



Diversity of macrofungi in Para rubber plantation of Wang Takien Subdistrict, Khao Saming District, Trat Province, Thailand

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Abstract

This research aimed to survey macrofungi in para rubber plantations, Wang Takien Subdistrict, Khao Saming District, Trat Province, to identify them using molecular biology method, and to investigate their role in the ecosystem with their edibility. Thirty-four macrofungi samples were collected in June 2024. Among these samples, the macrofungi could be classified into 7 groups based on morphological characteristics. The gilled fungi group is the most abundant (39.29%). Subsequently, twenty-eight samples with different fruiting body structures were selected to identify by molecular techniques. Polymerase chain reaction (PCR) was used to amplify the internal transcribed spacer (ITS), and nucleotide sequence analysis was performed. They could be classified into 2 phyla, 4 classes, 10 orders, 17 families, and 22 genera. Most of the macrofungi were classified into phylum Basidiomycota (85.71%) and family Polyporaceae (25%). Three samples (10.71%) could be classified only at the genus level. In addition, most macrofungi were saprotrophs (89.28%). Seven species of macrofungi (25%) were reported to be edible, namely *Auricularia cornea* (WK19), *Dacryopinax spathularia* (WK18), *Phallus lutescens* (WK20), *Sanguinoderma rugosum* (WK4), *Schizophyllum commune* (WK15), *Termitomyces cylindricus* (WK12), and *Trichaleurina javanica* (WK24). Only one poisonous macrofungus (3.57%), *Scleroderma xanthochroum* (WK17), has been reported. Most of the macrofungi in this study (71.43%) did not have any information on their edibility. The list of macrofungi species will be used to create the database of macrofungi diversity in this area.

Keywords – Diversity – ITS – Macrofungi – Para rubber plantation – Thailand

Introduction

The para rubber tree (*Hevea brasiliensis*) is an economically important plant that has led people to convert native forests into agricultural plantations, including in Wang Takien Subdistrict, Khao Saming District, Trat Province, Thailand. In this area, local farmers tend to convert their

native forests into para rubber plantations. The monocultural crops, such as para rubber plantations, have resulted in altered biodiversity in this area. Previous studies suggested that native forests showed higher diversity than plantation forests (Li et al. 2018). The changing in soil fungal community may be one explanation for this situation (Lan et al. 2020) and may affect macrofungi diversity. Macrofungi are present in several patterns of fruiting bodies that the naked eye can observe. The members of the kingdom Fungi, including macrofungi, are eukaryotes, heterotrophs, and produce spores for reproduction (Chandrasrikul et al. 2008). Macrofungi can be divided into 2 phyla consisting of Ascomycota and Basidiomycota based on their sexual spore, ascospore, and basidiospore, respectively (Chandrasrikul et al. 2008). They play an important role as decomposers (saprotroph), but some can cause disease in other organisms (pathotroph), and some can exchange resources with other organisms (symbiotroph) (Nguyen et al. 2016). Additionally, some species have been reported to be edible as a food resource and some could be cultivated for economic such as bag cultivation.

The agriculturists of a para rubber plantation at Wang Takien Subdistrict, Khao Saming District, Trat Province, Thailand, are seeking Forest Stewardship Council (FSC) certification, however, one of the requirements is to prepare a biodiversity database including macrofungal diversity in their para rubber plantation. Certification of FSC results in selling latex at higher prices.

The survey of macrofungi diversity has to collect and identify their species. Identification of macrofungi is usually based on their morphological structure and nucleotide sequence analysis. The molecular biology approach plays an important role in accurate identification at the species level (Cho et al. 2015, Zhou et al. 2016, Na et al. 2022).

The conserved region such as the internal transcribed spacer (ITS) region is usually selected for fungal identification (Raja et al. 2017, White et al. 1990). While considerable variation in ITS region is observed between species, they are highly conserved within species, and the large number of rRNA loci allows easy amplification of even small amounts of template DNA, which is ideal for species-level differentiation of fungi (White et al. 1990).

Therefore, this study aims to survey macrofungi in para rubber plantations at Wang Takien Subdistrict, Khao Saming District, Trat Province, to identify them using molecular biology method, and to investigate their role in the ecosystem with their edibility data. The list of macrofungi species will be used to create a database of macrofungi diversity in this area.

