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Original research article

Macrofungi Diversity in a Community Forest, Tha Mai District, Chanthaburi Province, Thailand

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ABSTRACT

This study aimed to survey macrofungi diversity in a community forest in Tha Mai District, Chanthaburi Province, Thailand, and to evaluate their trophic mode and edibility; the knowledge gained from this study will help to establish a macrofungi database as part of a biodiversity learning center for the community. Macrofungi sampling was performed in the area, with identification being done by morphological analysis and internal transcribed spacer (ITS) sequencing. The results showed that 18 unique macrofungi samples with diverse fruiting body characteristics were examined. These could be classified into 9 groups: (i) Ascomycetes fungi, (ii) Gilled fungi, (iii) Polypores and bracket fungi, (iv) Leather-bracket fungi, (v) Jelly fungi, (vi) Earthstar fungi, (vii) Stinkhorn fungi, (viii) Coral fungi, and (ix) Bird's nest fungi. The gilled fungi were the most common (27.78%). Subsequently, molecular identification by ITS region analysis was able to classify them into 2 phyla, 2 classes, 8 orders, 14 families, and 17 genera. The phylum Basidiomycota (83.33%) and the family Polyporaceae (22.22%) were the most predominant taxon and family, respectively, in this area. There were 6 samples (33.33%), the genus classification was reliable (95 to 98.5% similarity), but the species are yet to be confirmed. Most fungi acted as saprotrophs (94.44%) in the ecosystem of the community forest, and only 1 species (5.56%), *Termitomyces* sp. (KN20), functioned as a symbiotroph. There was however no pathotrophy documented in this study. Additionally, the edible macrofungi (16.67%) reported in this study

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include *Auricularia novozealandica* (KN40), *Phallus lutescens* (KN38), and *Termitomyces* sp. (KN20), with no report of any poisonous macrofungi in this area. However, the remaining macrofungi species (83.33%) have no information on edibility or toxicity.

Keywords: Community forest; Diversity; Identification; Macrofungi

1. Introduction

The community forest in this study is located in Khao Noi Sam Phan Temple of Song Phi Nong Subdistrict, Tha Mai District, Chanthaburi Province, Thailand. This area is under the supervision of the Song Phi Nong Subdistrict Municipality (Government agency) which has enacted a policy to establish a biodiversity learning center for the community.

The forest area is a lush tropical rainforest situated in a mountainous region, rich in a variety of flora and fauna including mushrooms (macrofungi). The macrofungal diversity is quite evident in this area as the soil is moist and the trees provide plenty of shade, allowing macrofungal fruiting bodies to thrive.

Macrofungi are eukaryotic organisms that develop fruiting bodies. A fruiting body is the structure responsible for producing spores; these fruiting bodies have a diverse range of characteristics and can be observed with the naked eye. Macrofungi belong to the kingdom Fungi and are divided into the phyla Ascomycota and Basidiomycota based on their sexual spore type.

Several studies of macrofungi diversity in community forests have previously been reported. The Brahakshetra community forest in Nepal explored macrofungi in 2 phyla, 8 orders, 21 families, and 30 genera [1]. The Teghari community forest in western Nepal reported 36 families of macrofungi diversity [2]. Additionally, the Thulo Ban community forest in Nepal reported 12 orders, 26 families, 14 genera [3].

In Thailand, the community forest in Sai Yok District, Kanchanaburi Province (Western Thailand) reported 15 macrofungi species. [4]. The community forest of Na Si Nuan Subdistrict, Maha Sarakham Province (North-eastern Thailand) explored 27 macrofungal species [5]. Subsequently, the Plant Genetic Conservation Area, a community forest in Chanthaburi Province (Eastern Thailand), observed 41 macrofungal samples which were classified into 2 phyla, 5 classes, 11 orders, 21 families, and 34 genera [6].

