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Research article

Biodiversity and Functional Distribution of Macrofungi from Plant Genetic Conservation Area, Chanthaburi Province, Thailand

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Abstract

Keywords

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plant genetic conservation area;

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The aims of this study were to investigate the biodiversity and functional distribution of macrofungi within the Plant Genetic Conservation Area of Rambhai Barni Rajabhat University, Chanthaburi Province, Thailand, and to identify the macrofungi by sequence analysis of their internal transcribed spacer (ITS) regions. One hundred and eighty-five macrofungi samples were collected from the survey routes in 2021. The macrofungi with different morphologies were selected to perform molecular identification by sequence analysis of ITS. A total of 41 samples of representative macrofungi were classified into 2 phyla, 5 classes, 11 orders, 21 families, and 34 genera. The macrofungi were found to be in the phylum Basidiomycota (35 taxa, 85.4%), the family Polyporaceae (10 taxa, 24.4%), and *Microporus xanthopus* was the most frequently found species in every month of samples collection. Their role in the ecosystem was saprotroph (40 taxa, 97.6%), symbiotroph (7 taxa, 17.0%), and pathotroph (4 taxa, 9.8%). In addition, there was no published information about the edibility of many of the macrofungi (29 taxa, 70.7%); however, some edible (8 taxa, 19.5%) and poisonous macrofungi (4 taxa, 9.8%) had previously been reported. Interestingly, some macrofungi samples need more investigation for further identification, and additional genes may be required for the study.

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1. Introduction

Macrofungi appear in different structures of the fruiting body and can be divided into 2 phyla based on sexual spore production. The phyla Basidiomycota and Ascomycota produce basidiospores on basidia and ascospores in asci, respectively. Macrofungi play an important role in the ecology as saprophytes but some are parasitic or symbiotic macrofungi. Additionally, some are edible and others are poisonous.

The identification of macrofungi should be based on both morphology and molecular study because closely related macrofungi species are difficult to differentiate by morphology alone, and thus easily confused. Therefore, a molecular technique such as sequence analysis was used to confirm their identity [1]. Conserved region, such as the internal transcribed spacer (ITS)region, has been targeted in this study.

Several studies of macrofungal diversity in Thailand were investigated [2-5] including the diversity of macrofungi within an oil palm plantation [6], within community forest [5], and within a para rubber plantation [7]. In Eastern Thailand (Trat province), the study of macrofungi in the phylum Ascomycota within a para rubber plantation revealed the presence of *Daldinia eschscholtzii, Cookeina sulcipes, Cookeina garethjonesii, Cookeina tricholoma, Trichoderma* sp. and *Xylaria* sp. [7]. However, the evidence of macrofungi diversity within a Plant Genetic Conservation Area has not yet been explored.

The Plant Genetic Conservation Area of Rambhai Barni Rajabhat University is a small lowland forest located in the northern part of Chanthaburi Province on the eastern Gulf of Thailand. The forest measures about 7.74 ha, and has an average height above sea level of about 300 m. The forest has temperatures in the range of 28.2-32.2°C with precipitation of 2,000 cm^3 per year. The forest is strongly influenced by temperature and precipitation variations due to the northeast monsoon in the dry season (November to April), and the southwest monsoon in the wet season (May to October). It includes diverse habitat types, including swamp forest, tropical rain forest, and freshwater canal areas that are used for youth recreation. Previous research on the diversity of flora in the conservation area revealed that there were 127 species present and the predominant trees were species such as *Horsfieldia irya, Anisoptera costata,* and *Aporosa nervosaa.* In terms of fauna, there were 73 bird species and 23 species of butterfly identified. The area is not only a natural habitat for life but also an invaluable resource for green learning education [8]. The biodiversity of the conservation areas will be further explored to widen the database of living organisms present and this will include macrofungi.

Therefore, the aims of this study were to report on the biodiversity of macrofungi in the Plant Genetic Conservation Area, Chanthaburi Province, Thailand, by identification based on sequence analysis of the internal transcribed spacer (ITS) regions, and by study of the functional distributions of macrofungi encountered.

