



Macrofungi Species in Para Rubber Plantation in Kaeng Hang Maeo District, Chanthaburi Province, Thailand

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Abstract

This study aimed to investigate the macrofungi species in a para rubber plantation in Kaeng Hang Maeo District, Chanthaburi Province, to identify them by a molecular method, and to evaluate their mode of life, and edibility data. Twenty-three macrofungi samples were collected, of which 16 macrofungi with different fruiting body forms were selected for identification. Based on macro-morphological characteristics, they were divided into 5 groups: (i) *Cordyceps*, *Xylaria*, and *Daldinia*, (ii) Earth tongues, (iii) Gilled fungi, (iv) Polypores and bracket fungi, and (v) Jelly fungi. The gilled fungi (37.5%) were mostly found in this area. Subsequently, the internal transcribed spacer (ITS) was amplified by a polymerase chain reaction (PCR), and its sequence was analyzed. The macrofungi were classified into 2 phyla, 4 classes, 5 orders, 9 families, and 15 genera. Most macrofungi were classified in the phylum Basidiomycota (75%) and the family Polyporaceae (31.25%). However, only 1 sample could not be identified at the species level, which was *Gerronema* sp. (KM12). Additionally, most of the macrofungi played a role as saprotrophs in the ecosystem (93.75%), and only 1 sample was found to be a pathotroph (6.25%). Although most macrofungi lacked edibility data (87.5%), edible macrofungi, including *Schizophyllum commune* (KM2) and *Dacryopinax spathularia* (KM3), were identified (12.5%). However, poisonous macrofungi were not reported in this study.

Keywords: Macrofungi, Para rubber plantation, Identification, ITS

1. Introduction

The para rubber (*Hevea brasiliensis*) plantation is a monoculture crop, which is grown worldwide, including in Thailand. The farm process and human activity revealed negative effects resulting in changes to the biodiversity and function of the organisms in this area [1]. Recently, the fungi associated with the rubber tree have been

reported [2]. They provide a checklist of fungi found on the para rubber tree, including 788 species and 179 taxa from 57 countries, including Thailand. These species are classified into 3 phyla. Firstly, phylum Ascomycota consists of 47 orders, 138 families, 439 genera, and 679 species. Secondly, phylum Basidiomycota consists of 21 orders, 40 families, 71 genera, and 97 species. Lastly, phylum Oomycota consists of 1 order, 2 families, 4 genera, and 14 species. This checklist includes the fungi that have previously been reported in the para rubber plantations of Thailand [3-5]. Seephueak et al. [3] reported fungi associated with the leaf litter of para rubber in Nakhon Si Thammarat and Songkhla Province in southern Thailand. They explored 447 species of fungi, including macrofungi such as *Xylaria* sp., and *Marasmius* sp. [3]. Additionally, the fungi on the decaying branch litter of the rubber tree were also explored. In addition, they studied in Nakhon Si Thammarat and Songkhla Province and reported 497 species of fungi. The macrofungi found in this study were *Xylaria* sp., *Dacryopinax spathularia*, *Hexagonia tenuis*, *Lactarius hygrophoides*, *Lentinus conatus*, *Lenzites elegans*, *Marasmius* sp., *Schizophyllum commune*, *Trametes scabosa*, and *Tremella fuciformis* [4]. Surawut et al. [5] have explored the diversity and identified some ascomycetes of the macrofungal species in para rubber plantations in Trat province, in eastern Thailand. They reported ascomycetes macrofungi which included *Daldinia eschscholtzii*, *Cookeina sulcipes*, and *C. tricholoma* [5].

The monoculture plantations of para rubber trees not only led to the loss of ecosystems [6], but also caused a reduction in biodiversity and an increase in the pathogen density within the ecosystem [7]. The plant diseases caused by pathogens affected tree health and loss of agricultural products such as latex from the para rubber tree [8], which results in the loss of income.

Most people in Kaeng Hang Maeo District, Chanthaburi Province, Thailand, are agriculturists of para rubber plantations, but these do not yield sufficient income. Extra income is obtained from supplementary crop cultivation in agricultural areas, by finding forest products, and foraging for mushrooms. Government agencies, in collaboration with local universities, recognize the importance of conserving the ecosystem and the well-being of the local people. Therefore, the study of biodiversity in this area was explored by researchers, including macrofungi diversity. Because the macrofungal diversity has not been studied before, the data from this study will be used as the database for macrofungi diversity in this area. Macrofungal species with edible properties are a nutritious food source for the community and supplement their income.

Macrofungi are classified as the kingdom Fungi. They are eukaryotic cells, heterotrophic organisms, and form fruiting bodies with several structures. Two types of sexual spores, ascospores, and basidiospores, are produced for reproduction, and these are also used for taxonomic classification. Therefore, macrofungi are divided into phylum Ascomycota and Basidiomycota [9]. Additionally, the role of macrofungi in natural habitats based on trophic modes includes saprotroph, symbiotroph, and pathotroph [10]. These roles are important in ecology and human benefits, especially their edible properties.

The factors associated with macrofungal diversity, including geography, climate, and vegetation, have been previously described. For example, one such study has revealed that the most diverse macrofungal species are produced in the rainy season [11]. Additionally, a comparison between native forests and plantation forests shows that the native forests have a greater diversity than their counterpart [12], which has been explained by Lan et al. [11], who suggested that converting native rainforests into agricultural areas, such as rubber plantations, results in the changing of the soil fungal community.