However, the community forest of Khao Noi Sam Phan Temple lacks biodiversity data for many organisms, including macrofungi. The classification and identification of fungi can be done by studying morphological characteristics. However, morphological identification requires specialized expertise, is time-consuming, and depends on the presence of intact fruiting body structures. Moreover, different species can present with very similar characteristics, which can lead to incorrect identification. Therefore, studying morphological characteristics may not be sufficient to identify macrofungal species. In this study, molecular techniques were used for the identification of macrofungi by analyzing the nucleotide sequences at the internal transcribed spacer (ITS). This method has high accuracy in identifying macrofungi species, takes a short time, and only uses a small number of samples. It uses the polymerase chain reaction (PCR) technique to amplify the target DNA region, which is then sequenced and compared against samples from an online database to identify the species and construct a phylogenetic tree [7]. However, the identification of macrofungal species still requires morphological data to be analyzed together with molecular biology data.

Therefore, the aim of this study was to investigate the diversity of macrofungi in the

community forest of Tha Mai District, Chanthaburi Province, Thailand, by morphological and molecular methods, and further, to evaluate the trophic mode and edibility of the species found therein.

2. Materials and Methods

2.1 Study site and macrofungi sampling

A survey of macrofungi was conducted on the nature trail of the community forest in Khao Noi Sam Phan Temple, Song Phi Nong Subdistrict, Tha Mai District, Chanthaburi Province (Fig. 1) (38.3 acres, latitude 12°40'02" N to 12°40'25" N and longitude 101°58'25" E to 101°58'38" E). The macrofungi were collected twice in June 2025 because this hiking trail was closed during the other months of the rainy season for safety reasons. Macrofungi specimens were found along the nature trails of the community forest, growing on decaying logs, rotting branches, and the ground. When macrofungi were found, photos were taken (Nikon D3400, Japan) to collect data on their habitat and fruiting body structure. Subsequently, the macrofungi samples were placed in a plastic box and transported to the laboratory. Once there, the sample boxes were labeled with a collection number and a paper scale was added for size comparison. A small amount of tissue was collected and put into a 1.5 ml microcentrifuge tube to extract DNA. If possible, the tissue sample was taken from the inner tissue, which was not exposed to the environment, to reduce contamination. A large amount of tissue was collected and put into a 1.5 ml microcentrifuge tube for tissue stocking in absolute ethanol. These were stored at -20ºC to preserve the tissue. The remaining samples were dried at 50ºC to preserve the fruiting body samples.

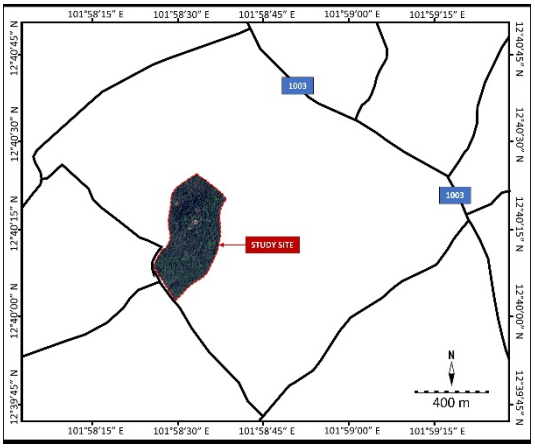


Fig. 1. Map of the community forest where macrofungi were collected in this study.

2.2 Morphological classification

The morphological characteristics of macrofungi were studied from both fresh samples and their photographs. The characteristics of the cap, gill, stalk, ring, volva, scales, and spores, as well as their habitat, were studied to provide preliminary data for classification by morphological characteristics.

The preliminary morphological data were compared with existing literature on the macrofungi of Thailand [8-10]. The macrofungi samples presented in different fruiting body patterns were selected to be identified by the molecular method.