2. Materials and Methods

2.1 Study area and macrofungi sample collection

The macrofungi were collected from the Plant Genetic Conservation Area (26.317 acres), Chanthaburi Province, Thailand (Latitude 12°39'50.58" N to 12°39'37.83" N and longitude 102° 6'22.03" E to 102° 6'33.07" E) (Figure 1). One hundred and eighty-five macrofungi samples were collected from the survey route from March 2021 to December 2021. The samples were placed in plastic boxes, and small pieces of the macrofungi were kept in absolute ethanol at -20ºC and stored

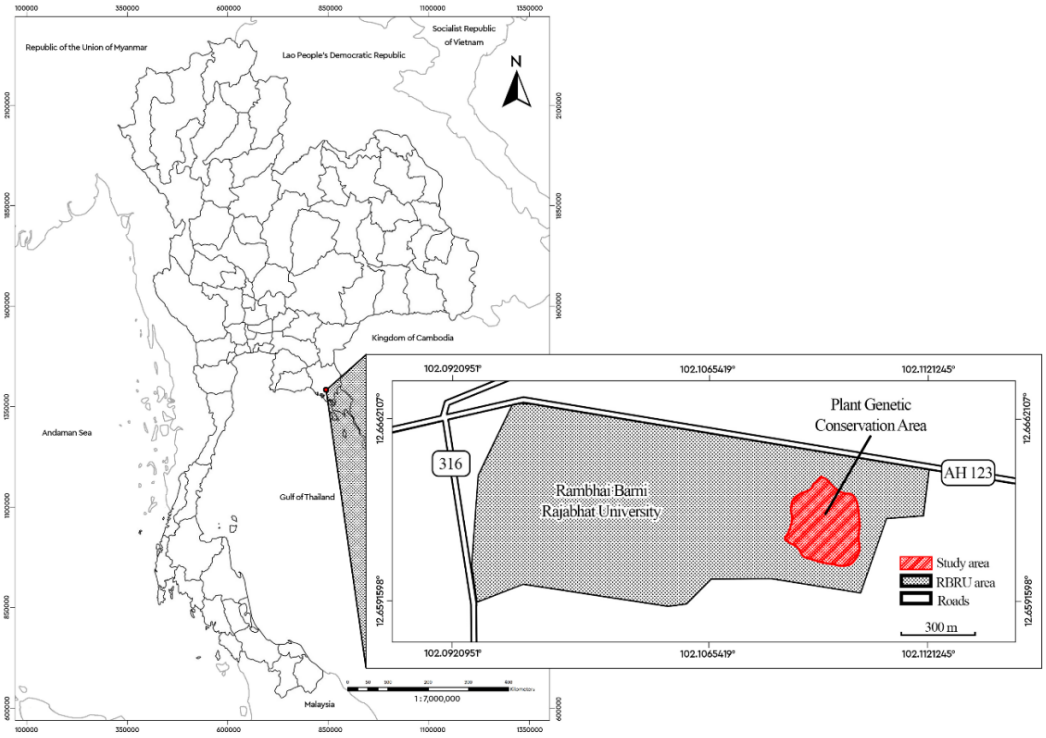

Figure 1. Map of Rambhai Barni Rajabhat University, Chanthaburi Province, Thailand in which the study plots of the Plant Genetic Conservation Area were located.

in the lysis buffer of the DNA extraction kit (Flavogen, Taiwan) for DNA extraction. The remaining fruiting bodies of the macrofungi were dried in an oven at 50ºC.

2.2 Morphological study

All macrofungi samples were classified by fruiting body characteristics as previously described [9-11] and by using the Index Fungorum system (www.indexfungorum.org). The representatives of different morphologies were selected for identification by molecular identification. Samples which showed a similarity of fruiting body were excluded.

2.3 DNA extraction and PCR amplification of ITS region

DNA extraction was performed as described by the manufacturer (Flavogen, Taiwan). Briefly, a piece of macrofungi in 200 µL of lysis buffer I (Flavogen kit) was ground with a micropestle and vortexed until it was homogenized (2-3 min). Proteinase K (20 µL) was added and incubated at 60ºC for 3 h. Subsequently, 200 µL of lysis buffer II was added and incubated at 70ºC for 10 min. Then, absolute ethanol was added and the mixture was vortexed. All lysates were transferred to the column that was provided with the kit, and the protocol according to the manual was followed. The internal transcribed spacer (ITS) (including ITS1 and ITS2) was used as a target for amplification by

polymerase chain reaction (PCR) with the primers, ITS1 (5'- TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [1]. The PCR mixtures were then made to a total volume of 20 µL that contained 6 µL of distilled water (Apsalagen, Thailand), 10 µL of 2x PCR master mix (Apsalagen, Thailand), 1 µL of each 10 µM primer, and 2 µL of template DNA. PCR amplification was performed in a thermal cycler under the following conditions: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 1 min, and lastly, a final extension at 72°C for 10 min. The PCR products (600-700 bp) were analyzed by 2% agarose gel electrophoresis with RedSafe (iNtRON biotechnology, Korea) at 100V for 30 min. The PCR products were purified using NucleoSpin Gel and PCR clean-up kit according to manufacturer's protocol (Macherey-Nagel, Germany).