The study of macrofungi diversity has identified unknown samples from the survey sites. The identification of macrofungi is usually based on their morphological characteristics and an analysis of their nucleotide sequences. The internal transcribed spacer (ITS) region has been targeted to amplify by polymerase chain reaction (PCR), DNA sequencing, and phylogenetic tree construction. These molecular biology methods can be used to confirm macrofungal identity.

Therefore, this study aims to investigate the species of macrofungi in the para rubber plantation at Kaeng Hang Maeo District, Chanthaburi Province, Thailand, and to identify them by the molecular method, and to evaluate their mode of life and edibility data.

2. Materials and Methods

2.1 Study site and collection of macrofungi samples

All macrofungi were collected from the Rubber farm at Kaeng Hang Maeo, Chanthaburi Province, Thailand. The weather in the Kaeng Hang Maeo area is hot and humid all year round. The temperatures generally vary from 19°C to 35°C. Twenty-three samples were collected in July 2024, during the rainy season. All the samples were collected, and a photo recording was taken with a Nikon D3400 from Japan at the GPS point where they were found (latitude 12°59'29" N to 12°59'40" N and longitude 101°53'13" E to 101°53'26" E) (Figure 1). They were transferred to sterilized brown plastic bags. The samples were labeled and split into 2 portions. Each sample's initial portion was cut into small pieces (1x1 cm) and kept in absolute ethanol at -20°C to avoid cross-

contamination until a molecular analysis was conducted. The other portion was air-dried at 50°C and stored in brown paper bags for further analysis.

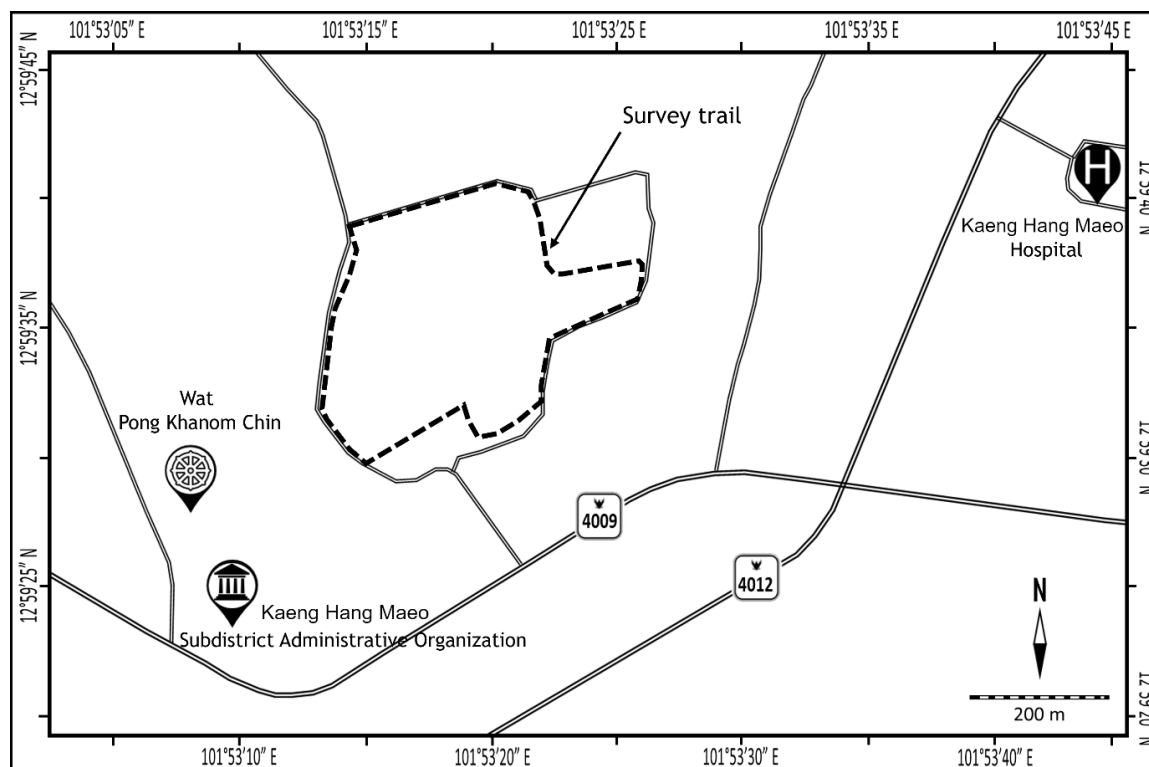


Figure 1 Map of para-rubber plantation farm in Kaeng Hang Maeo Subdistrict, Kaeng Hang Maeo District, in Chanthaburi Province where macrofungi were collected for this study.

2.2 Morphological classification

Macromorphological characteristics, including the coloration, structure of the fruiting body, gills configuration, cap morphology, annulus presence, and photographic documentation, were recorded in the field [13-15]. The macrofungi samples with different morphologies of their fruiting body pattern were selected and evaluated for species-level identification following molecular studies (those samples with a similar morphology were excluded).