2.3 DNA extraction

DNA extraction was performed using a commercial kit (Favorgen, Taiwan) according to the manufacturer's protocol. Before using the kit, the sample was prepared by placing 0.1 g of silicon dioxide (Sigma-Aldrich, USA) in a 1.5 ml microcentrifuge tube. Macrofungi tissue of equal size to the amount of silicon dioxide (0.05-0.1 g) was added. Then, 50 µl of lysis buffer (FATG1 buffer) was added and ground with a micropestle for 2-3 minutes or until the tissue was fine. Lysis buffer was added incrementally to a final volume of 200 µl. At

this point, the sample was ready for extraction.

2.4 ITS amplification and gel electrophoresis

The ITS region was amplified by PCR as previously described with primers ITS1 and ITS4 (ITS1:5'-TCCGTAGGTGAACCTGCGG-3'; ITS4: 5'-TCCTCCGCTTATTGATATGC-3') [6, 7]. Briefly, the PCR mixture consisted of DNA template (10 pg-1 µg), primers (0.5 µM), 1X PCR mastermix (Apsalagen, Thailand), with the final reaction volume adjusted to 20 µl using molecular-grade distilled water (Apsalagen, Thailand). The thermal cycler was set with the following conditions: initial denaturation at 95ºC for 3 min; 35 cycles of denaturation at 95ºC for 30 sec, annealing at 52ºC for 30 sec, extension at 72ºC for 1 min; and final extension at 72 ºC for 10 min [6].

The PCR product was examined by gel electrophoresis using 2% agarose gel in TAE buffer with nucleic acid staining dye or RedSafe (iNtRON, Korea) and run at 100 V for 30 min as previously described [6].

2.5 DNA sequencing and analysis of nucleotide sequence

The PCR products were sent to ATGC Co., Ltd. (Pathum Thani, Thailand) to purify and perform the DNA sequencing. Subsequently, the sequences were analyzed using the BLASTn (Basic Local Alignment Search Tool or nucleotide BLAST) program in the GenBank database of NCBI (National Center for Biotechnology Information) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and pairwise analysis in the MycoBank database (https://www.mycobank.org/page/Pairwise_alignment) to examine the %similarity or %identity of the fungal species. A phylogenetic tree was constructed based on nucleotide sequences of the ITS region from the macrofungal samples by the Neighbor-Joining method [11-13], using the MEGA X program [14].

2.6 Determination of taxonomy data, the role in ecology, and edibility property

The taxonomic data and the current name of macrofungi were searched in the Index Fungorum system (www.indexfungorum.org) and GenBank (https://www.ncbi.nlm.nih.gov). The edibility and trophic mode were searched for in previously published data and FUNGuild (https:// github.com/UMNFuN/FUNGuild) [15].

3. Results and Discussion

3.1 Macrofungi samples and morphological classification

In total, 40 macrofungi samples were collected from the nature trail of the community forest. Their morphological characteristics were studied and classified. It was found that some macrofungi samples had similar morphological characteristics. Therefore, from the original 40, 18 samples with different fruiting body characteristics were selected.

The preliminary classification results using morphological characteristics found that these macrofungi could be classified into 9 groups: (i) Ascomycetes fungi, collection numbers KN25, KN32, and KN39, with various fruiting body shapes, such as club-like with hard texture (KN25 and KN32), convex plate-shaped without stalk, attached to substate (KN39), all of which produce ascospore spores; (ii) Gilled fungi, consisting of KN1, KN3, KN12, KN17, and KN20, with a cap, soft texture, with gills under the cap; (iii) Polypores and bracket fungi, consisting of KN16, KN26, KN31, and KN37, exhibiting shelf-, bracket-, or fan-shaped fruiting bodies, with tough and hard texture, with pores under the cap; (iv) Leather-bracket fungi, consisting of KN35, with a fruiting body resembling a bracket or fan, with no stalk, with a thin and hard texture, with a smooth back of the cap; (v) Jelly fungi, consisting of KN40, with soft and slimy fruiting body, elastic like a rubber sheet; (vi) Earthstars fungi, consisting of KN19, with

round fruiting body, like an egg or a pear, and the outer shell is broken off in the shape of a star; (vii) Stinkhorn fungi, consisting of KN38, with a fruiting body resembling an animal horn, with a stem that is porous and very soft, with a net covering the stem like a skirt; (viii) Coral fungi, consisting of KN23, with a fruiting body resembling coral; (ix) Bird's nest fungi, consisting of KN29, with a fruiting body resembling a bird's nest, with something like an egg, round and flat, placed at the bottom of the nest.