2.4 DNA sequencing and pairwise analysis

DNA sequencing was performed at ATGC company (Pathum Thani, Thailand). The obtained sequences of macrofungi were analyzed with the BioEdit program, and the percent similarities of macrofungi samples were analyzed by pairwise similarity of partial ITS sequences in the MycoBank database (https://www.mycobank.org/page/Pairwise_alignment) and BLASTn in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.5 Phylogenetic analysis

The ITS sequences of 41 samples were analyzed using the Neighbor-Joining method to generate an evolutionary tree [12]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10,000 replicates) was shown next to the branches [13]. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [14]. The ITS phylogeny involved 85 nucleotide sequences. Evolutionary analyses were conducted in MEGA X [15].

2.6 The role of macrofungi in ecology and their edibility

The roles of the representative macrofungi, their ecology, and their edible properties were searched for in previously published data and FUNGuild (https://github.com/UMNFuN/FUNGuild) [16]. The roles of macrofungi were defined based on three trophic modes: (i) pathotrophs, which receive nutrients by harming host cells, (ii) saprotrophs, which receive nutrients by breaking down dead host cells, and (iii) symbiotrophs, which receive nutrients by exchanging resources with host cells [16].

3. Results and Discussion

One hundred and eighty-five macrofungi samples were collected from the survey routes of Plant Genetic Conservation Area, Chanthaburi province, Thailand in 2021. Only 41 macrofungi samples with different morphology of fruiting bodies were selected to be identified by ITS region. The ITS amplification by PCR method revealed PCR products between 600-700 bp. The obtained sequences were BLASTn searched in the GenBank and the MycoBank database by pairwise similarity with closely related reference sequences, as described in Table 1.

Table 1. The pairwise similarity of partial ITS sequences of macrofungi with closest matches based on MycoBank and *GenBank results

Family	Collection No.	Best Match (Accession No.)		GenBank Accession Number ITS
		ITS	Similarity (%)	
Phylum Ascomycota				
Sarcoscyphaceae	H13	*Cookeina garethjonesii* (KY094617)	100.0	OM442971
Sarcoscyphaceae	B7	*Cookeina sulcipes* (KY094620)	100.0	MZ221608
Sarcoscyphaceae	B5	*Cookeina tricholoma* (AF394026)	99.8	MZ221762
Hypocreaceae	H6	*Trichoderma pezizoides* (DQ835513)	98.6	OL519515
Xylariaceae	G6	*Xylaria allantoidea* (GU324743)	99.8	OL477337
Xylariaceae	G3	*Xylaria papulis* (GU300100)	99.8	OL687382
Phylum Basidiomycota				
Agaricaceae	G11	*Heinemannomyces splendidissima* (HM488760)	99.8	OL518941
Agaricaceae	H8	*Lepiota thrombophora* (EU681779)	97.3	OL546654
Agaricaceae	K2	*Micropsalliota furfuracea* (KJ849235)	98.5	OM455512
Agaricaceae	I11	*Micropsalliota subalba* (HM436646)	97.9	OP522030
Entolomataceae	F38	*Entoloma omiense* (LT716036)	99.4	OP522010
Inocybaceae	K1	*Inocybe parvisquamulosa* (KT329447)	97.6	OP522012
Lyophyllaceae	G33	*Termitomyces* sp. (AB073501)	100.0	OP529828
Marasmiaceae	I13	*Crinipellis malesiana* (FJ167628)	99.5	OL629256
Marasmiaceae	F16	*Marasmius guyanensis* (EU935554)	99.6	OP522027
Marasmiaceae	H20	*Marasmius palmivorus* (JQ653425)	99.9	OM442970

Table 1. The pairwise similarity of partial ITS sequences of macrofungi with closest matches based on MycoBank and *GenBank results (continued)

Family	Collection No.	Best Match (Accession No.)		GenBank Accession Number ITS
		ITS	Similarity (%)	
Marasmiaceae	H29	*Marasmiellus scandens* (MN794137)*	97.5	OP522022
Marasmiaceae	G25	*Trogia benghalensis* (KU647630)	97.8	OP522023
Physalacriaceae	G12	*Cyptotrama asprata* (KY649460)	99.5	OL518952
Pleurotaceae	G13	*Hohenbuehelia grisea* (MF150036)	99.9	OP535887
Psathyrellaceae	G16	*Psathyrella singeri* (MG734718)	98.2	OL518973
Schizophyllaceae	D8	*Schizophyllum commune* (KU042974)	100.0	MZ230230
Auriculariaceae	G39	*Auricularia cornea* (KM884963)	99.3	OL661641
Sclerodermataceae	J19	*Scleroderma xanthochroum* (EU718126)	99.6	OP522021
Geastraceae	H28	*Geastrum mirabile* (AB509620)	100.0	OL546797
Ganodermataceae	G19	*Amauroderma rugosum* (KJ531666)	99.0	OL477338
Ganodermataceae	H22	*Ganoderma williamsianum* (MG279168)	100.0	OP522020
Phanerochaetaceae	I6	*Oxychaete* sp. (KX752596)	100.0	OM456127
Polyporaceae	J11	*Funalia caperata* (KP757738)	100.0	OL629609
Polyporaceae	J12	*Coriolopsis dendriformis* (KC867409)	99.3	OL636129
Polyporaceae	G18	*Microporus ochrotinctus* (AJ537405)	100.0	OP522011
Polyporaceae	H11	*Favolus* sp. (KM385435)	100	OP522006
Polyporaceae	L2	*Hexagonia glabra* (KX900637)	99.8	OP522019
Polyporaceae	L17	*Lentinus* sp. (KT956126)	99.2	OP522028
Polyporaceae	F35	*Melanoderma* sp. (KM521268)	97.6	OL583980

