sequence analysis.

Therefore, the main purpose of this study was to report on the diversity of macrofungi in the nature trails of Namtok Phlio National Park, Chanthaburi Province, Thailand, their role in the ecology, and their edibility.

2. Materials and Methods

2.1 Survey of macrofungi and sample collection

The macrofungi samples were collected from the nature trail (1.2 km) of Namtok Phlio National Park (26.317 acres), Chanthaburi Province, Thailand (Latitude 12°31’52” N to

12°31'43" N and longitude 102°11'00" E to 102°10'48" E) in June 2023 (Figure 1). Fifty-eight macrofungi samples were collected and photographs were taken to observe their morphology and habitat. The fruiting bodies were kept in a plastic box, and their tissue was stored in absolute ethanol at -20ºC for DNA extraction. The remaining fruiting body was dried in an oven at 50ºC.

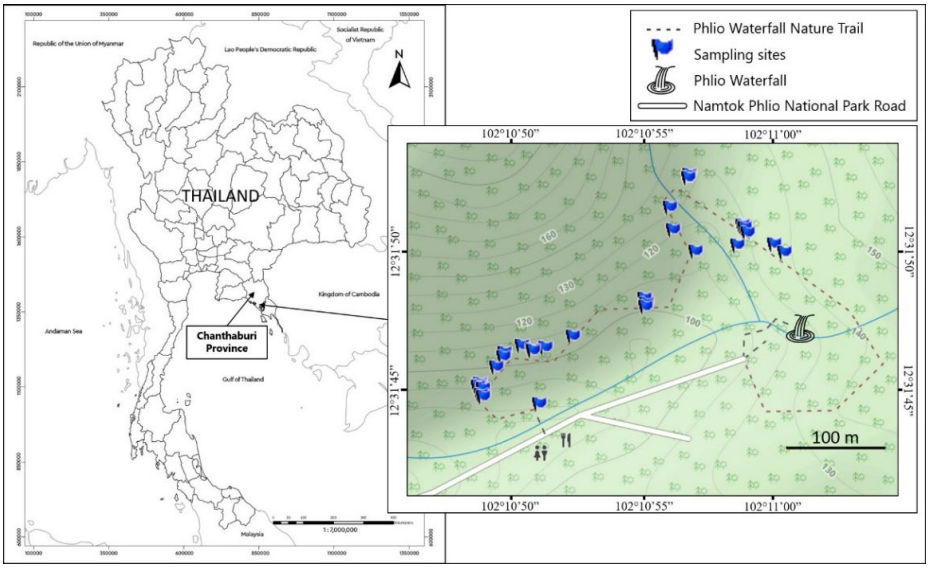

Figure 1. The nature trail of Namtok Phlio National Park, Chanthaburi Province, Thailand, where macrofungi samples were collected.

2.2 Morphology investigation

The fruiting body characteristics were observed as previously described (Desjardin et al., 2004; Chandrasrikul et al., 2008; Chandrasrikul et al., 2011) and by using the Index Fungorum system (www.indexfungorum.org). The macrofungi with different fruiting bodies were selected for identification by molecular identification (a similarity morphology was excluded).

2.3 DNA extraction and ITS amplification by PCR

The DNA of macrofungi was extracted using a commercial kit (Favorgen, Taiwan). The primer, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4(5'-TCCTCCGCTTATTGATA TGC-3') (Raja et al., 2017) were used to amplify the ITS region using PCR method. PCR amplification was accomplished in 20 µL volume. The reaction mixture consisted of 10 pg of genomic DNA in 1×PCR master mix (Apsalagen, Thailand), and 0.5 µM of each primer. Sterile distilled water (Apsalagen, Thailand) was added to adjust the volume to 20 µL. ITS amplification was carried out in a thermal cycler apparatus. Cycling conditions were as follows: 95º C for 3 min, followed by 35 cycles at 95º C for 30 s, 52º C for 30 s, 72º C for 1 min, and finalized at 72ºC for 10 min. Electrophoresis was used for PCR product analysis using 2% agarose gel with RedSafe (iNtRONbiotechnology, Korea) at 100 V for 30 min (Surawut et al., 2023).

2.4 DNA sequencing and ITS region analysis

The purification of PCR products and DNA sequencing were performed at the ATGC Company (Pathum Thani, Thailand). The obtained sequences of macrofungi were analyzed with the BioEdit program (version 7.2.5) and the percent similarity of macrofungi samples was analyzed by BLASTn in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The ITS sequences of 28 samples were analyzed using the Neighbor-Joining method to generate an evolutionary tree (Saitou & Nei, 1987). The percentage of replicate trees in which the associated taxa were clustered together in the bootstrap test (10,000 replicates) was shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004). The ITS phylogeny involved 66 nucleotide sequences. Evolutionary analyses were conducted in MEGA (version X) (Kumar et al., 2018).