The results found that the gilled fungi were the most common, with 5 samples (27.78%), followed by polypores and bracket fungi, with 4 samples each (22.22%), then ascomycetes fungi, with 3 samples (16.67%), followed by the leather-bracket fungi, jelly fungi, earth star fungi, stinkhorn fungi, coral fungi, and bird's nest fungi, with only 1 sample each (5.56%). Subsequently, all 18 macrofungi samples were identified using DNA sequencing.

3.2 Molecular identification

The ITS PCR products were analyzed by gel electrophoresis. PCR products of 500-600 bp were observed (Fig. 2). Subsequently, the nucleotide sequence of the fungal samples was used for pairwise analysis and the %similarity is shown in Table 1. Additionally, the nucleotide sequences from this study have been submitted to NCBI and are provided here with the sequence accession number (Table 1). A phylogenetic tree was constructed to determine the genetic relatedness of these macrofungi, as shown in Fig. 3. The best reference matches (≥ 98.5% similarity) could be considered for species-level identification in fungi, the samples with a similarity of 95 to 98.5% could be considered for genus-level identification depending on supporting evidence, but samples with a similarity <95% should not be identified at the genus and species level from only the ITS region; further sequencing of other genes may be required for identification. In this study, twelve samples (66.67%) had ≥ 98.5% similarity and species-level identification could be confirmed, except for KN23 and KN20, which best matched species in families Clavariaceae and *Termitomyces*, respectively. Six samples (33.33%) had 95 to 98.5% similarity, including KN25, KN3, KN17, KN12, KN19, and KN26. For these samples, the genus identification is reliable, but the species-level identification could not be confirmed. However, further sequencing of additional genes, such as the nuclear large subunit of ribosomal DNA, may be required to confirm the species of these fungi [16, 17].

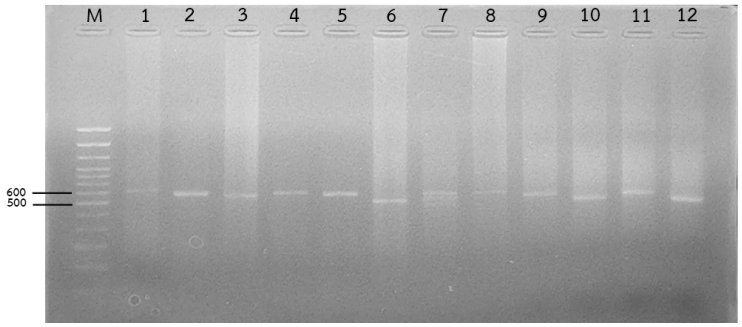


Fig. 2. Representative gel electrophoresis results to examine PCR products from the ITS amplification by PCR. M: 100 bp DNA Ladder (Bio-Tech Rabbit, Germany); Lane 1: KN3 code, size 600 bp; Lane 2: KN12 code, size 600 bp; Lane 3: KN17 code, size 600 bp; Lane 4: KN19 code, size 600 bp; Lane 5: KN20 code, size 600 bp; Lane 6: KN23 code, size 500 bp; Lane 7: KN26 code, size 600 bp; Lane 8: KN29 code, size 600 bp; Lane 9: KN31 code, size 600 bp; Lane 10: KN32 code, size 600 bp; Lane 11: KN35 code, size 600 bp; Lane 12: KN37 code, size 500 bp.

Table 1. Taxonomy data, pairwise analysis, mode of life, and edibility of macrofungi collected from community forest, Tha Mai District, Chanthaburi Province, Thailand.