Table 1. The pairwise similarity of partial ITS sequences of macrofungi with closest matches based on MycoBank and *GenBank results (continued)

Family	Collection No.	Best Match (Accession No.)		GenBank Accession Number ITS
		ITS	Similarity (%)	
Polyporaceae	E8	*Microporus xanthopus* (KT273357)	100.0	MZ221237
Polyporaceae	E3	*Picipes* sp. (KX900074)*	99.4	MZ229893
Polyporaceae	L1	*Trametes elegans* (MF377436)	99.8	OM276859
Stereaceae	J6	*Stereum hirsutum* (KP715550)	98.7	OL531456
Dacrymycetaceae	F46	*Dacryopinax spathularia* (AY854070)	100.0	OL639170
Tremellaceae	H18	*Tremella fuciformis* (FJ501580)	99.3	OL477326

Non-asterisk: accession number from MycoBank
Asterisk (*): accession number from GenBank

Practically, a sample with a sequence identity of ≥ 97.0% match with a published sequence that sample can be assigned at species-level identification. A sample with sequence identity in the range of 90.0-96.9% that matches with a published sequence can be assigned at genus-level identification. However, in the study of fungal taxonomy, sample with lower than 98.0% similarity may be different species from the published sequence. As shown in this study, many fungi (collection numbers H8, I11, K1, H29, G25, and F35) were shown to have less than 98.0% similarity (Table 1). While the G33, I6, H11, L17, and E3 shared ITS sequence similarity higher than 99.0% (except for F35, which showed 97.6%) with unknown species of macrofungi. Therefore, these samples need more investigation for identification in the future.

The evolution and relationship of each macrofungal in this study were explored by phylogeny based on ITS sequences as shown in Figure 2. The dominant order was Agaricales (Family Agaricaceae, Psathyrellaceae, Lyophyllaceae, Entolomataceae, Inocybaceae, Marasmiaceae, Physalacriaceae, Pleurotaceae, and Schizophyllaceae) and Polyporales (Family Phanerochaetaceae, Ganodermataceae and Polyporaceae), respectively. The families in Polyporales revealed the close relationships in each family, and Polyporaceae showed the most diverse taxa. Additionally, the families in Agaricales showed evolution and relationship characteristics of the family. Pleurotaceae, Lyophyllaceae, and Marasmiaceae were closely related to each other and present in the same cluster. Together, Physalacriaceae, Psathyrellaceae, Agaricaceae, and Schizophyllaceae were also shown in the same cluster. However, some families of Agaricales were closely related to families in other orders such as Inocybaceae (order Agaricales), showing a close relationship with Sclerodermataceae (order Boletales) and Entolomataceae (order Agaricales), revealing a close relationship with Dacrymycetaceae (order Dacrymycetales) (Figure 2).

In Thailand, the rainy season starts in June and lasts until the end of October. However, in Chanthaburi province located in Eastern Thailand which has a tropical climate, the summer rains are more abundant. Therefore, diverse taxa of macrofungi were found this study, starting in May and lasting until July 2021 (May, June, and July found 36, 28, and 20 taxa, respectively) (Table 2). The climate may probably support the growth and development of the fungal fruiting bodies in the