2.5 The taxonomy data, ecology role, and edibility of macrofungi

The role of the identified macrofungi in ecology and their edibility were searched in previously published data and FUNGuild (https://github.com/UMNFuN/FUNGuild) (Nguyen et al., 2016). Trophic modes were used to define their roles in the ecology: (i) pathotrophs (PA) obtain nutrients by harming their host cells, (ii) saprotrophs (SA) obtain nutrients by breaking down dead host cells, and (iii) symbiotrophs (SM) obtain nutrients by exchanging resources with their host cells (Nguyen et al., 2016). The taxonomy data of identified macrofungi was searched in Index Fungorum (https://www.indexfungorum. org).

3. Results and Discussion

Fifty-eight macrofungi samples were collected from the nature trail of Namtok Phlio National Park, Chanthaburi Province, Thailand in June 2023. Twenty-eight macrofungi samples with different fruiting body structures were selected (a similar morphology was excluded). They were classified by morphology and thus divided into 8 groups: (i) cup or disclike fungi (collection numbers P9, P40, and P49), (ii) gilled fungi (P2, P4, P5, P7, P8, P10, P11, P15, P18, P30, P31, and P48), (iii) polypore and bracket fungi (P1, P13, P16, P26, P33, P34, and P58), (iv) puffball and earthstar fungi (P6 and P29), (v) bird's nest fungi (P37), (vi) crust and parchment fungi (P17), (vii) jelly fungi (P3), and (viii) leather-bracket fungi (P20). The results suggested that the gilled fungi (42.9%) were found mostly in the nature trail of Namtok Phlio National Park, followed by polypore and bracket fungi, cup or disclike fungi, and puff ball and earthstar at 25.0%, 10.7%, and 7.1%, respectively. Each group of the bird's nest fungi, crust and parchment fungi, jelly fungi, and leather-bracket fungi was found at 3.6%. These 28 macrofungi samples were selected to be identified by the molecular identification method. The ITS region was amplified by the PCR method with ITS1 and ITS4 primers. The size of PCR products observed was between 600-750 bp (Figure 2).

The ITS sequences of the macrofungi were compared with the sequences in the GenBank database by BLASTn for identification. The base-pair matches with the closely related reference sequences described in Table 1.

The macrofungi samples from the nature trail of Namtok Phlio National Park, Chanthaburi Province, Thailand were classified into 2 phyla, 3 classes, 9 orders, 18 families, and 23 genera. Three samples were classified in the phylum Ascomycota (10.7%)

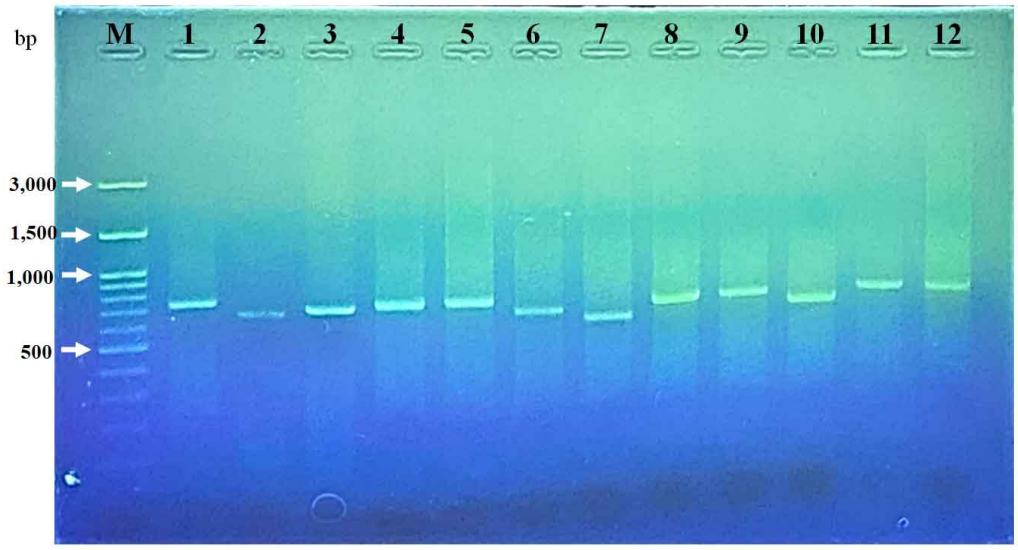

Figure 2. Agarose gel electrophoresis of polymerase chain reaction (PCR) products from amplification of internal transcribed spacer (ITS) region by PCR method. M: 100 bp DNA ladder; 1: Collection No. P7; 2: Collection No. P26; 3: Collection No. P4; 4: Collection No. P13; 5: Collection No. P15; 6: Collection No. P16; 7: Collection No. P17; 8: Collection No. P18; 9: Collection No. P29; 10: Collection No. P20; 11: Collection No. P37; and 12: Collection No. P58

while the remaining macrofungi samples were found in the phylum Basidiomycota (89.3%) (Table 1 and Figure 3). Figure 4 showed representatives of macrofungi found in the nature trail of Namtok Phlio National Park. The family with the highest number of samples (and thus of the highest diversity) was Polyporaceae (6 samples, 21.4%), while 4 samples (14.3%) were found in the Marasmiaceae and 2 samples per family (7.1%) were found in the Omphalotaceae, and Sarcoscyphaceae. One sample per family (3.57 %) was found in the Hypocreaceae, Agaricaceae, Entolomataceae, Mycenaceae, Nidulariaceae, Pleurotaceae, Porotheleaceae, Psathyrellaceae, Aporpiaceae, Auriculariaceae, Sclerodermataceae, Geastraceae, Meripilaceae, and Stereaceae (Table 1). In this study, five macrofungi samples (21.4%) could not be identified at the species level by using only ITS. These were *Agaricus* sp. (P5), *Entoloma* sp. (P15), *Mycena* sp. (P2), *Marasmiellus* sp. (P11), *Clitocybula* sp. (P8), and one sample of Polyporaceae family (P34) could not be identified at the genus level (Table 1).