2.3 DNA extraction

The extraction of deoxyribonucleic acid (DNA) was conducted utilizing a modified methodology [16]. Briefly, a segment of fresh basidiocarp was placed into a 1.5 ml microcentrifuge tube along with 0.1g of silicon dioxide (Sigma-Aldrich, USA). The lysis buffer was added and ground with a micro pestle. The tube was subsequently incubated at ambient temperature for 15 min. Following this, 150 μ l of potassium acetate (pH 4.8) was added and thoroughly mixed to neutralize the reaction. Thereafter, the tube was centrifuged at 10,000 rpm for 1 min, and the supernatant was transferred into a new tube. An equivalent volume of isopropanol was introduced to the supernatant to facilitate nucleic acid precipitation. The suspension was mixed by tube inversion and centrifuged at 15,000 rpm for 2 min. Subsequently, the supernatant was discarded, and the DNA pellet was rinsed with 300 μ l of 70% ethanol, followed by centrifuging at 10,000 rpm for 1 min. The supernatant was removed, and the DNA pellet was allowed to air dry at room temperature for 30 min. Finally, 50 μ l of molecular-grade distilled water (Apsalagen, Thailand) was added to the DNA pellet to prepare a DNA suspension, which was then stored at -20°C before utilization in a polymerase chain reaction (PCR) amplification.

2.4 ITS region amplification by PCR

The internal transcribed spacer (ITS) was amplified using ITS1/ITS4 primer pairs [17]. The PCR reactions were accomplished in a total volume of 20 μ l, consisting of 10 pg of genomic DNA, 1 $\times$ PCR master mix (Apsalagen, Thailand), 0.5 μ M of each primer, and molecular-grade water (Apsalagen, Thailand). The PCR

amplification was performed in a thermal cycler. The amplification reactions were carried out as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation, annealing, and extension at 95°C for 30 sec, 52°C for 30 sec, and 72°C for 1 min, respectively. Finally, the final extension was carried out at 72°C for 10 min. Electrophoresis was used for PCR product analysis using 2% agarose gel with RedSafe (iNtRONbiotechnology, Korea) at 100 V for 30 min [9].

2.5 ITS sequence analysis and taxonomy data

The PCR products were purified and sequenced (DNA sequencing) at the ATGC company (Pathum Thani, Thailand). The sequences of macrofungi samples that were obtained were analyzed and trimmed using the BioEdit program (version 7.2.5). The pair of sense stand and anti-sense stand sequences were aligned using ClustalW (<https://www.genome.jp/tools-bin/clustalw>) before being analyzed by BLASTn in GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to investigate the percentage similarity of unknown macrofungi with the sequence in the database. Sequences generated in this study were deposited in GenBank, and they are listed in Table 1. The ITS sequences of 23 samples were analyzed using the Neighbor-Joining method to generate an evolutionary tree [18]. 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. The evolutionary distances were computed using the Maximum Composite Likelihood method [19]. The ITS phylogeny involved 18 and 39 nucleotide sequences for the phylum Ascomycota and Basidiomycota, respectively. Evolutionary analyses were conducted in MEGA (version 12) [20]. The taxonomy data and the current name of the macrofungi were searched by the Index Fungorum system (www.indexfungorum.org) and GenBank.

2.6 The ecological role and edibility properties of macrofungi

The ecology role and their edible properties were searched in previously published data and FUNGuild (<https://github.com/UMNFun/FUNGuild>) [10]. The trophic modes consisting of pathotroph (PA), saprotroph (SA), and symbiotroph (SM) that were receiving nutrients by harming host cells, breaking down dead host cells, and exchanging resources with host cells, respectively, were used to define the role of macrofungi in ecology [10].

3. Results and Discussion

Twenty-three macrofungi samples were collected from a para-rubber plantation in Kaeng Hang Maeo District, Chanthaburi Province, Thailand, in July 2024. Sixteen samples with different structures of their fruiting bodies were selected for classification by morphology and molecular identification. The macro-morphology characteristics were used to divide these macrofungi into 5 groups: (i) *Cordyceps*, *Xylaria*, and *Daldinia* (collection number KM4, KM15, and KM16), (ii) Earth tongues (KM7), (iii) Gilled fungi (KM2, KM5, KM11, KM12, KM13, and KM23), (iv) Polypores and bracket fungi (KM6, KM10, KM14, KM18, and KM20), and (v) Jelly fungi (KM3). These results indicated that the gilled fungi (6 samples, 37.5%) were mostly found in a para-rubber plantation. Subsequently, polypores and bracket fungi group were found in 5 samples (31.25%), *Cordyceps*, *Xylaria*, and *Daldinia* group were found in 3 samples (18.75%), respectively. Each group of earth tongues and jelly fungi was found in only 1 sample (6.25%).

These 16 macrofungi with several fruiting bodies were identified by ITS sequence analysis. Firstly, the ITS region was amplified by the PCR method, and the PCR products were analyzed by gel electrophoresis. The size of the PCR products ranged between 650-1000 base pairs (bp), and DNA sequencing was performed. Secondly, the ITS sequences of macrofungi were subjected to pairwise analysis by BLASTn in the GenBank database. The percentage similarity with closely related reference sequences is presented in Table 1. Thirdly, the phylogenetic trees were constructed based on nucleotide sequences of the ITS region, as shown in Figures 2-3. The evolution and relationship of macrofungi samples were explored separately for samples that were classified in phyla Ascomycota and Basidiomycota, as shown in Figures 2 and 3, respectively. The dominant orders for Ascomycota and Basidiomycota were Xylariales and Agaricales, respectively. However, Agaricales was the dominant order in this study. Additionally, the families in Agaricales showed evolution and relationship characteristics of the family. Marasmiaceae, Omphalotaceae, Schizophyllaceae, and Porothleaceae were closely related to each other and present in the same cluster. Moreover, at the family level, Polyporaceae showed the most diverse taxa (Figure 3).