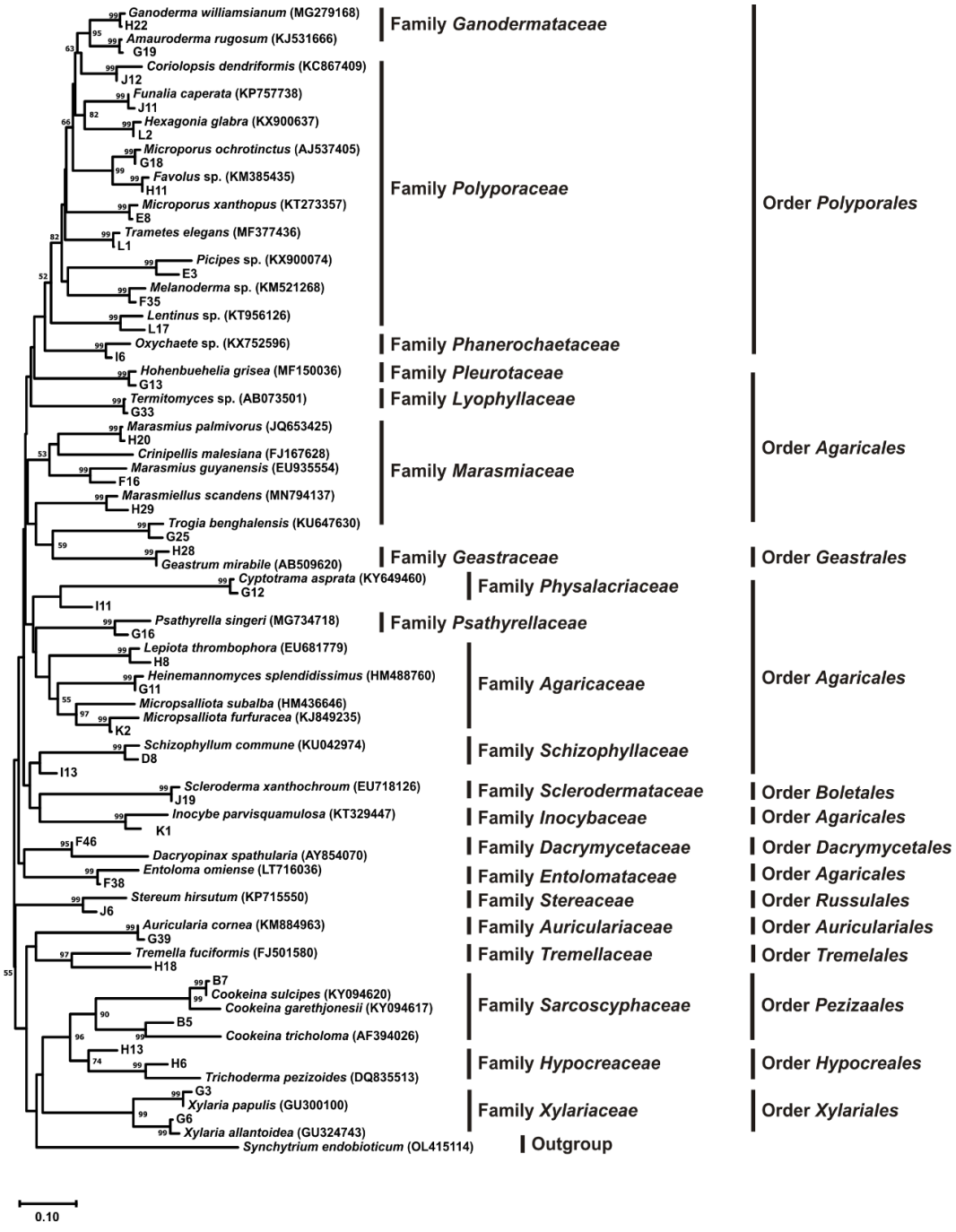

Figure 2. Phylogenetic tree based on ITS sequences of macrofungi in this study. Family level taxa and order level taxa indicated to the right are those accepted in this study.

Table 2. Monthly occurrences of macrofungi collected in Plant Genetic Conservation Area, Chanthaburi province, Thailand, with their modes of life and edibility

Phylum	Class	Order	Family	Scientific Name (collection number)	Month										Mode of Life	Edibility
					3	4	5	6	7	8	9	10	11	12		
Ascomycota	Pezizomycetes	Pezizales	Sarcoscyphaceae	*Cookeina garethjonesii* (H13)			x	x							SA	
Ascomycota	Pezizomycetes	Pezizales	Sarcoscyphaceae	*Cookeina sulcipes* (B7)			x	x	x		x				SA	E
Ascomycota	Pezizomycetes	Pezizales	Sarcoscyphaceae	*Cookeina tricoloma* (B5)			x	x	x		x	x			SA	E
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma pezizoidez* (H6)		x	x	x			x	x		x	SA	
Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Xylaria allantoidea* (G6)	x	x	x	x						x	SA SM	
Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Xylaria papulis* (G3)		x	x	x	x					x	SA SM	
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Heinemannomyces splendidissima* (G11)				x							SA	
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Lepiota thrombophora* (H8)			x	x	x	x					SA	P
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Micropsalliota furfuracea* (K2)				x	x				x	x	SA	
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Micropsalliota subalba* (I11)				x		x	x				SA	
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Entoloma omiense* (F38)			x		x						SA	P
Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	*Inocybe parvisquamulosa* (K1)			x						x	x	SA SM	P
Basidiomycota	Agaricomycetes	Agaricales	Lyophyllaceae	*Termitomyces* sp. (G33)			x	x	x			x			SA SM	E