Normally, morphological characteristics and the sequence analysis are used to identify macrofungi. The ITS is a conserved region that is commonly used for fungal identification. However, some fungal species cannot be identified by only the ITS region. Therefore, several genes such as nuclear large subunit rDNA (LSU), nuclear small subunit rDNA (SSU), translation elongation factor 1-alpha (*tef1-*α), RNA polymerase II second largest subunit (*rpb2*), *tub*, *act*, and *cox* gene were used to identify fungi at the species level (Raja et al., 2017).

Table 1. Taxonomy data, BLASTn results, mode of life and edibility of macrofungi collected from the nature trail of Namtok Phlio National Park

Phylum	Class	Order	Family	Scientific name (Collection number)	Best Match (Accession No.)		GenBank Accession No. in this study	Mode of life	Edibility
					ITS	Similarity (%)			
Ascomycota	Pezizomycetes	Pezizales	Sarcoscyphaceae	*Cookeina speciosa* (P9)	*Cookeina speciosa* (PP375111)	100.00%	PP789878	SA	E
Ascomycota	Pezizomycetes	Pezizales	Sarcoscyphaceae	*Cookeina tricholoma* (P40)	*Cookeina tricholoma* (KY094619)	100.00%	PP763494	SA	E
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma pezizoides* (P49)	*Trichoderma pezizoides* (MW659098)	100.00%	PP777368	SA	
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Agaricus* sp. (P5)	*Agaricus* sp. (U975111)	97.12%	PP789872	SA	
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Entoloma* sp. (P15)	*Entoloma* sp. (PP357326)	90.60%	PP789885	SA	P
Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Crinipellis nigrolamellata* (P10)	*Crinipellis nigrolamellata* (MT946364)	87.40%	PP789879	SA	
Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Marasmius guyanensis* (P48)	*Marasmius guyanensis* (PP622170)	99.68%	PP763759	SA	
Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Marasmius nummularius* (P30)	*Marasmius nummularius* (EU935493)	97.62%	PP789738	SA	
Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Marasmius tenuissimus* (P7)	*Marasmius tenuissimus* (EU935568)	99.36%	PP789877	SA	
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena* sp. (P2)	*Mycena* sp. (KP012834)	98.06%	PP789866	SA	
Basidiomycota	Agaricomycetes	Agaricales	Nidulariaceae	*Cyathus subglobisporus* (P37)	*Cyathus subglobisporus* (OM831394)	100.00%	PP763704	SA	
Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	*Gymnopus hirtelloides* (P31)	*Gymnopus hirtelloides* (MF100975)	98.65%	PP790223	SA	

Table 1. Taxonomy data, BLASTn results, mode of life and edibility of macrofungi collected from the nature trail of Namtok Phlio National Park (continued)

Phylum	Class	Order	Family	Scientific name (Collection number)	Best Match (Accession No.)		GenBank Accession No. in this study	Mode of life	Edibility
					ITS	Similarity (%)			
Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	*Marasmiellus* sp. (P11)	*Marasmiellus* sp. (MN483261)	100.00%	PP763708	SA	
Basidiomycota	Agaricomycetes	Agaricales	Pleurotaceae	*Hohenbuehelia leiospora* (P4)	*Hohenbuehelia leiospora* (EF409738)	92.53%	PP789869	SA	
Basidiomycota	Agaricomycetes	Agaricales	Porotheleaceae	*Clitocybula* sp. (P8)	*Clitocybula* sp. (OQ147048)	99.61%	PP789880	SA	
Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	*Candolleomyces candolleanus* (P18)	*Candolleomyces candolleanus* (OP022009)	99.13%	PP789886	SA	
Basidiomycota	Agaricomycetes	Auriculariales	Aporpiaceae	*Protohydnum sclerodontium* (P17)	*Protohydnum sclerodontium* (KC422643)	99.38%	PP789740	SA	
Basidiomycota	Agaricomycetes	Auriculariales	Auriculariaceae	*Auricularia thailandica* (P3)	*Auricularia thailandica* (LC373484)	99.58%	PP789868	SA	E
Basidiomycota	Agaricomycetes	Boletales	Sclerodermataceae	*Scleroderma xanthochroum* (P29)	*Scleroderma xanthochroum* (EU718126)	99.65%	PP789893	SM	P
Basidiomycota	Agaricomycetes	Geastrales	Geastraceae	*Geastrum saccatum* (P6)	*Geastrum saccatum* (KT273360)	98.62%	PP790219	SA	
Basidiomycota	Agaricomycetes	Hymenochaetales	Meripilaceae	*Rigidoporus ginkgonis* (P33)	*Rigidoporus ginkgonis* (MK269256)	99.74%	PP790210	PA	
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Polyporaceae sp. (P34)	*Polyporaceae* sp. (MZ354992)	100.00%	PP789890	SA	
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Coriolopsis retropicta* (P16)	*Coriolopsis retropicta* (OL771752)	99.55%	PP789882	SA	