The macrofungi samples from the para-rubber plantation in Kaeng Hang Maeo District, Chanthaburi Province, Thailand could be classified into 2 phyla, 4 classes, 5 orders, 9 families, and 15 genera. Four samples were classified in the phylum Ascomycota (25%), while the remaining samples were in the phylum Basidiomycota (12 samples, 75%). The family Polyporaceae showed the most species diversity (5 samples, 31.25%); Omphalotaceae, Malasmiaceae, and Xylariaceae were found in 2 samples per family (12.5%), meanwhile, Hypoxylaceae, Leotiaceae, Porothleaceae, Schizophyllaceae, and Dacrymycetaceae were found only in 1 sample per family (6.25%) (Table 1).

The high representation of macrofungi classified in the phylum Basidiomycota was also found in previously published data from studies conducted in agricultural areas, including oil palm (*Elaeis guineensis* Jacq.) plantations and para rubber plantations. The macrofungi in oil palm plantations belong to Basidiomycota (90.99%) and Ascomycota (9.01%) [21]. Meanwhile, the macrofungi in the para rubber plantation are also classified in the phylum Basidiomycota more than in Ascomycota [22, 23]. These studies suggest that the high representation of Basidiomycota may depend on environmental factors such as rainfall, humidity, and temperature, especially during the rainy season, as moisture is one of the most important factors contributing to the formation of fruiting bodies in macrofungi. Therefore, macrofungi are frequently observed when they grow in optimal conditions.

The family Polyporaceae (31.25%) was the most frequently found in this study. The important characteristic of macrofungi in this family was their texture. It was tough and hard like cork or wood, causing it to be inedible for animals and humans. Due to these characteristics, they had a long life in all seasons and were frequently observed [24, 25]. However, the studies of macrofungi diversity in the para rubber plantation in Nigeria revealed that the family Tricholomataceae was the most represented taxon. Based on their macromorphology, agaric fungi or gilled fungi (52%) were the most defined [22], which correlated with the results in this study. The gilled fungi (37.5%) were mostly found in the para rubber plantation in Kaeng Hang Maeo District, Chanthaburi Province, Thailand.

Therefore, the morphology of a fungus is a result of adaptation to its habitat and environmental conditions; for example, fungi growing on decaying wood develop sturdy structures to firmly attach to the wood substratum, jelly fungi have gelatinous tissue to retain moisture during dry periods, and gilled or highly-poroid mushrooms increase spore-dispersal efficiency in humid conditions, all of which reflect the ecological adaptation strategies of macrofungi [26].

However, 1 macrofungal sample (6.25%) could not be identified at the species level, which was *Gerronema* sp. (KM12) (Table 1). The morphological study and the nucleotide sequence analysis were combined to determine the macrofungal species. Although the conserved region, such as ITS was usually selected for fungal identification by molecular biology methods, some fungal species could not be identified by only the ITS region. Therefore, some other genes were used to identify fungal species [17]. Previously published data reported *Gerronema* with 3 new taxa and 1 new record from China by using the multi-locus dataset that consists of ITS and a nuclear large subunit rDNA (LSU) [27]. The combined genes, ITS and LSU, for sequence analysis may be required for species identification of *Gerronema* sp. (KM12) as previously described [27].

Among macrofungi samples in this study, *Daldinia eschscholtzii*, *Xylaria tericola*, *X. feejeensis*, *Schizophyllum commune*, *Earliella scabrosa*, *Dacryopinax spathularia* [2, 4, 22, 23], and *Marasmius haematocephalus* [28] have been previously reported in para rubber plantations. Therefore, these species were expected to be found in this study region. In contrast, *Microglossum capitatum*, *Tetrapyrgos nigripes*, *Collybiopsis menehune*, *Gymnopus hirtellus*, *Gerronema* sp., *Funalia aspera*, *Trametes sanguinea*, *Neofomitella quangxiensis*, and *Hexagonia glabra* have not yet been reported in this plantation area. Although *T. nigripes* has not been found in a para rubber plantation before, it has been reported in an oil palm plantation in Southern Thailand [21].

The role of macrofungi in ecology was studied in a para rubber plantation area. Most of the macrofungi found in this study play a major role as saprotrophs (15 samples, 93.75%). Rubber is a deciduous tree, and the nutrient cycling pathway in the rubber plantation ecosystem was contributed to by plant litter [29]. The high proportion of saprotrophic fungi found in the rubber plantation can be explained by several ecological factors, such as the composition of litter components and environmental factors such as humidity and temperature, which may affect wood decomposition and the level of fungal diversity [30]. The rubber plantation contains a large amount of organic matter, which serves as a food source for saprotrophic fungi. In addition, the high humidity and shaded environment within the plantation promote decomposition processes, allowing these fungi to grow well. Moreover, it has been reported that the soil in rubber plantations tends to be acidic and rich in carbon, which are conditions that favor decomposing fungi. These combined factors contribute to the high abundance of saprotrophic fungi observed in this ecosystem. The important role of saprophytic macrofungi in the para rubber plantation has been described in this study. The macrofungi associated with leaf litter and decaying branch litter of the para rubber tree have also been reported [3, 4].

In this study, the pathotroph was found in only 1 sample (6.25%), *Earliella scabrosa* (KM18), which has previously been reported as a plant pathogen [31] and associated with trunk rot of the para rubber tree [32]. The *E. scabrosa* (KM18) may have spread and developed as a pathogen of the rubber tree in this study site. Moreover, the presence of *E. scabrosa* (KM18) as a pathotroph may be caused by a reduction of biodiversity that results in an increase of pathogen density within the rubber plantation ecosystem [7]. However, macrofungi with the role of symbiotroph were not found in this study (Table 1).