Table 2. Monthly occurrences of macrofungi collected in Plant Genetic Conservation Area, Chanthaburi province, Thailand, with their modes of life and edibility (continued)

Phylum	Class	Order	Family	Scientific Name (collection number)	Month										Mode of Life	Edibility
					3	4	5	6	7	8	9	10	11	12		
Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Crinipellis malesiana* (I13)			x			x	x				SA	
Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Marasmius guyanensis* (F16)			x		x		x	x			PA SA SM	
Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Marasmius palmivorus* (H20)				x	x	x		x			PA SA SM	
Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Marasmiellus scandens* (H29)			x		x	x					PA SA	
Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	*Trogia benghalensis* (G25)			x	x	x			x			SA	
Basidiomycota	Agaricomycetes	Agaricales	Physalacriaceae	*Cyptotrama asprata* (G12)			x	x							SA	
Basidiomycota	Agaricomycetes	Agaricales	Pleurotaceae	*Hohenbuehelia grisea* (G13)			x	x	x						SA	
Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	*Psathyrella singeri* (G16)				x	x						SA	
Basidiomycota	Agaricomycetes	Agaricales	Schizophyllaceae	*Schizophylum commune* (D8)	x		x				x	x			SA	E
Basidiomycota	Agaricomycetes	Auriculariales	Auriculariaceae	*Auricularia cornea* (G39)			x	x	x	x	x	x			SA	E
Basidiomycota	Agaricomycetes	Boletales	Sclerodermataceae	*Scleroderma xanthochroum* (J19)		x	x				x	x	x	x	SM	P
Basidiomycota	Agaricomycetes	Geastrales	Geastraceae	*Geastrum mirabile* (H28)		x	x	x							SA	

Table 2. Monthly occurrences of macrofungi collected in Plant Genetic Conservation Area, Chanthaburi province, Thailand, with their modes of life and edibility (continued)

Phylum	Class	Order	Family	Scientific name (collection number)	Month										Mode of life	Edibility
					3	4	5	6	7	8	9	10	11	12		
Basidiomycota	Agaricomycetes	Polyporeales	Ganodermataceae	*Amauroderma rugosum* (G19)	x	x	x	x				x	x	x	SA	E
Basidiomycota	Agaricomycetes	Polyporeales	Ganodermataceae	*Ganoderma williamsianum* (H22)		x	x	x	x			x	x	x	PA SA	
Basidiomycota	Agaricomycetes	Polyporeales	Phanerochaetaceae	*Oxychaete* sp. (I6)		x	x							x	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	*Funalia caperata* (J11)	x	x	x				x	x	x	x	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	*Coriolopsis dendriformis* (J12)		x	x	x	x	x	x	x		x	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	*Microporus ochrotinctus* (G18)	x	x	x	x			x	x	x	x	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	*Favolus* sp. (H11)	x	x	x	x		x		x		x	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	*Hexagonia glabra* (L2)	x	x	x		x					x	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	*Lentinus* sp. (L17)			x							x	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	*Melanoderma* sp. (F35)		x	x								SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	*Microporus xanthopus* (E8)	x	x	x	x	x	x	x	x	x	x	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	*Picipes* sp. (E3)	x	x	x	x					x	x	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	*Trametes elegans* (L1)	x	x	x				x	x	x	x	SA	

Table 2. Monthly occurrences of macrofungi collected in Plant Genetic Conservation Area, Chanthaburi province, Thailand, with their modes of life and edibility (continued)

Phylum	Class	Order	Family	Scientific Name (collection number)	Month										Mode of Life	Edibility
					3	4	5	6	7	8	9	10	11	12		
Basidiomycota	Agaricomycetes	Russulales	Stereaceae	*Stereum hirsutum* (J6)		x	x	x	x	x	x	x	x	x	SA	
Basidiomycota	Dacrymycetes	Dacrymycetales	Dacrymycetaceae	*Dacryopinax spathularia* (F46)			x	x							SA	E
Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	*Tremella fuciformis* (H18)			x	x	x						SA	E
		Monthly total taxa richness			10	18	36	28	20	10	15	18	11	19		

Abbreviations: Mode of life: PA =pathotroph, SA = saprotroph, SM=symbiotroph;
Edibility: E= edible macrofungi, P= poisonous macrofungi;
Month: 3=March, 4=April, 5=May, 6=June, 7=July, 8=August, 9=September, 10=October, 11=November, 12=December
x=found

Plant Genetic Conservation Area at these times. Similarly, Enow [17] revealed that higher macrofungi diversity was obtained during the rainy season.