Table 1. Taxonomy data, BLASTn results, mode of life and edibility of macrofungi collected from the nature trail of Namtok Phlio National Park (continued)

Phylum	Class	Order	Family	Scientific name (Collection number)	Best Match (Accession No.)		GenBank Accession No. in this study	Mode of life	Edibility
					ITS	Similarity (%)			
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Favolus roseus* (P13)	*Favolus roseus* (KR049231)	99.43%	PP767377	SA	
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Ganoderma nasalanense* (P1)	*Ganoderma nasalanense* (MK345441)	100.00%	PP789865	PA	
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Ganoderma williamsianum* (P26)	*Ganoderma williamsianum* (MG279169)	99.82%	PP789889	PA	
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Microporus xanthopus* (P58)	*Microporus xanthopus* (KX580186)	100.00%	PP767378	SA	
Basidiomycota	Agaricomycetes	Russulales	Stereaceae	*Stereum ostrea* (P20)	*Stereum ostrea* (MH121193)	100.00%	PP789887	SA	

Abbreviations: Mode of life: PA =pathotroph, SA = saprotroph, SM=symbiotroph
Edibility: E= edible macrofungi, P= poisonous macrofungi

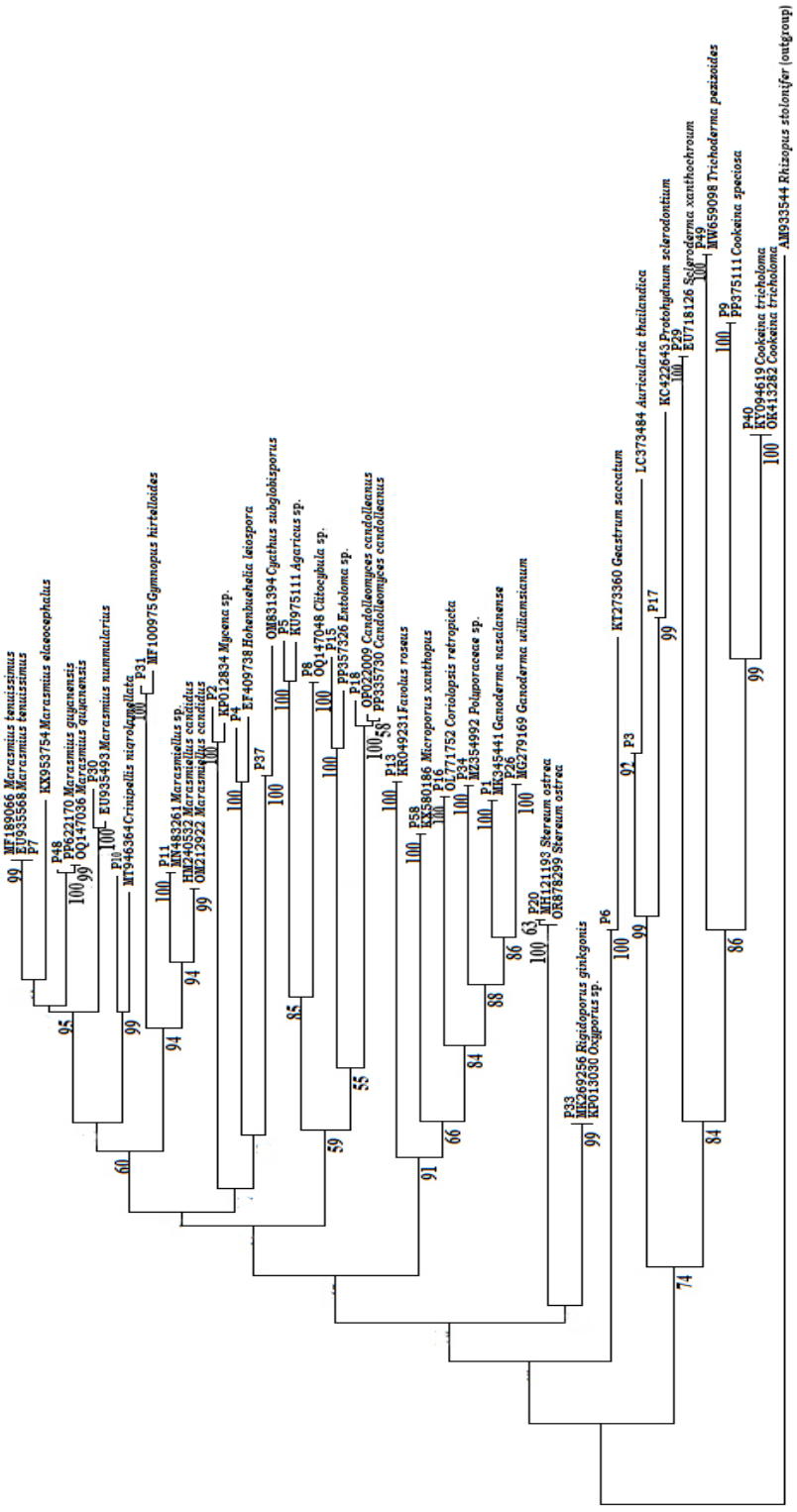

Figure 3. Phylogenetic tree based on internal transcribed spacer (ITS) sequences of macrofungi in the nature trail of Namtok Phlio National Park (The bootstrap values below 50% are not shown in the node.)