Materials & Methods

Macrofungi sampling and study area

A survey of macrofungi was conducted in the para rubber plantation at Wang Takien Subdistrict, Khao Saming District, Trat Province, Thailand (Fig. 1) (20,510 acres, Latitude 12°24'17" N to 12°30'57" N and longitude 102°25'58" E to 102°33'45" E). The specimens were collected in June 2024 by survey along the rubber plantation rows. When macrofungi were found, photos were taken with a camera (Nikon D3400, Japan) to document their habitats and the characteristics of the fruiting bodies. Then, specimens were collected in a plastic box, transported to the laboratory, and the sample box was labeled collection number and was added a paper scale for size comparison. A small amount of tissue was collected and put into a 1.5 ml microcentrifuge tube for DNA extraction. If possible, the inner tissue was collected, 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, and absolute ethanol was added until the tissue was completely covered. It was stored at -20°C to preserve the tissue. The remaining samples were dried at 60°C to preserve the fruiting body samples.

Study of morphological characteristics

The morphological characteristics of macrofungi were studied as previously described and classified by their morphology (Chandrasrikul et al. 2011, 2008, Desjardin et al. 2004), such as the

cap, gill, stalk, ring, volva, scales, and spore as well as their habitat where the macrofungi grew. The preliminary morphological data were compared with the several books or checklist on the diversity of macrofungi in Thailand (Chandrasrikul et al. 2011, 2008, Desjardin et al. 2004). The macrofungi samples presented in different fruiting body patterns were selected to identify by molecular method (a similarity morphology was excluded).

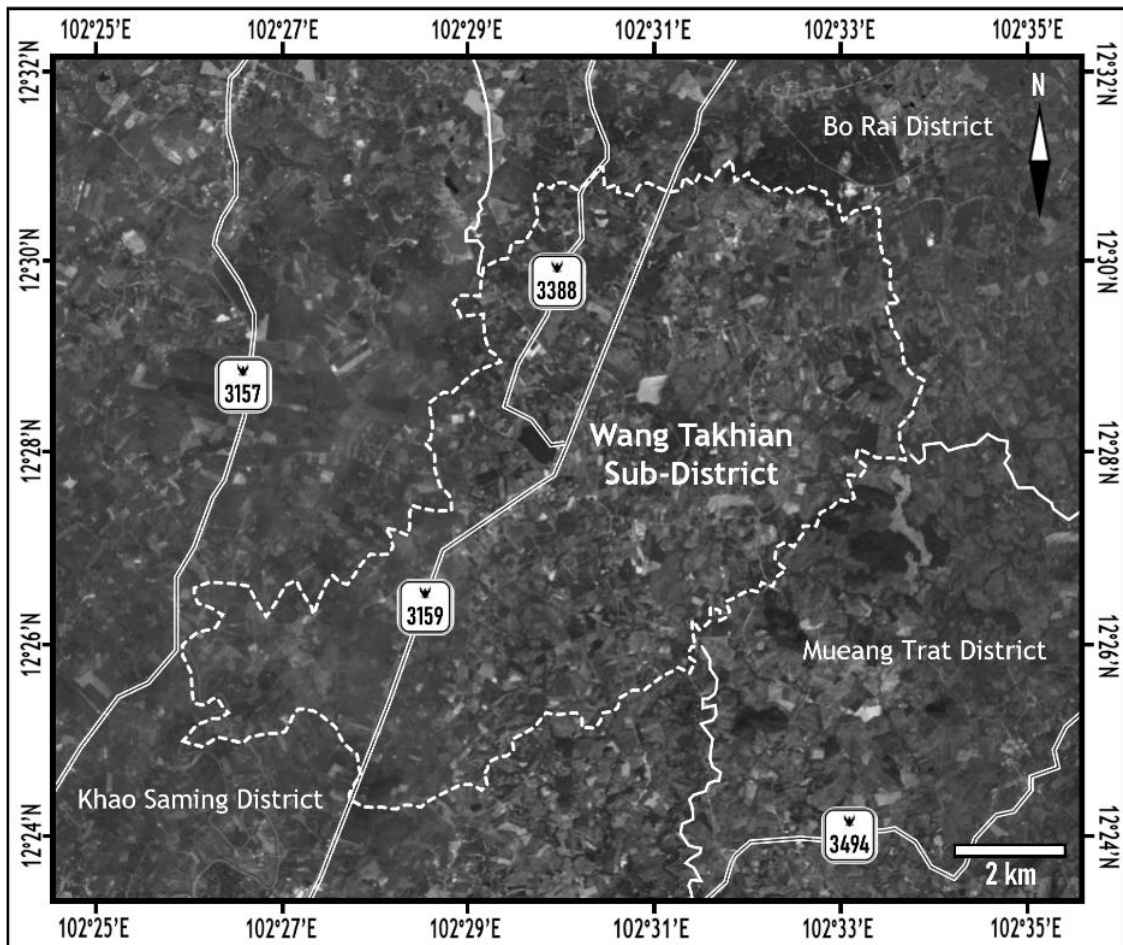


Fig. 1 – Map of para rubber plantation area in Wang Takhien Subdistrict, Khao Saming District, Trat Province, Thailand where macrofungi sampling in this study.