The macrofungi from Plant Genetic Conservation Area, Chanthaburi province could be classified into 2 phyla, 5 classes, 11 orders, 21 families, and 34 genera. Six taxa were classified in the phylum Ascomycota (14. 6%), while the remaining macrofungi taxa were in the phylum Basidiomycota (35 taxa, 85.4%) (Table 2, Figure 3). The Polyporaceae demonstrated the most diverse taxa (10 taxa, 24.4%) while the families Marasmiaceae, Agaricaceae, and Sarcoscyphaceae found 5 (12.2%), 4 (9.8%), and 3 (7.3%) taxa, respectively. The Xylariaceae and Ganodermataceae had 2 taxa per family (4.9%), whilst the Hypocreaceae, Entolomataceae, Inocybaceae, Lyophyllaceae, Physalacriaceae, Pleurotaceae, Physalacriaceae, Schizophyllaceae, Auriculariaceae, Sclerodermataceae, Geastraceae, Phanerochaetaceae, Stereaceae, Dacrymycetaceae, and Tremellaceae families were found to have only 1 taxon per family (2.4%) (Table 2, Figure 3).

Microporus xanthopus was mostly found in every month of sample collection (10 months) while *Stereum hirsutum, Coriolopsis dendriformis,* and *Microporus ochrotinctus* were found in 9, 8, and 8 months of sample collection, respectively. The most frequent taxa found in this study were classified as Polyporaceae and Stereaceae. The members of these families were shaped like shelves or brackets, and were tough and hard like cork or wood, and unsuitable for edibility. They play an important role in the biogeochemical cycles and are relatively common in tropical forest regions [18, 19]. Due to these properties, *Microporus xanthopus* could adapt and have a long life in all seasons, which was mostly found in this study.

Nowadays, macrofungi identification using only morphological characteristics cannot classify some closely related species, and the molecular identification by sequence analysis is used to support and confirm the morphological data. Although the internal transcribed spacer (ITS) sequence has commonly been used for macrofungi identification, some fungi cannot be identified at the species level [1, 5, 20].

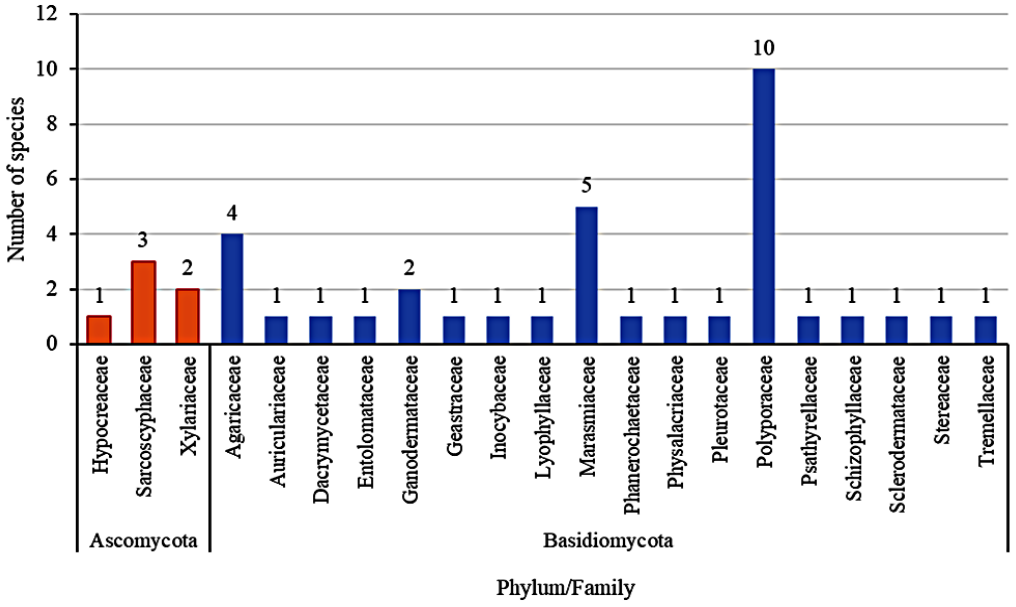

Figure 3. Macrofungi family distribution in the Plant Genetic Conservation Area, Chanthaburi Province, Thailand

Several genes have been used as additional molecular markers for fungal identification including LSU (nuclear large subunit rDNA), SSU (nuclear small subunit rDNA), TEF1-alpha (translation elongation factor 1-alpha), TUB (tubulin), and RPB2 (DNA-directed RNA polymerase II subunit 2) [1]. According to Cho *et al*. [21], *Amanita* species were identified by sequence analyses of ITS and LSU. The macrofungi were identified as *Amanita fulva, A. eijii, A. volvata*, *A. manginiana, A. pallidorosea*, *A. rubescens, A. supjunquillea,* and the new records were *Amanita caesareoides*, *A. girseoturcosa*, *A. imazekii*, *A. Sepiacea*; however, 2 samples of macrofungi were unknown species.