Phylum	Class	Order	Family	Scientific name (Collection number)	Best Match (Accession No.)		GenBank Accession No. in this study	Mode of life	Edibility
					ITS (MycoBank)	Similarity (%)			
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma viride* (KN39)	*Trichoderma viride* (DQ835513)	98.93	PV565621	SA	
Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Astrocystis pseudomirabilis* (KN25)	*Astrocystis pseudomirabilis* (NR_182737)	95.90	PV565609	SA	
Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Xylaria feejeensis* (KN32)	*Xylaria feejeensis* (MH370737)	99.59	PV566945	SA	
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Micropsalliota rubrobrunnescens* (KN3)	*Micropsalliota rubrobrunnescens* (HM436627)	98.01	PV565600	SA	
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavariaceae sp. (KN23)	Clavariaceae sp. (MK427063)	99.17	PV565607	SA	
Basidiomycota	Agaricomycetes	Agaricales	Lyophyllaceae	*Termitomyces* sp (KN20)	*Termitomyces* sp. (LC481556)	100	PV565606	SM	E
Basidiomycota	Agaricomycetes	Agaricales	Malasmiaceae	*Marasmius guyanensis* (KN1)	*Marasmius guyanensis* (EU935553)	98.72	PV565582	SA	
Basidiomycota	Agaricomycetes	Agaricales	Nidulariaceae	*Cyathus subglobisporus* (KN29)	*Cyathus subglobisporus* (NR_137683)	100	PV565611	SA	
Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	*Collybiopsis polygramma* (KN17)	*Collybiopsis polygramma* (OR751617)	96.21	PV565605	SA	
Basidiomycota	Agaricomycetes	Agaricales	Porotheleaceae	*Trogia benghalensis* (KN12)	*Trogia benghalensis* (NR_155910)	97.98	PV565599	SA	
Basidiomycota	Agaricomycetes	Auriculariales	Auriculariaceae	*Auricularia novozealandica* (KN40)	*Auricularia novozealandica* (NR_191053)	99.11	PV565622	SA	E
Basidiomycota	Agaricomycetes	Geastrales	Geastraceae	*Geastrum saccatum* (KN19)	*Geastrum saccatum* (MH856502)	96.95	PV565603	SA	
Basidiomycota	Agaricomycetes	Phallales	Phallaceae	*Phallus lutescens* (KN38)	*Phallus lutescens* (MN131079)	99.44	PV565620	SA	E
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	*Favolus tenuiculus* (KN16)	*Favolus tenuiculus* (OR354979)	99.66	PV565601	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	*Funalia aspera* (KN31)	*Funalia aspera* (KC867352)	100	PV565612	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	*Microporus subaffinis* (KN26)	*Microporus subaffinis* (MK269126)	98.40	PV565608	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	*Trametes meyenii* (KN37)	*Trametes meyenii* (PV108208)	99.70	PV565617	SA	
Basidiomycota	Agaricomycetes	Russulales	Stereaceae	*Stereum ostrea* (KN35)	*Stereum ostrea* (MT214938)	99.56	PV565618	SA	

Abbreviations: Mode of life: PA = Pathotroph, SA = Saprotroph, SM = Symbiotroph; Edibility: E = Edible macrofungi, P = Poisonous macrofungi.