DNA extraction and PCR amplification

Placed 0.1 g of silicon dioxide (Sigma-aldrich, USA) in a 1.5 ml microcentrifuge tube. Added macrofungi tissue of equal size to the amount of silicon dioxide (approximately 0.05-0.1 g). Added 50 μ l of lysis buffer and ground with a micropestle for 2-3 minutes or until the tissue was fine. Continue doing this until the added lysis buffer reaches a volume of 500 μ l. Subsequently, stand for this tube at room temperature for 10-15 min. Added 150 μ l of potassium acetate solution (pH 4.8) and mix briefly with a vortex mixer. Centrifuge at 10,000 rpm for 1 min. Collected the supernatant and transfer it to a new tube. Then, centrifuge the tube again at 10,000 rpm for 1 min. Transferred the supernatant to a new tube (record the volume in μ l) and added an equal volume of isopropyl alcohol. Inverting the tube briefly and centrifuging it at 15,000 rpm for 2 minutes, then discarding the supernatant. Washed the DNA pellet by adding 300 μ l of 70% ethanol, centrifuging at 10,000 rpm for 1 minute, then discard the supernatant gently. Dry the DNA pellet for 30 min or until dry, and dissolve the DNA pellet with 50 μ l of distilled water (Apsalagen, Thailand). Stored the DNA at -70°C until used in the PCR reaction (Liu et al. 2000).

The PCR mixture was prepared by adding a final concentration of 10 pg-1 μ g DNA template, 0.5 μ M primer ITS1, and primer ITS4, 1X PCR mastermix (Apsalagen, Thailand), and adjusting

the volume with distilled water to 20 µl (Raja et al. 2017, Surawut et al. 2023). The ITS amplification was performed in the thermal cycler under the following conditions: initial denaturation at 95°C for 3 min; 35 cycles of denaturation at 95°C for 30 sec, annealing at 52°C for 30 sec, extension at 72°C for 1 min; and final extension at 72 °C for 10 min (Surawut et al. 2023).

The PCR product was examined by gel electrophoresis method using 2% agarose gel in TAE buffer with nucleic acid staining dye or RedSafe (iNtRON, Korea). The 5 µl of PCR product mix with loading dye (Biotechrabbit, Germany) was loaded into the well of gel on an electrophoresis machine and run at 100 V for 30 min. To observe and identify the size of the DNA band of the PCR product, the gel was exposed to a UV-transilluminator by comparing it with the standard DNA (Biotechrabbit, Germany) (Surawut et al. 2023).

Sequence analysis of ITS region

The PCR products were sent to ATGC Co., Ltd. (Pathum Thani, Thailand) to purify the PCR product and analyze the nucleotide sequence by DNA sequencing. When the analysis results are received, the incorrect nucleotide sequences were edited using the BioEdit program. 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>) to determine the %similarity or %identity of the fungal species. The ITS nucleotide sequences from the macrofungi samples were analyzed by the Neighbor-Joining method to construct a phylogenetic tree (Felsenstein 1985, Saitou & Nei 1987, Tamura et al. 2004) using the MEGA X program (Kumar et al. 2018).

Determination of taxonomy data

The taxonomy 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/>).

Determination of ecological role and edibility status

Macrofungi samples were classified according to their roles in the ecosystem and human benefits. They were searched from previously published data and with the FUNGuild program (<https://github.com/UMNFuN/FUNGuild>) (Nguyen et al. 2016). The roles of macrofungi in the ecosystem were divided into 3 groups: saprotroph (SA), pathotroph (PA), and symbiotroph (SM). Additionally, the role of macrofungi in human benefits consists of 3 groups that were reported to be edible (Edible macrofungi: E), to be poisonous (Poisonous macrofungi: P), and that were not reported the edibility properties (Unknown data).