The edibility of macrofungi has been described in this study. Two macrofungi species (12.5%), *Schizophyllum commune* (KM2) [33] and *Dacryopinax spathularia* (KM3) [34] have previously been reported as edible macrofungi. However, the poisonous macrofungal species was not found in this study. Furthermore, it was not possible to identify the remaining macrofungal species because of the lack of data on edibility (14 samples, 87.5%) (Table 1).

Table 1 Taxonomy data, BLASTn results, modes of life, and edibility of macrofungi collected from para rubber plantation at Kaeng Hang Maeo 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	Similarity (%)			
Ascomycota	Sordariomycetes	Xylariales	Hypoxylaceae	<i>Daldinia eschscholtzii</i> (KM4)	<i>Daldinia eschscholtzii</i> (KC895542)	99.45%	PQ506024	SA	
Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	<i>Xylaria terricola</i> (KM15)	<i>Xylaria terricola</i> (MW659104)	99.52%	PQ507591	SA	
Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	<i>Xylaria feejeensis</i> (KM16)	<i>Xylaria feejeensis</i> (JQ862701)	100%	PQ507592	SA	
Ascomycota	Leotiomycetes	Leotiales	Leotiaceae	<i>Microglossum capitatum</i> (KM7)	<i>Microglossum capitatum</i> (PP982473)	99.53%	PQ507471	SA	
Basidiomycota	Agaricomycetes	Agaricales	Malasmiaceae	<i>Marasmius haematocephalus</i> (KM5)	<i>Marasmius haematocephalus</i> (MK532785)	100%	PQ506029	SA	
Basidiomycota	Agaricomycetes	Agaricales	Malasmiaceae	<i>Tetrapyrgos nigripes</i> (KM23)	<i>Tetrapyrgos nigripes</i> (MK532785)	98.06%	PQ507768	SA	
Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	<i>Collybiopsis menhune</i> (KM13)	<i>Collybiopsis menhune</i> (OR751618)	100%	PQ507589	SA	
Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	<i>Gymnopus hirtellus</i> (KM11)	<i>Gymnopus hirtellus</i> (QQ597075)	98.41%	PQ507483	SA	
Basidiomycota	Agaricomycetes	Agaricales	Porothelaceae	<i>Gerronema</i> sp. (KM12)	<i>Gerronema</i> sp. (KR002880)	100%	PQ507588	SA	
Basidiomycota	Agaricomycetes	Agaricales	Schizophyllaceae	<i>Schizophyllum commune</i> (KM2)	<i>Schizophyllum commune</i> (MG437405)	100%	PQ506022	SA	E
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	<i>Funalia aspera</i> (KM6)	<i>Funalia aspera</i> (PP622251)	99.02%	PQ507460	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	<i>Trametes sanguinea</i> (KM10)	<i>Trametes sanguinea</i> (MK269258)	99.80%	PQ507482	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	<i>Neofomitella guangxiensis</i> (KM14)	<i>Neofomitella guangxiensis</i> (OR885685)	100%	PQ507590	SA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	<i>Earliella scabrosa</i> (KM18)	<i>Earliella scabrosa</i> (PP582912)	99.81%	PQ507713	PA	
Basidiomycota	Agaricomycetes	Polyporeales	Polyporaceae	<i>Hexagonia glabra</i> (KM20)	<i>Hexagonia glabra</i> (OK642181)	100%	PQ507767	SA	
Basidiomycota	Dacrymycetes	Dacrymycetales	Dacrymycetaceae	<i>Dacryopinax spathularia</i> (KM3)	<i>Dacryopinax spathularia</i> (MW577304)	99.73%	PQ506021	SA	E