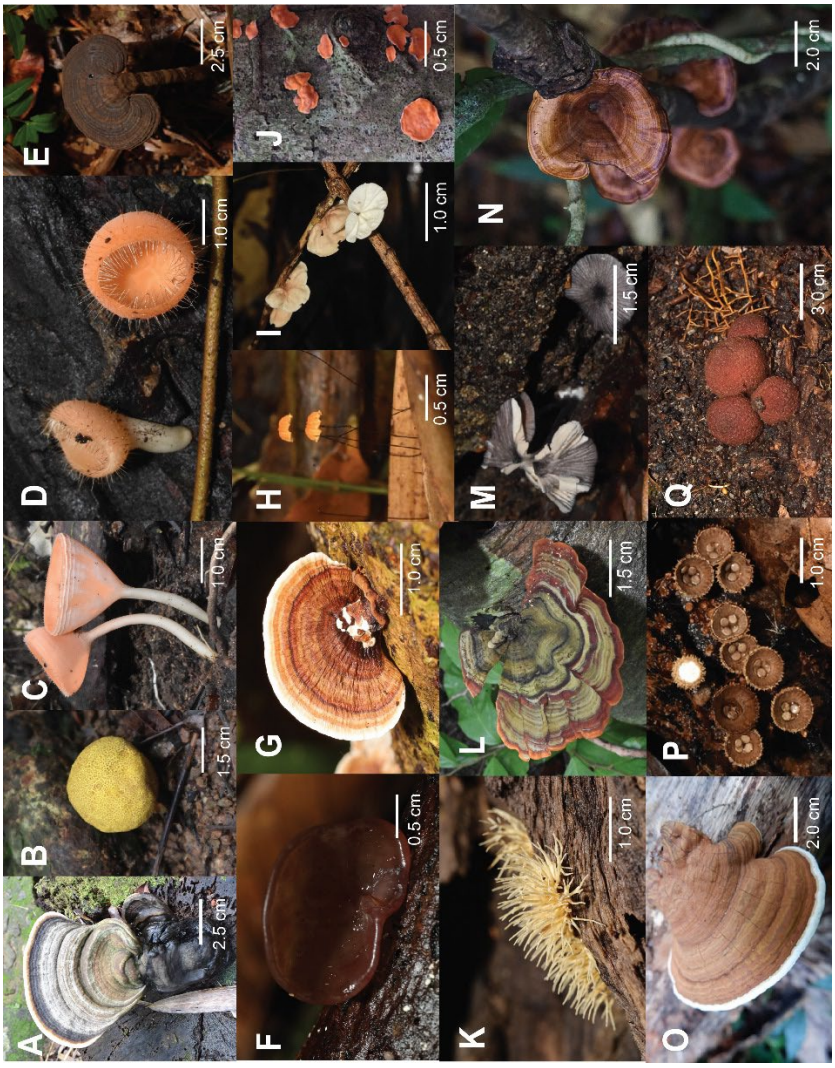

Figure 4. Representatives of macrofungi found in the nature trail of Namtok Phlio National Park. A: *Ganoderma nasalanense*; B: *Scleroderma xanthochroum*; C: *Cookeina speciosa*; D: *C. tricoloma*; E: Polyporaceae sp.; F: *Auricularia thailandica*; G: *Coriolopsis retropicta*; H: *Marasmius guyanensis*; I: *Marasmius tenuissimus*; J: *Trichoderma pezizoides*; K: *Protohydnum sclerodontium*; L: *Stereum ostrea*; M: *Entoloma* sp.; N: *Microporus xanthopus*; O: *G. williamsianum*; P: *Cyathus subglobisporus*; Q: *Geastrum saccatum*

Wang & Bau (2024) reported new species of *Agaricus* from Northeast China. They used 3 loci for molecular identification; ITS, LSU, and *tef1* gene. Elliott et al. (2020) showed that the identification of genus *Entoloma* used several gene analyses including the ITS, LSU, *rpb2*, and mitochondrial small subunit (mtSSU), and they reported a new species from northern Thailand as *Entoloma sequestratum*. Liu et al. (2022) reported four new species of *Mycena* by sequence analysis of ITS, RNA polymerase II largest subunit (*rpb1*), and *tef1*. The identification of a member of Marasmiaceae including *Marasmiellus* sp. was reported and 3 loci were used for analysis: the ITS, LSU, and mtSSU (Amoako-Attah et al., 2020). Additionally, identification of the genus *Clitocybula* (Antonín et al., 2019) was performed using sequence analysis of the ITS and LSU. In the present study, one sample of macrofungi in Namtok Phlio National Park (Collection number P34, Figure 4E) was classified in the Polyporaceae family but could not be classified down to the genera. The member of Polyporaceae reported that sequence analysis of the ITS, LSU, tef1, and rpb1 genes was necessary for the classification of the polypore fungi (Polyporaceae) at the species level, therefore, unidentified polypore fungi (P34) in this study should be further analyzed for these genes (Mao et al., 2023). These studies suggested that some macrofungi samples could not be identified at the species level in this study, and the sequence analysis of additional genes may be required.