Results

Para rubber plantation area located at Wang Takien Subdistrict, Khao Saming District, Trat Province, Thailand was a survey site for macrofungi sampling. Thirty-four macrofungi specimens were collected and studied for differentiation of their fruiting body by morphological characteristics. Twenty-eight samples with the presence of different fruiting bodies were classified into 7 groups: (i) Ascomycetes fungi (collection number WK1, WK2, WK3, and WK24), (ii) Gilled fungi (WK12, WK13, WK14, WK15, WK22, WK25, WK26, WK27, WK28, WK29, and WK30), (iii) Jelly fungi (WK18 and WK19), (iv) Puffballs fungi (WK17), (v) Stinkhorns fungi (WK20), (vi) Polypores and bracket fungi (WK4, WK5, WK6, WK7, WK8, WK9, WK10, and WK11), and (vii) Leather-bracket fungi (WK16). Gilled fungi were the most diverse fruiting bodies with 11 samples (39.29%). The polypore and bracket fungi, the ascomycetes fungi, and the jelly fungi consisted of 8 samples (28.57%), 4 samples (14.29%), and 2 samples (7.14%), respectively. Meanwhile, the puffballs fungi, the stinkhorns fungi, and the leather-bracket fungi contained only 1 sample (3.57%) per group. These 28 samples with different fruiting bodies were selected for molecular identification.

Internal transcribed spacer (ITS) was used as a target to amplify by Polymerase Chain Reaction (PCR), a conserved region that is used for fungal identification, to analyze nucleotide

sequence in this study. The PCR products were analyzed by agarose gel electrophoresis and revealed product size between 550-700 bp (Fig. 2). Subsequently, the nucleotide sequences of ITS region from several macrofungi samples were analyzed by BLAST (Basic Local Alignment Tool) or nucleotide BLAST (BLASTn), and the percent similarity with best match reference sequence in the database (GenBank) was provided in Table 1. The Phylogenetic tree was constructed based on their ITS sequences as shown in Fig. 3. The taxonomy data, the current name, and the ITS accession number of these macrofungi are presented in Table 1. The molecular identification of macrofungi samples could be classified into 2 phyla, 4 classes, 10 orders, 17 families, and 22 genera. The phylum Ascomycota consists of 4 samples (14.29%), while the remaining could be classified in phylum Basidiomycota 24 samples (85.71%). The family Polyporaceae was the most diverse macrofungi species (7 samples, 25%). The Entolomataceae, Malasmiaceae, and Schizophyllaceae revealed 3 samples (10.71%), 3 samples (10.71%), and 2 samples (7.14%), respectively. Meanwhile, Auriculariaceae, Dacrymycetaceae, Ganodermataceae, Hypocreaceae, Hypoxylaceae, Lyophyllaceae, Phallaceae, Physalacriaceae, Porotheleaceae, Pyronemataceae, Sclerodermataceae, Stereaceae, and Xylariaceae found only 1 sample (3.57%) per family (Table 1 and Fig. 3). However, three macrofungi samples (10.71%) could not be identified at the species level including *Marasmius* sp. (WK29), *Gerronema* sp. (WK25), and *Picipes* sp. (WK10) (Table 1 and Fig. 3).