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

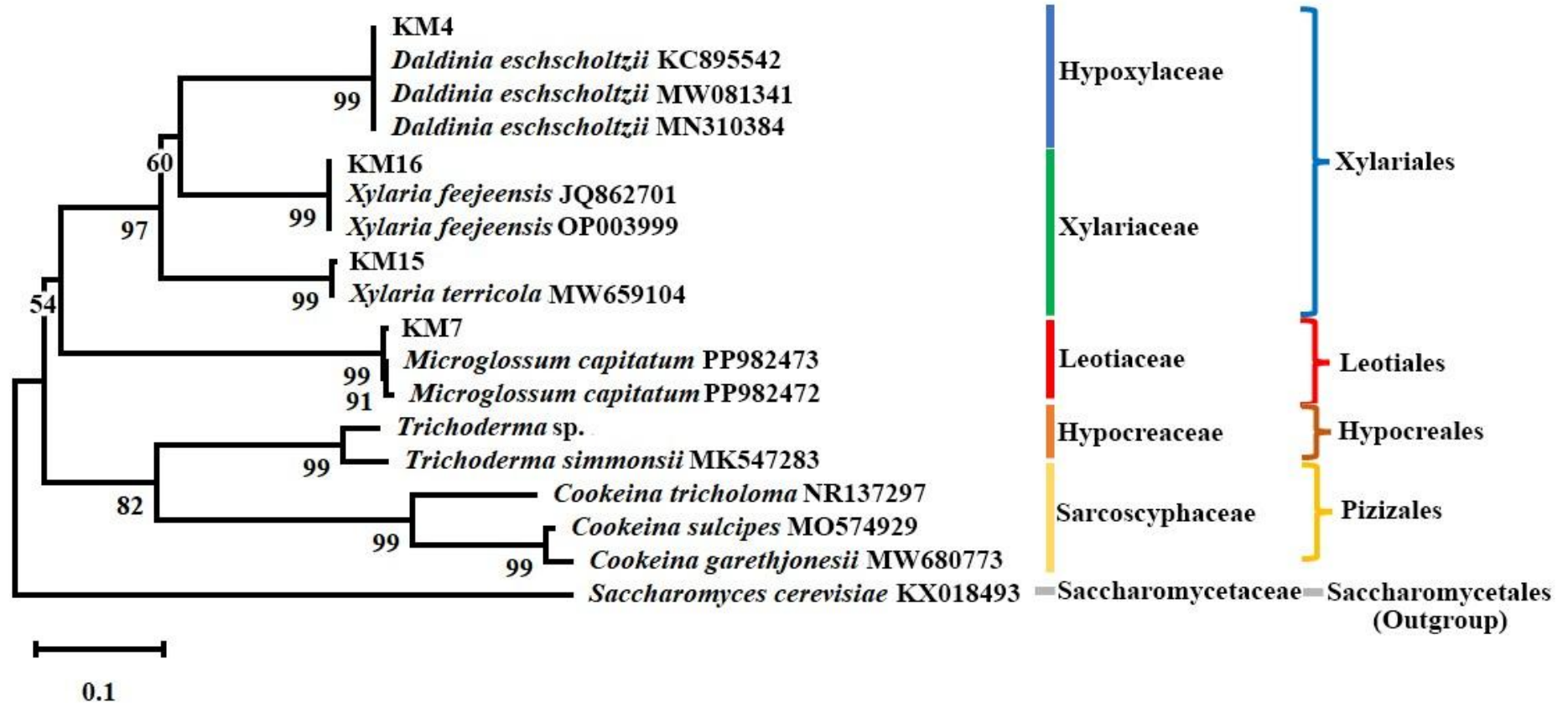


Figure 2 Phylogenetic tree based on ITS sequences of macrofungi in this study that are classified in the phylum Ascomycota. The tree was constructed with sequences of the ITS region, with 463 nucleotides aligned using the maximum likelihood (ML) method and the K2 + G + I substitution model. The bootstrap analysis was performed with 10,000 replicates. The scale represents the number of substitutions per site. Evolutionary analyses were conducted in MEGA12. Bootstrap values lower than 50 are not shown.

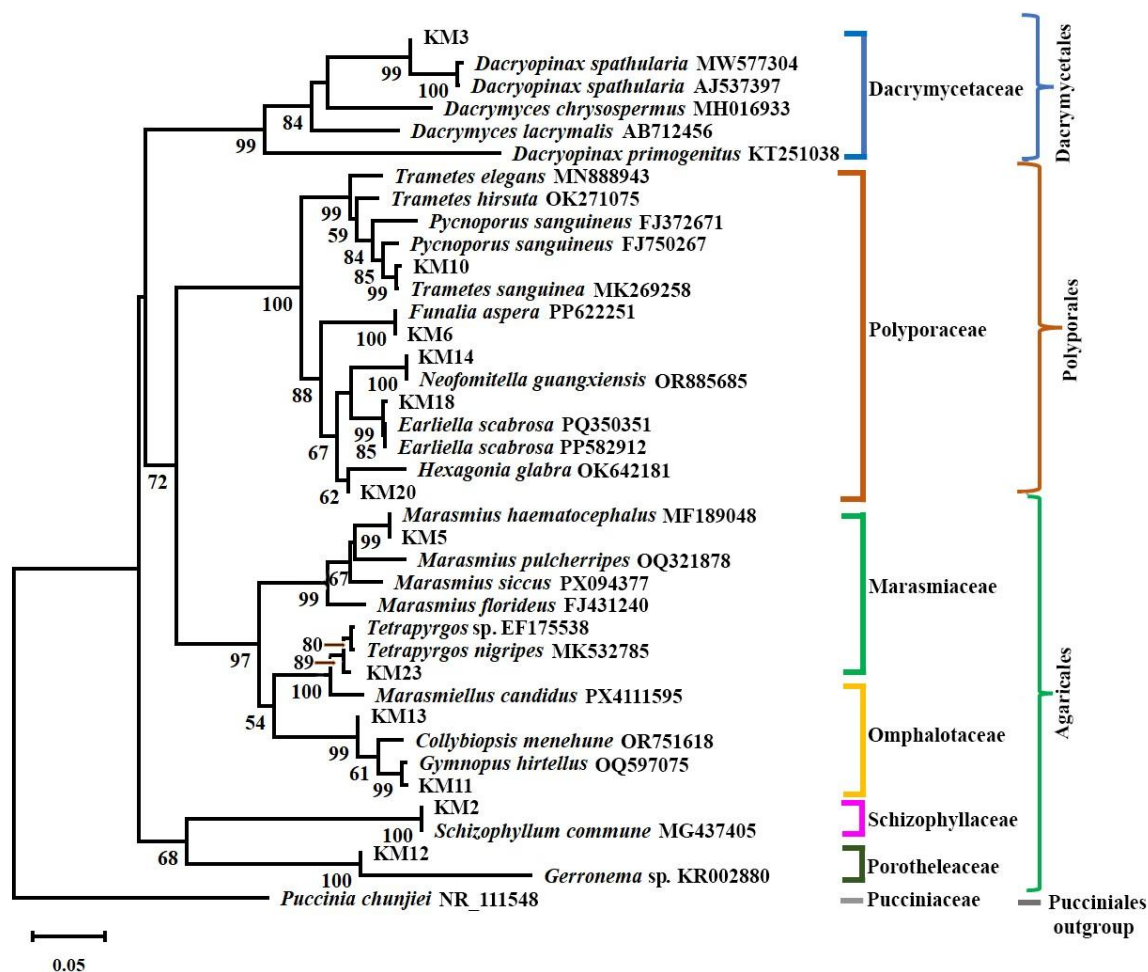


Figure 3 Phylogenetic tree based on ITS sequences of macrofungi in this study that are classified in the phylum Basidiomycota. The tree was constructed with sequences of the ITS region, with 358 nucleotides aligned using the maximum likelihood (ML) method and the T92+G+I substitution model. The bootstrap analysis was performed with 10,000 replicates. The scale represents the number of substitutions per site. Evolutionary analyses were conducted in MEGA12. Bootstrap values lower than 50 are not shown.