In the present study, many macrofungi (collection numbers H8, I11, K1, H29, G25, and F35) revealed less than 98.0% similarity (Table 1). While the G33, I6, H11, L17, and E3 shared the ITS sequence similarities of higher than 99.0% (except for F35, which showed 97.6%) with unknown species of macrofungi. Therefore, these samples were probably novel species. This suggested that sequence analysis of additional genes such as ITS, LSU, TEF1-alpha, and RPB2 was required. These had previously been used to study phylogeny and species identification of *Lentinus* sp. [22], while ITS and LSU had been used for *Melanoderma* sp. classification [23]. In addition, sequences analyses of SSU, TUB, TEF1-alpha, RPB1, and RPB2 were used for phylogeny and classification of *Picipes* sp. [24].

The roles of macrofungi in the ecology of Plant Genetic Conservation Area showed that most macrofungal taxa played the important role of saprotrophs (40 taxa, 97.6%). However, symbiotrophs (7 taxa, 17.0%) such as *Termitomyces* sp. and pathotrophs (4 taxa, 9.8%) such as *Ganoderma williamsianum* were also explored (Table 2). However, the mode of life data in Table 2 was based on the database in FUNGuild (https://github. com/UMNFuN/FUNGuild). If the previously published data indicates more than 1 mode of life, as in the case of *Termitomyces* sp. which was reported as being both saprotroph (SA) and symbiotroph (SM), then as a result FUNGuild showed 2 modes of life (SA and SM in *Termitomyces* sp.)

Additionally, the macrofungi identified in this study were previously reported as being edible, and include were *Cookeina sulcipes* [25], *Cookeina tricoloma* [26], *Amauroderma rugosum* [27, 28], *Termitomyces* sp. [29], *Schizophylum commune* [30], *Auricularia cornea* [31-34], *Dacryopinax spathularia* [35, 36] and *Tremella fuciformis* [37, 38] (8 taxa, 19.5%). Moreover, some of these edible macrofungi had medicinal properties [39] (Table 2, Figure 4). In contrast, the poisonous macrofungi consisted of *Entoloma omiense* [40, 41], *Lepiota thrombophora* [42-44], *Inocybe parvisquamulosa* [45], and *Scleroderma xanthochroum* [46] (4 taxa, 9.76%). There was no data on the edibility of the remaining taxa of macrofungi in this study (29 taxa, 70.73%) (Table 2).

These results of the present study are similar to the report of Kassim *et al*. [47], who investigated macrofungi at Sungai Kangkawat Research Station, Imbank Canyon Conservation Area, Sabah, Malaysia (a tropical rainforest). Of the 104 macrofungi samples identified, the most common belonged to phylum Basidiomycota (91.2%), and family Polyporaceae (21.6%). Most of them were saprophytic fungi (58.5%).

4. Conclusions

The biodiversity and functional distribution of macrofungi within the Plant Genetic Conservation Area of Rambhai Barni Rajabhat University, Chanthaburi Province, Thailand, were reported in this study. One hundred and eighty-five macrofungi samples were collected from the survey routes in 2021. The molecular identification by sequence analysis of ITS was used for the identification. The macrofungi samples (41 samples with a different fruiting bodies) were classified into 2 phyla, 5 classes, 11 orders, 21 families, and 34 genera, and the most common were in the phylum

Figure 4. Photographs of macrofungi found in this study that had previously been reported to be edible.
A: *Cookeina tricoloma*; B: *Cookeina sulcipes*; C: *Amauroderma rugosum*;
D: *Termitomyces* sp.; E: *Auricularia cornea*; F: *Schizophylum commune*;
G: *Dacryopinax spathularia*; H: *Tremella fuciformis*

Basidiomycota (35 taxa, 85.4%), the family Polyporaceae (10 taxa, 24.4%), and played the role of saprotroph (40 taxa, 97.6%). In addition, for most of studied species (29 taxa, 70.7%), no edibility data were available. However, edible (8 taxa, 19.5%) and poisonous (4 taxa, 9.8%) macrofungi were reported. Interestingly, *Microporus xanthopus* was the most frequently found in every month of sample collection, and some macrofungi samples will need more investigation in order to be identified, and additional genes will be needed. These results of our study provided a useful database of the macrofungi diversity in this area, together with the information on their roles and edibility, provides a foundation for further studies for mushroom cultivation, beneficial bioactive compounds, and medicinal applications.

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