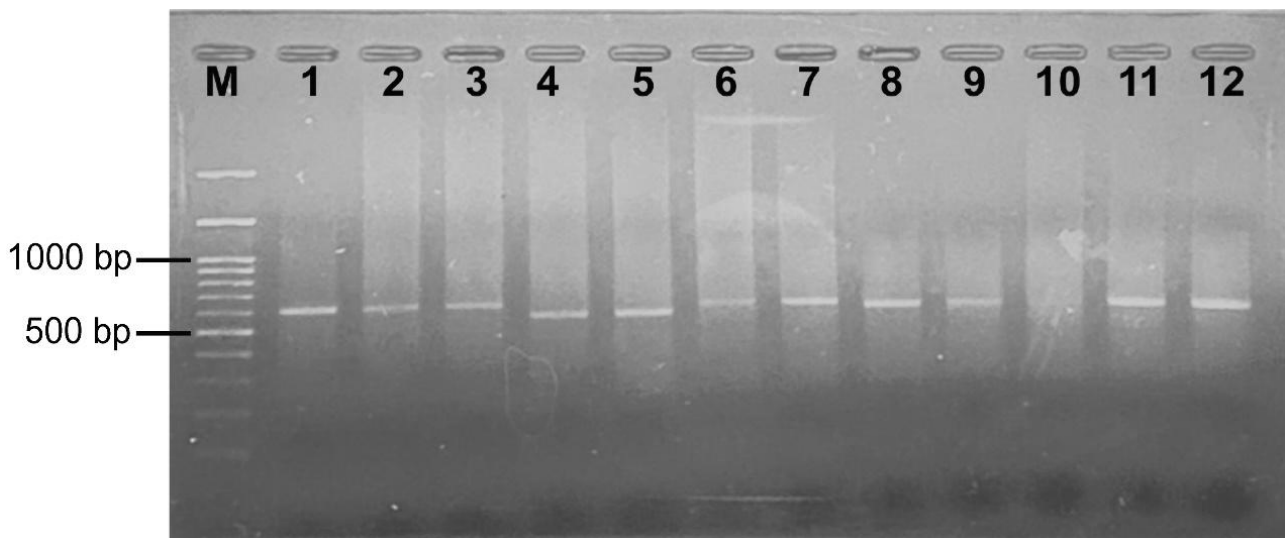


Fig. 2 – PCR product of ITS region by agarose gel electrophoresis analysis. Lane M 100 bp DNA ladder. Lane 1 Collection No. WK1. Lane 2 Collection No. WK2. Lane 3 Collection No. WK3. Lane 4 Collection No. WK4. Lane 5 Collection No. WK5. Lane 6 Collection No. WK6. Lane 7 Collection No. WK7. Lane 8 Collection No. WK8. Lane 9 Collection No. WK9. Lane 10 Collection No. WK10. Lane 11 Collection No. WK11. Lane 12 Collection No. WK12.

The role of macrofungi in the ecology of the para rubber area was investigated. Most of them play an important role as the saprotroph (SA) (25 samples, 89.28%), while, the pathotroph (PA) found only 1 sample (3.57%) including *Ganoderma williamsianum* (WK5), and the symbiotroph (SM) found 2 samples (7.14%) consist of *Termitomyces cylindricus* (WK12) and *Scleroderma xanthochroum* (WK17).

Additionally, the macrofungi found in this study had previously been reported to be edible revealing 7 samples (25%) included *Auricularia cornea* (WK19), *Dacryopinax spathularia* (WK18), *Phallus lutescens* (WK20), *Sanguinoderma rugosum* (WK4), *Schizophyllum commune* (WK15), *Termitomyces cylindricus* (WK12), and *Trichaleurina javanica* (WK24). In contrast, the poisonous macrofungi, *Scleroderma xanthochroum* (WK17), has been reported in this study only in

1 sample (3.57%) (Table 1 and Fig. 4). However, there were no edibility data of the remaining macrofungi samples (20 samples, 71.41%) (Table 1).