Subsequently, the ecological role of macrofungi in Namtok Phlio National Park indicated that most macrofungal species played an important role as saprotrophs (24 samples, 85.71%); however, symbiotrophs such as *Scleroderma xanthochroum* (1 sample, 3.6%) and pathotrophs such as *Ganoderma nasalanense, G. williamsianum* and *Rigidoporus ginkgonis* (3 samples, 10.7%) were also found (Table 1). Additionally, the species of macrofungi in this study that had been previously reported for edible included *Au. thailandica* (Bandara et al., 2017), *C. speciosa* (Putra et al., 2022), and *C. tricoloma* (Putra et al., 2022), (3 samples, 10.7%). Among these edible macrofungi, *C. tricoloma* has previously been evidenced to possess medicinal properties. It acts as an antinociceptive and immunomodulator (Kusuma et al., 2020). In contrast, the poisonous macrofungi consisting of *Entoloma* sp. (Aoki et al., 2020; Elliott et al., 2020) and *S. xanthochroum* (Sato et al., 2019), (2 samples, 7.1%) were explored. However, data relating to the edibility of the remaining species of macrofungi in this study (23 samples, 82.14 %) was not found (Figure 4).

The results of this study correlated with Surawut et al. (2023), who revealed the biodiversity of macrofungi from the Plant Genetic Conservation Area, Chanthaburi Province, Thailand. They classified macrofungi samples (a total of 41 samples) into 2 phyla, 5 classes, 11 orders, 21 families, and 34 genera. The family Polyporaceae was the most diverse species (24.4%) and the saprotroph (97.6%) had the most ecology role. Additionally, the investigation of edibility revealed a number of edible species (*A. rugosum*, *Au. cornea*, *C. sulcipes*, *C. tricoloma*, *D. spathularia*, *Termitomyces* sp., *Tremella fuciformis,* and *S. commune*) and some poisonous macrofungi (*Entoloma omiense*, *Inocybe parvisquamulosa*, *Lepiota thrombophora* and *S. xanthochroum*). The Plant Genetic Conservation Area and Namtok Phlio National Park are located in Chantaburi province where the same climate resulted in the diversity of macrofungi species being quite similar.

Additionally, the macrofungi of Sungai Kangkawat Research Station, Imbank Canyon Conservation Area, Sabah, Malaysia were explored. This area is a rainforest similar to Namtok Phlio National Park. The diversity of macrofungi indicated that the most common was in the phylum Basidiomycota (91.2%), and family Polyporaceae (21.6%). Most of them played the ecological role of saprotrophs (58.5%) (Kassim et al., 2020). Subsequently, Ahmadni et al. (2023) revealed the macrofungal diversity in Perlis State

Park, Perlis, Malaysia. They explored a total of 69 species of macrofungi. Most of them (59 species) were classified in Phylum Basidiomycota. However, this study identified the macrofungi based on morphology only, so they were classified into 8 groups, designated as cup, gilled, shelf and bracket, bolete, coral, jelly, earthball and puffball, and tooth fungi. The gilled fungi contained the most diverse species (20 samples) which correlated with the result of this study that revealed the gilled fungi as the most diverse species (12 samples, 42.9%). Macrofungi diversity in Columbia and Pakistan has recently been reported. Zambrano-Forero et al. (2023) revealed 164 taxa of macrofungi distributed in 15 orders in Columbia with eighteen records in a doubtful taxa section because they used only ITS region for molecular identification that may not be sufficient for taxonomic classification. Meanwhile, Zeb et al. (2023) identified 51 macrofungi species in Pakistan that were classified into 22 families and 37 genera. Among the identified species, 32 were saprotrophs, 7 were pathotrophs, 6 were both saprotrophs and pathotrophs, and 6 were mycorrhiza or symbiotrophs. Four new species were reported in this study. Therefore, these reports suggested that similar factors such as climate, type of forest, and area location result in similar macrofungi diversity. Differences in these factors have led to variations in macrofungal diversity.