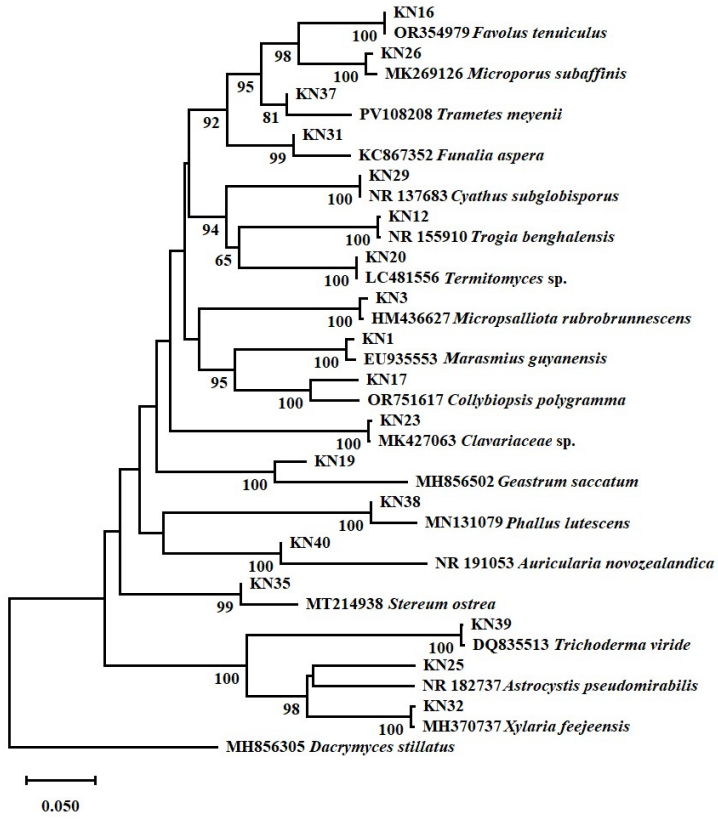


Fig. 3. Phylogenetic tree based on ITS sequences of macrofungi in this study.

It was found that the macrofungi samples could be classified into 2 phyla, 2 classes, 8 orders, 14 families, and 17 genera. The phylum Ascomycota was found in 3 samples (16.67%), while the phylum Basidiomycota found in 15 samples, (83.33%), with the Polyporaceae family having the highest diversity, with 4 samples (22.22%), followed by the Xylariaceae family, with 2 samples (11.11%), while the Agaricaceae, Auriculariaceae, Clavariaceae, Geastraceae, Hypocreaceae, Lyophyllaceae, Malasmiaceae, Nidulariaceae, Omphalotaceae, Phallaceae, Porotheleaceae, and Stereaceae families had only 1 sample each (5.56%).

Polyporaceae was the family that was most species-rich in this study, which correlated with several studies. Polyporaceae was the most frequently observed in the Teghari community forest in western Nepal [2] and in the Plant Genetic Conservation Forest in eastern Thailand (Chanthaburi Province, the same province as in this study) [6]. Polypore fungi are classified as belonging to the Polyporaceae family, with fruiting bodies that look like a shelf, bracket, or fan, with tough texture, and pores under the cap. Due to this tough texture, these fungi are considered unsuitable for eating, hardly digestible, resulting in a long life and easy observation of the fruiting body in its natural habitat.

Several studies of macrofungi diversity in community forests have previously been reported. The Brahakshetra community forest in Nepal reported macrofungi in 2 phyla, 8 orders, 21 families, and 30 genera. Here, the order Agaricales was the most frequently observed, and symbiotrophy, in the form of ectomycorrhiza, was the trophic mode most observed [1]. In the Teghari community

forest in western Nepal, 36 families of macrofungi were reported. Here, the family Polyporaceae was the most frequently observed and saprotrophy was the trophic mode most observed [2]. In the Thulo Ban Community Forest in Nepal, 56 macrofungi species belonging to 12 orders, 26 families, and 14 genera were reported. Here, the family Russulaceae was the most frequently observed [3].

In Thailand, a number of studies have reported on the macrofungal diversity in community forests. In the community forest at Sai Yok District, Kanchanaburi Province (Western Thailand), 15 macrofungi species were reported, 13 saprotrophic species and 2 symbiotrophic species [4]. In the community forest of Na Si Nuan Sub-District, Maha Sarakham Province (North-eastern Thailand), 27 macrofungal species were found, with Russulaceae being the family with the most species reported [5]. In the Plant Genetic Conservation Area, a community forest in Chanthaburi Province (Eastern Thailand), 41 macrofungal samples were taken, that were classified into 2 phyla, 5 classes, 11 orders, 21 families, and 34 genera. The Polyporaceae was the largest family, and saprotrophy was the most frequently observed trophic mode [6].