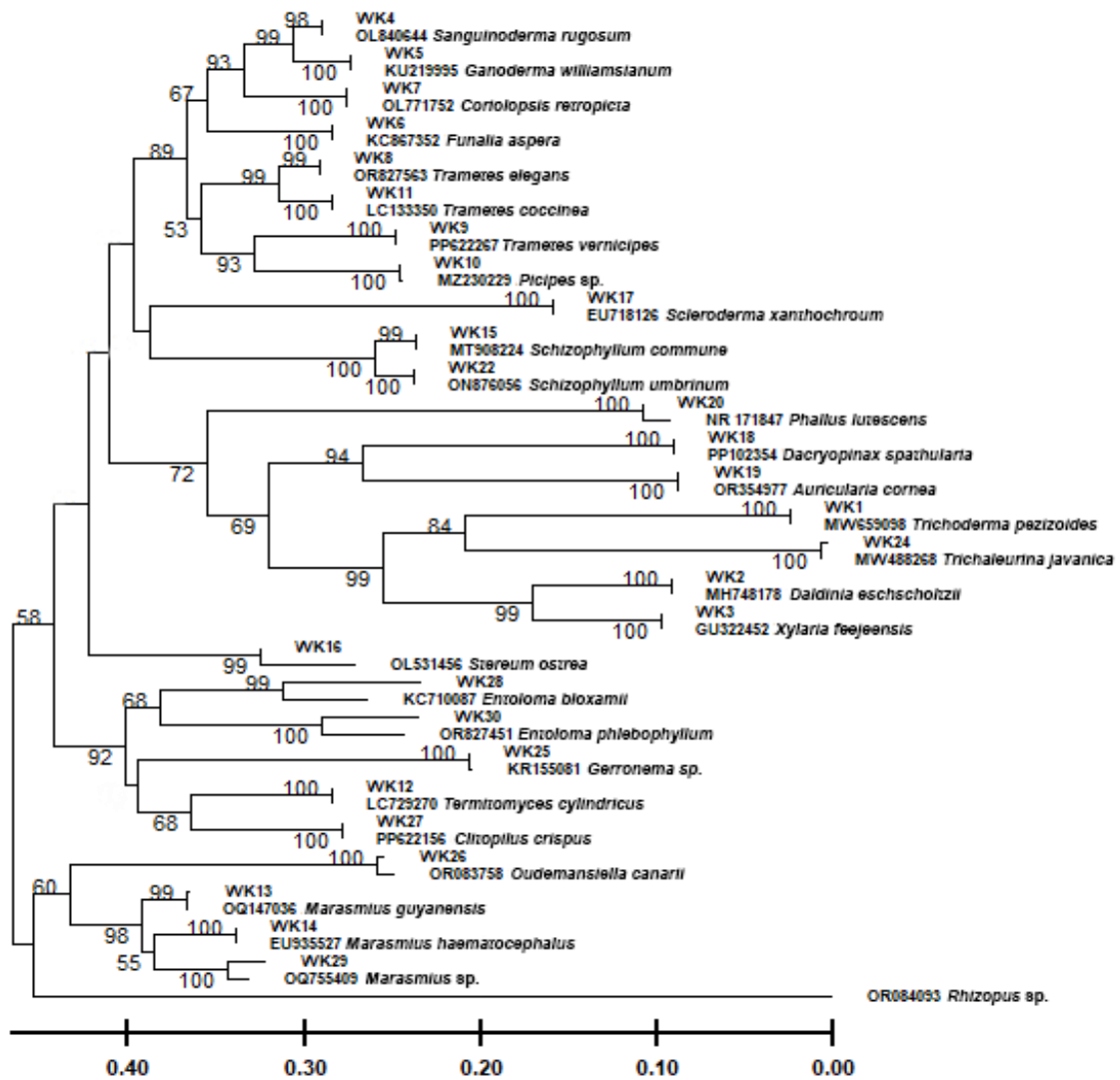


Fig. 3 – Phylogenetic tree based on ITS sequences of macrofungi in para rubber plantation at Wang Takien Subdistrict, Khao Saming District, Trat Province, Thailand

Table 1 Taxonomy data, BLASTn results, mode of lives, and edibility of macrofungi collected from para rubber plantation at Wang Takien Subdistrict, Khao Saming District, Trat Province, Thailand.

Phylum	Class	Order	Family	Scientific name (Collection number)	Best Match (Accession No.)		Mode of life	Edibility	
					ITS	Similarity (%)			
Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	<i>Trichaleurina javanica</i> (WK24)	<i>Trichaleurina javanica</i> (MW488268)	99.81	PV056378	SA	E
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	<i>Trichoderma pezizoides</i> (WK1)	<i>Trichoderma pezizoides</i> (MW659098)	100	PV052342	SA	
Ascomycota	Sordariomycetes	Xylariales	Hypoxylaceae	<i>Daldinia eschscholtzii</i> (WK2)	<i>Daldinia eschscholtzii</i> (MH748178)	100	PV052343	SA	
Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	<i>Xylaria feejeensis</i> (WK3)	<i>Xylaria feejeensis</i> (GU322452)	100	PV052344	SA	
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	<i>Clitopilus crispus</i> (WK27)	<i>Clitopilus crispus</i> (PP622156)	99.70	PV052377	SA	
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	<i>Entoloma bloxamii</i> (WK28)	<i>Entoloma bloxamii</i> (\KC710087)	83.83	PV053538	SA	
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	<i>Entoloma phlebophyllum</i> (WK30)	<i>Entoloma phlebophyllum</i> (OR827451)	87.26	PV053539	SA	
Basidiomycota	Agaricomycetes	Agaricales	Lyophyllaceae	<i>Termitomyces cylindricus</i> (WK12)	<i>Termitomyces cylindricus</i> (LC729270)	100	PV052354	SM	E
Basidiomycota	Agaricomycetes	Agaricales	Malasmiaceae	<i>Marasmius guyanensis</i> (WK13)	<i>Marasmius guyanensis</i> (OQ147036)	100	PV052353	SA	
Basidiomycota	Agaricomycetes	Agaricales	Malasmiaceae	<i>Marasmius haematocephalus</i> (WK14)	<i>Marasmius haematocephalus</i> (EU935527)	100	PV052355	SA	
Basidiomycota	Agaricomycetes	Agaricales	Malasmiaceae	<i>Marasmius</i> sp. (WK29)	<i>Marasmius</i> sp. (OQ755409)	92.41	PV052378	SA	
Basidiomycota	Agaricomycetes	Agaricales	Physalacriaceae	<i>Oudemansiella canarii</i> (WK26)	<i>Oudemansiella canarii</i> (OR083758)	97.92	PV052376	SA	
Basidiomycota	Agaricomycetes	Agaricales	Porothleaceae	<i>Gerronema</i> sp. (WK25)	<i>Gerronema</i> sp. (KR155081)	99.86	PV052375	SA	
Basidiomycota	Agaricomycetes	Agaricales	Schizophyllaceae	<i>Schizophyllum commune</i> (WK15)	<i>Schizophyllum commune</i> (MT908224)	99.84	PV052356	SA	E
Basidiomycota	Agaricomycetes	Agaricales	Schizophyllaceae	<i>Schizophyllum umbrinum</i> (WK22)	<i>Schizophyllum umbrinum</i> (ON876056)	99.67	PV052374	SA	
Basidiomycota	Agaricomycetes	Auriculariales	Auriculariaceae	<i>Auricularia cornea</i> (WK19)	<i>Auricularia cornea</i> (OR354977)	99.66	PV052363	SA	E
Basidiomycota	Agaricomycetes	Boletales	Sclerodermataceae	<i>Scleroderma xanthochroum</i> (WK17)	<i>Scleroderma xanthochroum</i> (EU718126)	99.62	PV052360	SM	P
Basidiomycota	Agaricomycetes	Phallales	Phallaceae	<i>Phallus lutescens</i> (WK20)	<i>Phallus lutescens</i> (NR_171847)	99.17	PV052364	SA	E