4. Conclusions

The biodiversity of macrofungi within the nature trail of Namtok Phlio National Park, Chanthaburi Province, Thailand in 2023 was reported in this study. Twenty-eight macrofungi samples with different fruiting bodies were selected and classified by their morphology and sequence analysis. The result found that the gilled fungi were the most diverse species (42.9%). Subsequently, the macrofungi were identified by analysis of the ITS region. They were classified into 2 phyla, 3 classes, 9 orders, 18 families, and 23 genera, and most commonly found in the phylum Basidiomycota (89.3%), the family Polyporaceae (21.4%), and most played the role of saprotroph (85.7%). Additionally, there was no information available on the edibility of most samples (82.1%). However, the edible macrofungi *Au. thailandica, C. speciosa*, and *C. tricoloma* (10.7%) were reported. In contrast, the poisonous macrofungi *Entoloma* sp. and *S. xanthochroum* (7.1%) were discovered. Moreover, some macrofungi samples need more investigation for molecular identification by analysis of the additional genes. Therefore, these results were used as part of the creation of a database of the macrofungi diversity in the nature trail of Namtok Phlio National Park. The diverse macrofungi need to be further studied for better understanding of their ecological roles and edibility, their potential for commercial cultivation, and their potential use in medicines and foods and their bioactive compound extracts.

5. Acknowledgements

This work was financially supported by the Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG) in the fiscal year 2023 and Rambhai Barni Rajabhat University and Thailand Science Research and Innovation (Grant number 1117/2566). We would like to thank Panatda Plaenkong and Pornpailin Thanyaponl for the preparation of material for the field trip, macrofungi sampling, and photography. Additionally, we thank Chawanrat Somnuek and Phisut Kanboon for their cooperation with the Head of Namtok Phlio National Park and document preparation.

Finally, we would like to thank Namtok Phlio National Park, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment.

6. Conflicts of Interest

The authors declare no conflicts of interest.

ORCID

Saowapha Surawut https://orcid.org/0000-0001-5672-0240

Sorasak Nak-eiam https://orcid.org/0000-0001-7921-6311

Chutapa Kunsook https://orcid.org/0000-0001-6646-7234

Pornpimon Kanjanavas https://orcid.org/0009-0001-2252-5161

References

Ahmadni, A. S. A., Kamar, N. A. S., Hadi, M. A., & Terhem, R. (2023). Assessment of macrofungi diversity in Perlis State Park, Perlis, Malaysia. *Journal of Wildlife and Biodiversity,* 8(1), 298-307, https://doi.org/10.5281/zenodo.10206965

Amoako-Attah, I., Shahin, A. S., Aime, M. C., Odamtten, G. T., Cornelius, E., Nyaku, S. T., Kumi-Asare, E., Yahaya, H. B., & Bailey, B. A. (2020). Identification and characterization of fungi causing thread blight diseases on cacao in Ghana. *Plant Disease*, 104(11), 3033-3042. https://doi.org/10.1094/PDIS-03-20-0565-RE

Antonín, V., Borovička, J., Holec, J., Piltaver, A., & Kolařík, M. (2019). Taxonomic update of *Clitocybula* sensu lato with a new generic classification. *Fungal Biology,* 123(6), 431-447. https://doi.org/10.1016/j.funbio.2019.03.004

Aoki, W., Watanabe, M., Watanabe, M., Kobayashi, N., Terajima, J., Sugita-Konishi, Y., Kondo, K., & Hara-kudo, Y. (2020). Discrimination between edible and poisonous mushrooms among Japanese *Entoloma sarcopum* and related species based on phylogenetic analysis and insertion/deletion patterns of nucleotide sequences of the cytochrome oxidase 1 gene. *Genes & Genetic Systems,* 95(3), 133-139. https://doi.org/10.1266/ggs.19-00032

Bandara, A.R., Karunarathna, S.C., Mortimer, P.E., Hyde, K.D., Khan, S., Kakumyan, P., & Xu, J. (2017). First successful domestication and determination of nutritional and antioxidant properties of the red ear mushroom *Auricularia thailandica* (Auriculariales, Basidiomycota). *Mycological Progress,* 16(5), 1029-1039. https://doi.org/10.1007/s11557-017-1344-7

Chandrasrikul, A., Suwanarit, P., Sangwanit, U., Morinaga, T., Nishizawa, Y., & Murakami, Y. (2008). *Diversity of mushroom and macrofungi in Thailand.* Kasetsart University Press.

Chandrasrikul, A., Suwanarit, P., Sangwanit, U., Lumyong, S., Payapanon, A., Sanoamuang, N., Pukahuta, C., Petcharat, V., Sardsud, U., Duengkae, K., Klinhom U., Thongkantha, S., & Tongklam, S. (2011). *Checklist of mushrooms (Basidiomycetes) in Thailand.* Office of Natural Resources and Environmental Policy and Planning.

Department of National Park. (2024, Jan 1). *Namtok Phlio National Park*. https://portal.dnp.go.th/Content/nationalpark?contentId=963

Desjardin, D. E., Flegel, T. W., & Boonpratuang, T. (2004). Basidiomycetes. In: E. G. B. Jones, M. Tanticharoen & K. Dd. Hyde (Eds.). *Thai fungal diversity* (pp. 37-49). BIOTEC-Thailand Publishing.