3.3 Trophic mode and edibility

How an organism obtains the energy and nutrients it needs to survive, i.e., its trophic mode, can typically be categorized as saprotrophic, symbiotrophic, or pathotrophic. Saprotrophs break down dead host tissue, symbiotrophs exchange resources with host cells in a cooperative manner, and pathotrophs parasitize host cells to receive nutrients [15].

In this study, the role of macrofungi in the ecosystem of the community forest was explored. Most of the fungi found were saprotrophs, totaling 17 species (94.44%). Only 1 species of symbiotrophic fungi was found (5.56%), namely *Termitomyces* sp. (KN20). Finally, no pathotrophic fungi were reported (Table 1).

Several studies on macrofungi diversity normally state that macrofungi fill the important role of saprotrophs in food webs [2, 4, 6]. This perspective aligns with the findings of the present study, as macrofungi are able to act as effective decomposers by secreting enzymes to digest organic matter, especially lignin and cellulose, as these compounds represent the primary structural components of plant debris. However, some community forests have found that symbiotrophic macrofungi are mostly observed acting as ectomycorrhiza [1].

Concerning edibility, 3 species (16.67%), namely, *Auricularia novozealandica* (KN40) [18], *Phallus lutescens* (KN38) [19], and *Termitomyces* sp. (KN20) [20], were found to be edible. Of these three, none were found to be toxic (Table 1 and Figs. 3-4). However, the remaining 15 macrofungi species have no information available on their edibility or toxicity (Table 1).

4. Conclusion

Eighteen macrofungi samples with different fruiting body characteristics were explored in a community forest located in Tha Mai District, Chanthaburi Province, Thailand. The preliminary classification using morphological characteristics found that gilled fungi were the most common (27.78%). Subsequently, molecular identification by nucleotide sequence analysis of the ITS could classify them into 2 phyla, 2 classes, 8 orders, 14 families, and 17 genera. The phylum Basidiomycota (83.33%) and family Polyporaceae (22.22%) were the most commonly found in this area. There were 6 samples (33.33%), including KN25, KN3, KN17, KN12, KN19, and KN26, for which the genus was reliable (95 to 98.5% similarity), but the species is yet to be confirmed. Most fungi acted as saprotrophs (94.44%) in the ecosystem of the community forest. Additionally, the edible macrofungi

(16.67%) reported in this study include *Auricularia novozealandica* (KN40), *Phallus lutescens* (KN38), and *Termitomyces* sp. (KN20), with no report of any poisonous macrofungi in this area. These results will serve as a database of macrofungi biodiversity, as part of establishing a biodiversity learning center for the local community, which will aid in the utilization of edible macrofungi as a food source and for commercial cultivation.



Fig. 4. Macrofungi found in community forest, Tha Mai District, Chanthaburi Province, Thailand.
A: *Marasmius guyanensis* (KN1) B: *Micropsalliota rubrobrunnescens* (KN3)
C: *Trogia benghalensis* (KN12) D: *Favolus tenuiculus* (KN16)
E: *Collybiopsis polygramma* (KN17) F: *Geastrum saccatum* (KN19)
G: *Termitomyces* sp. (KN20) H: *Clavariaceae* sp. (KN23)
I: *Astrocystis pseudomirabilis* (KN25) J: *Microporus subaffinis* (KN26)
K: *Cyathus subglobisporus* (KN29) L: *Funalia aspera* (KN31)
M: *Xylaria feejeensis* (KN32) N: *Stereum ostrea* (KN35)
O: *Trametes meyenii* (KN37) P: *Phallus lutescens* (KN38)
Q: *Trichoderma viride* (KN39) R: *Auricularia novozealandica* (KN40)

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