Table 1 Continued

Phylum	Class	Order	Family	Scientific name (Collection number)	Best Match (Accession No.)		GenBank Accession No. in this study	Mode of life	Edibility
					ITS	Similarity (%)			
Basidiomycota	Agaricomycetes	Polyporales	Ganodermataceae	<i>Sanguinoderma rugosum</i> (WK4)	<i>Sanguinoderma rugosum</i> (OL840644)	100	PV052345	SA	E
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	<i>Ganoderma williamsianum</i> (WK5)	<i>Ganoderma williamsianum</i> (KU219995)	99.84	PV052346	PA	
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	<i>Coriolopsis retropicta</i> (WK7)	<i>Coriolopsis retropicta</i> (OL771752)	99.46	PV052348	SA	
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	<i>Funalia aspera</i> (WK6)	<i>Funalia aspera</i> (KC867352)	100	PV052347	SA	
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	<i>Picipes</i> sp. (WK10)	<i>Picipes</i> sp. (MZ230229)	100	PV052351	SA	
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	<i>Trametes elegans</i> (WK8)	<i>Trametes elegans</i> (OR827563)	100	PV052349	SA	
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	<i>Trametes vernicipes</i> (WK9)	<i>Trametes vernicipes</i> (PP622267)	99.69	PV052350	SA	
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	<i>Trametes coccinea</i> (WK11)	<i>Trametes coccinea</i> (LC133350)	100	PV052352	SA	
Basidiomycota	Agaricomycetes	Russulales	Stereaceae	<i>Stereum ostrea</i> (WK16)	<i>Stereum ostrea</i> (OL531456)	100	PV052357	SA	
Basidiomycota	Dacrymycetes	Dacrymycetales	Dacrymycetaceae	<i>Dacryopinax spathularia</i> (WK18)	<i>Dacryopinax spathularia</i> (PP102354)	99.59	PV052361	SA	E

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



Fig. 4 – Photographs of macrofungi found in para rubber plantation at Wang Takien Subdistrict, Khao Saming District, Trat Province, Thailand. A *Auricularia cornea* (WK19). B *Dacryopinax spathularia* (WK18). C *Phallus lutescens* (WK20). D *Sanguinoderma rugosum* (WK4). E *Schizophyllum commune* (WK15). F *Termitomyces cylindricus* (WK12). G *Trichaleurina javanica* (WK24). H *Scleroderma xanthochroum* (WK17). A–G: Edible macrofungi. H Poisonous macrofungi.

Discussion

The survey of macrofungi in the para rubber plantation area, Wang Takien Subdistrict, Khao Saming District, Trat Province, Thailand by collecting samples, studying morphological characteristics, and identifying species by molecular biology method using the PCR technique together with the sequence analysis of the ITS region, normally, the nucleotide sequence of the organism that gives a percentage of similarity (%similarity or %identity) greater than or equal to 97.0% when compared to the nucleotide sequence in the database, we will be able to identify the

organism at the species-level. As for the nucleotide sequence of the organism that gives %similarity in the range of 90.0-96.9%, we can be identified them at the genus-level. However, in the study of fungal taxonomy, if it is found that the nucleotide sequence of the fungus that gives a %similarity less than 98.0% when compared to the nucleotide sequence in the database. This may mean that this fungal sample is different from the species in the database and may be a new species (Surawut et al. 2023).

For example, in this study, some fungi (collection number WK26, WK28, WK29 and WK30) were found to have a %similarity of less than 98.0%, while WK25 and WK10 had a %similarity of more than 99.0% with fungi that could be identified only by genus (unknown species in the database). Therefore, these fungal samples found in this study should be studied for other nucleotide position in order to be able to correctly identify the species.