Figure 4 Photographs of macrofungi found in the para rubber plantation at Kaeng Hang Maeo District, Chanthaburi Province.

A: *Schizophyllum commune* (KM2)

C: *Daldinia eschscholtzii* (KM4)

E: *Funalia aspera* (KM6)

G: *Trametes sanguinea* (KM10)

I: *Gerronema* sp. (KM12)

K: *Neofomitella guangxiensis* (KM14)

M: *Xylaria feejeensis* (KM16)

O: *Hexagonia glabra* (KM20)

B: *Dacryopinax spathularia* (KM3)

D: *Marasmius haematocephalus* (KM5)

F: *Microglossum capitatum* (KM7)

H: *Gymnopus hirtellus* (KM11)

J: *Collybiopsis menhune* (KM13)

L: *Xylaria terricola* (KM15)

N: *Earliella scabrosa* (KM18)

P: *Tetrapyrgos nigripes* (KM23)

Although only 2 species of the edible macrofungi were reported, they are valuable to the community, especially *S. commune*. This species is usually found in tropical and subtropical regions and is associated with the para rubber tree [2]. This fungus has been reported to contain many bioactive compounds and nutritional values that benefit people, such as anti-cancer, anti-oxidant, and anti-microbial properties [33]. It is collected from nature as a source of food in community forests and agricultural areas, including para rubber plantations. Moreover, *S. commune* was studied for commercial cultivation worldwide [33]. The finding of *S. commune* in a para rubber plantation is evidence that the physical and biological factors in this agricultural area are suitable for its cultivation. Therefore, the development of *S. commune* cultivation in the para rubber plantation area may be an additional source of food and extra income for the community in Kaeng Hang Maeo, Chanthaburi Province.

Several studies of macrofungi diversity in agricultural areas, including para rubber plantations, have previously been reported. In 2010, Osemwegie et al. [22] revealed the diversity of macrofungi in rubber plantations and in a forest in Nigeria. A total of 435 macrofungi samples were collected. Macrofungal species were identified and classified into 4 classes, 9 orders, and 28 families. The family Tricholomataceae is the most representative taxon. Based on their macromorphology, agaric fungi or gilled fungi (52%) and polypore fungi (31%) were the most defined in this study [22]. In 2011, Osemwegie and Okhuoya [23] revealed macrofungal diversity in rubber plantations in Nigeria. A total of 333 fruiting bodies were collected and identified. They were classified in the phylum Basidiomycota and family Tricholomataceae as the most representative taxa, while they also play a role as saprotrophs, making up 66% of the total samples [23].

In Thailand, the diversity of macrofungi in oil palm plantations has been explored in southern Thailand, including Nakhon Si Thammarat Province and Trang Province. One hundred and eleven macrofungi taxa were identified and could be classified into the phyla Ascomycota (10 taxa) and Basidiomycota (101 taxa). The most diverse species were *Marasmius* (15 species) [21]. Surawut et al. [5] have explored some ascomycetes macrofungal species in para rubber plantations in Trat Province, eastern Thailand. They analyzed sequences of both the ITS (internal transcribed spacer) and LSU (nuclear large subunit ribosomal DNA) regions to identify macrofungi samples. The ascomycetes macrofungi have been reported to consist of *Daldinia eschscholtzii*, *Cookeina sulcipes*, *C. garethjonesii*, and *C. tricholoma*. Two unidentified species, *Trichoderma* sp. (RP1) and *Xylaria* sp. (RP6), have been reported [5]. In addition, other ascomycete fungi in para rubber plantations in Thailand have been reported. Macrofungal species as well as microfungi have been previously described. Senwanna et al. [32] reported that Dothideomycetes are dominant among ascomycete fungi in para rubber plantations. Most taxa found in this study have been recorded in Thailand [32]. Recently, the fungal species of Dothideomycetes (Ascomycetes fungi) was reported as a new species of para rubber in China [35].

The factors related to climate, geography, altitudinal range, and vegetation that influence macrofungal diversity and fungal community have been described in several studies. The study of macrofungi diversity in western Thailand (Kanchanaburi province) revealed that the rainy season results in greater macrofungi diversity than in the dry season [36]. Yusran et al. [37] explored macrofungi diversity across 3 altitudinal ranges in Indonesia. They found that elevations of <500, 500-1500, and >1500 m above sea level (asl) revealed 77, 117, and 142 species, respectively. They showed that the altitudinal range of macrofungi diversity [37]. Tie et al. [38] revealed macrofungi diversity in 4 different vegetation types in China. The vegetation community types I, III, II, and IV found in 404, 231, 158, and 39 macrofungi species, respectively. The community type I has *Quercus aliena* as a dominant plant, which provides a suitable environment for macrofungi growth, such as high relative humidity. They suggested that the type of vegetation community influences the diversity of macrofungal species [38].