Elliott, T. F., Nelsen, D. J., Karunarathna, S. C., & Stephenson, S. L. (2020). *Entoloma sequestratum*, a new species from northern Thailand, and a worldwide key to sequestrate taxa of *Entoloma* (Entolomataceae). *Fungal Systemtatics and Evolution,* 6, 253-263. https://doi.org/10.3114/fuse.2020.06.12

Kassim, M. H. S. B., Wasti, I. G., Subari, I. A. A. M. H., Ganesan, T. A., Tang, P. L., Chong, C. C., Subramaniam, N., & Seelan, J. S. S. (2020). Macrofungi of Sungai Kangkawat Research Station, Imbak Canyon Conservation Area, Sabah, Malaysia. *Malayan Nature Journal*, 72(3), 371-388.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547-1549. https://doi.org/10.1093/molbev/msy096

Kumla, J., Suwannarach, N., Sujarit, K., Penkhrue, W., Kakumyan, P., Jatuwong, K., Vadthanarat, S., & Lumyong, S. (2020). Cultivation of mushrooms and their lignocellulolytic enzyme production through the utilization of agro-industrial waste. *Molecules*, 25(12), 1-41. https://doi.org/10.3390/molecules25122811

Kusuma, H.I., Harnelly, E., Thomy, Z., Fitra, M. A., & Samingan, S. (2020). Exploration of potential wild medicinal mushrooms from Pocut Meurah Intan forest park, Aceh, Indonesia. *Jurnal Natural,* 20(3), 66-73. http://doi.org/10.24815/jn.v20i3.16916

Liu, Z., Ge, Y., Zeng, H., Cheng, X., & Na, Q. (2022). Four new species of *Mycena* sect. *Calodontes* (Agaricales, Mycenaceae) from northeast China. *MycoKeys,* 93, 23-56. https://doi.org/10.3897/mycokeys.93.86580

Mao, W-L., Wu, Y-D., Liu, H-G., Yuan, Y., & Dai, Y-C. (2023). A contribution to *Porogramme* (Polyporaceae, Agaricomycetes) and related genera. *IMA Fungus,* 14, Article 5. https://doi.org/10.1186/s43008-023-00110-z

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J. Schilling, J. S., & Kennedy, P. G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, 20, 241-248. https://doi.org/10.1016/j.funeco.2015.06.006

Putra, I. P., Nurdebyandaru, N., Amelya, M. P., & Hermawan, R. (2022). Current checklist of local names and utilization information of Indonesian wild mushrooms. *Journal of Tropical Biodiversity and Biotechnology*, 7(3), Article jtbb.71407. https://doi.org/10.22146/jtbb.71407

Raja, H. A., Miller, A. N., Pearce, C. J., & Oberlies, N. H. (2017). Fungal identification using molecular tools: A primer for the natural products research community. *Journal of Natural Products*, 80(3), 756-770. https://doi.org/10.1021/acs.jnatprod.6b01085

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4(4), 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Sato, Y., Tomonari, H., Kaneko, Y., & Yo, K. (2019). Mushroom poisoning with *Scleroderma albidum*: a case report with review of the literature. *Acute Medicine and Surgery*, 7(1), Article e460. https://doi.org/10.1002/ams2.460

Surawut, S., Nak-eiam, S., Kunsook, C., Kamhaengkul, L., Kanjanavas, P., & Yasawong, M. (2021). Diversity and the molecular identification of some ascomycetes macrofungi found in the para rubber plantation, Thailand. *Journal of Biochemical Technology*, 12(4), 50-56. https://doi.org/10.51847/Mqnb3fKVWB

Surawut, S., Kunsook, C., Nak-eiam, S., Khamchatra, N.-M, Bhudharak, S., Phontharod, W., Boonmee, O., Yasawong, M., & Kanjanavas, P. (2023). Biodiversity and functional distribution of macrofungi from Plant Genetic Conservation Area, Chanthaburi

Province, Thailand. *Current Applied Science and Technology*, 23(5), 1-19. https://doi.org/10.55003/cast.2023.05.23.003

Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences of the United States of America*, 101(30), 11030-11035. https://doi.org/10.1073/pnas.0404206101

Thongklang, N., Keokanngeun, L., Taliam, W., & Hyde, K. D. (2020). Cultivation of a wild strain of *Auricularia cornea* from Thailand. *Current Research in Environmental and Applied Mycology*, 10(1), 120-130. https://doi.org/10.5943/cream/10/1/13

Wang, S-E., & Bau, T. (2024). Six new species of *Agaricus* (Agaricaceae, Agaricales) from Northeast China. *Journal of Fungi*, 10(1), Article 59. https://doi.org/10.3390/jof10010059

Zambrano-Forero, C. J., Dávila-Giraldo, L. R., Motato-Vásquez, V., Villanueva, P. X., Rondón-Barragán, I. S., & Murillo-Arango, W. (2023). Diversity and distribution of macrofungi (Ascomycota and Basidiomycota) in Tolima, a Department of the Colombian Andes: an annotated checklist. *Biodiversity Data Journal*, 11, Article e104307. https://doi.org/10.3897/BDJ.11.e104307

Zeb, M., Ullah, A., Ullah, F., Haq, A., Ullah, I., Badshah, L., & Haq, M. A. (2023). Diversity and biological characteristics of macrofungi of district Bajaur, a remote area of Pakistan in the Hindu Kush range. *Heliyon*, 9(7), Article e17818. https://doi.org/10.1016/j.heliyon.2023.e17818