Currently, identifying macrofungi by using only morphological characteristics may not be able to identify closely related fungi that have very similar morphology. Therefore, molecular identification by nucleotide sequence analysis is one method used in combined with morphological studies in order to correctly identify the species. Although the ITS region is commonly used to identify fungal species, some fungi could not be identified the species by only ITS region (Raja et al. 2017, White et al. 1990). Nowadays, multiple gene loci are used together to identify fungal species, such as nuclear large subunit rDNA (LSU), nuclear small subunit rDNA (SSU), translation elongation factor 1-alpha (*tefl- α*), RNA polymerase II second largest subunit (*rpb2*), beta-tubulin (*tub*), actin (*act*) and cyclooxygenase gene (*cox*). For example, Cho et al. (2015) evident the identification of *Amanita* sp. by analyzing the nucleotide sequences of the ITS and LSU loci together and new species were reported such as *Amanita caesareoides*.

In this study, some samples (WK26, WK28, WK29, and WK30) revealed %similarity of less than 98.0% and some could not be identified at the species level, namely *Gerronema* sp. (WK25), *Marasmius* sp. (WK29) and *Picipes* sp. (WK10). These fungal samples may be new species. Therefore, further nucleotide sequence analysis in other loci should be carried out. For example, species identification of the genus *Gerronema*, two loci, ITS and LSU, were reported to be used (Na et al. 2022). Meanwhile, the identification of the family Marasmiaceae, including the genus *Marasmius*, three loci, ITS, LSU, and mtSSU, were studied (Amoako-Attah et al. 2020). In addition, a study in *Picipes* sp. found that SSU, *tub*, *tefl- α* , *rpb1*, and *rpb2* loci were combined analyzed for identification (Zhou et al. 2016). Therefore, further study of these fungi samples in other loci combined with the ITS locus may enable more accurate identification of these samples.

The diversity of macrofungi is influenced by many factors, such as topography, altitude from sea level, forest type and vegetation, weather, temperature and humidity, etc. Trat Province is located in the Eastern Thailand. It has a tropical climate with heavy rainfall. In this study, samples were collected in June, which is the beginning of the rainy season. Therefore, the study found a large variety of macrofungi. The largest group of gilled fungi (39.29%) was found, and the Polyporaceae had the highest variety of fungi (25%). It is similar to the study of fungal diversity in Kanchanaburi Province, Thailand, which reported that fungal diversity was higher in the rainy season than in the dry season (Sutjaritvorakul et al. 2017). In addition, para rubber plantations were created by converting native forests with several plants into monoculture farms. Therefore, the diversity of fungal species in the area was affected by the changes in the land conditions for agriculture. Li et al. (2018) conducted a study comparing the diversity of fungi in native forests and forests that were converted to agricultural areas in the Greater Mekong Subregion. It was found that the native forest areas had a higher diversity of macrofungi than the areas that were converted for agriculture. Lan et al. (2020) explained that the transformation of the native forest into a rubber plantation has resulted in changes in the population of soil fungi, including macrofungi. It was found that most soil fungi belong to the phylum Ascomycota. It can be seen that monoculture, such as rubber plantations, and human agricultural activities have negatively affected the biodiversity and activities of soil fungi, which has resulted in the loss of nutrient cycling and the interaction of fungi with other organisms. However, changes in the population of soil fungi due to agriculture are not all disadvantages, as there have been reports of the discovery of new species of fungi in rubber

plantations (Xu et al. 2024). These new species of fungi may be beneficial to humans in other ways, such as the production of bioactive compounds and medicinal substances.

In conclusion, the data from this study reveal the diversity of macrofungi in para rubber plantations at Wang Takhian Subdistrict, Khao Saming District, Trat Province, Thailand by identifying fungal species mainly by molecular biology. This results in the identification of fungal species being accurate and reliable for the use of the obtained data to create a fungal biodiversity database in para rubber plantations to be used in the assessment of the FSC standards of para rubber farmers in Wang Takhian Subdistrict, who are members of the Trat Rubber Co-operative Limited. If the certification is received, farmers will be able to sell their latex at a higher price. They will also be able to share knowledge about the types of edible and poisonous macrofungi found in the para rubber plantations to monitor the collection of poisonous mushrooms for consumption. Macrofungi that are reported to be edible can also be collected for cooking or sold to generate additional income for farmers. Additionally, this study indicated that the study of biodiversity is not only for academic progress but also has an impact on human life and economics.

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