Li et al. [12] studied macrofungi diversity in the Greater Mekong Subregion. They found greater macrofungi diversity in native forests than in comparable plantation forests [12]. Lan et al. [11] suggested that converting native rainforests into agricultural areas, such as rubber plantations, results in changes in the fungal community. They revealed that the most abundant soil fungi in the rubber plantation were the phylum Ascomycota [11]. These studies indicated that the monoculture of para rubber trees and human activities revealed negative effects resulting in the diversity and function of the fungi community being changed, which affects nutrient cycling and other organism interactions [1]. Interestingly, the fungi community changes in para rubber plantations may result in the occurrence of a novel fungal species, and this has attracted the interest of researchers [35]. In contrast, the presence of macrofungi as a pathogen of the rubber tree may be caused by a reduction of biodiversity that results in an increase of pathogen density within the rubber plantation ecosystem [7].

The study of fungal diversity in rubber plantation areas using molecular techniques provides important insights and implications for biodiversity conservation in agroecosystems. Notably, changes in land-use from natural forest to rubber plantations significantly alter fungal community structure, particularly reducing the abundance of plant-associated and mycorrhizal fungi, while increasing saprotrophic fungi and soil- or root-dwelling species [39]. Furthermore, understanding the fungal community composition and microbial network structure highlights that sustainable management of rubber plantations must consider below-ground microorganisms, not just above-ground plants [40]. In terms of biodiversity conservation, the findings demonstrate that even monoculture rubber plantations can play a vital role in maintaining fungal diversity, which is essential for ecosystem functions such as the decomposition of organic matter. This study also indicates that conventional management practices that disregard microbial communities, such as heavy chemical use or soil washing, may compromise ecosystem

functions, including nutrient cycling and system resilience. Therefore, conserving biodiversity in agroecosystems like rubber plantations should integrate management strategies that support below-ground fungi, including preserving litter layers, maintaining fungal food plants, and adopting agricultural practices that promote symbiotic and decomposing fungi. This perspective emphasizes that biodiversity encompasses not only above-ground flora and fauna, but also the microbiome, which plays a critical role in the sustainability of agroecosystem functions.

Therefore, these research findings on macrofungal diversity in para rubber plantation areas using molecular techniques provide important insights and implications that can guide sustainable land-use management and the utilization of biological resources in Chanthaburi Province and similar tropical regions. Key recommendations include promoting agroforestry systems or intercropping with rubber trees, such as planting understory crops or cover crops. These practices increase plant diversity and provide additional resources for soil fungi, thereby enhancing the complexity of soil fungal communities and improving the overall health of the soil ecosystem [41]. Additionally, maintaining the litter layer and minimizing soil disturbance, such as leaving leaf litter, twigs, and bark on the plantation floor, is crucial, as organic matter serves as a food source for decomposing fungi and supports soil fertility. Reducing the use of heavy agrochemicals and limiting soil erosion further helps preserve below-ground microbial communities [39]. Monitoring and assessing soil fungal communities should be integrated into rubber plantation management using molecular techniques (e.g., ITS sequencing) to track how different management practices affect fungal communities. This information can then inform locally adapted land-use strategies in Chanthaburi Province and other similar tropical regions. Furthermore, raising awareness among rubber growers about the role of soil microorganisms is essential. Training sessions or workshops can help farmers understand that soil fungal diversity is a vital component of overall biodiversity and plays a key role in maintaining soil quality and the long-term sustainability of rubber plantations.

4. Conclusion

The macrofungi species in the para rubber plantation have been explored in this study. The gilled fungi were the species most commonly found in this area on the basis of macro-morphological characteristics. Sixteen macrofungi samples with different fruiting body structures were selected for molecular identification by ITS sequence analysis. They were classified into 2 phyla, 4 classes, 5 orders, 9 families, and 15 genera. Most of the macrofungi samples were classified in the phylum Basidiomycota and the family Polyporaceae. However, there was 1 sample that could not be identified at the species level. Additionally, most of the samples played a major role as saprotrophs, with only 1 pathotroph, *Earliella scabrosa*, that was reported to cause disease in the rubber tree. Edible macrofungi, including *Schizophyllum commune* and *Dacryopinax spathularia* were reported in this study, especially *S. commune*, which has been reported to be associated with the para rubber tree and to contain many beneficial bioactive compounds. Moreover, *S. commune* has previously been studied for commercial cultivation. The finding of *S. commune* in a para rubber plantation is evidence that the physical and biological factors in this agricultural area are suitable for the cultivation of this fungus. Therefore, the addition of *S. commune* cultivation in the para rubber plantation area may be another source of food and extra income for the community in Kaeng Hang Maeo, Chanthaburi Province. The data from this study will be used as a database for macrofungi in this area, including the mode of life in ecology, and edible properties. These will provide further studies of edible macrofungi, such as mushroom cultivation, and medical uses for health. Additionally, this information can then be used to develop locally adapted land-use strategies in Chanthaburi Province and other similar tropical regions. Furthermore, raising awareness among rubber growers about the role of soil microorganisms is essential as they play a key role in maintaining soil quality and the long-term sustainability of rubber plantations.

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6. Author contributions

SS, CK, SN, NA, CS, PS, and WN designed experiments; CK and SN designed the survey trail; SS, PK, CK, WP, OK, NA, and PS performed experiments; SS, PK, and CK prepared the manuscript. All authors discussed the results and commented on the manuscript.

7. Conflict of interests

The authors declare that there were no conflicts of interest.

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