IDENTIFICATION AND DIVERSITY OF MUSHROOMS OF PUALRENG WILDLIFE SANCTUARY IN MIZORAM, INDIA.

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY

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Identification and Diversity of Mushrooms of Pualreng Wildlife Sanctuary in Mizoram, India.

By

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Submitted

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CERTIFICATE

This is to certify that Mr. Benjamin Lalbiakmawia has submitted the M.Phil dissertation entitled "Identification and Diversity of Mushroom of Pualreng Wildlife Sanctuary in Mizoram" under my supervision, for the requirement of the award of the Degree of Master of Philosophy in the Department of Environmental Science, Mizoram University, Aizawl. The work is authentic, the content of the thesis is the original work of the Research Scholar and the nature and presentation of the work are the first of its kind in Mizoram. It is further certified that no portion(s) or parts of the content of the thesis has been submitted for any degree in Mizoram University or any other University or Institute. He is allowed to submit the thesis for examination and for the award of the Master of Philosophy in Environmental Science.

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DECLARATION

I, Mr. Benjamin Lalbiakmawia, hereby declare that the subject matter of this dissertation entitled "Identification and Diversity of Mushroom of Pualreng Wildlife Sanctuary in Mizoram" is the original record of the work done by me, that the contents of this dissertation did not form basis of award of any previous degree to me or to the best of my knowledge, to anybody else and that the dissertation has not been submitted by me for any research degree to any other University or Institute.

This is being submitted to the Mizoram University for the award of the Degree of Master of Philosophy in the Department of Environmental Science.

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1. INTRODUCTION:

Mushroom are the fruiting bodies of fungi, the main body of the fungi are generally buried inside the earth or inside decomposing matter as mycelium. According to Chang and Miles (1992), and Das (2010), they are regarded as the visible part of fungi with distinctive carpophores which represent the reproductive stage in the life cycles of some Ascomycetes and Basidiomycota. Biological diversity (biodiversity) encompasses the variety of life forms occurring in nature, from the ecosystem to the genetic level, as a result of evolutionary history (Wilson 1992). The idea that fungi form a kingdom distinct from plants and animals gradually became accepted only after Whittaker (1969). The mushroom diversity and effect of disturbance on mushroom and the ecosystem on a whole are poorly understood. It is important to understand the importance of these microscopic organisms as they play an important part in nutrient recycling and are indispensible chain in the ecosystem. According to Straatsma *et al* (2001), as a result of its sudden appearance in nature, characteristics morphology and colour mushroom carpophore gains more attention than its mycelia (vegetative stage). Onuoha and Obi-Adumanya (2010) also state that their fleshy, spore-bearing fruiting bodies grow on soil or wood substrates whereas some exist in mycorrhizal relationship with trees.

Inventory of macro fungal inhabitants in different natural and human-influenced ecosystems broadens our knowledge on their usefulness (Ammatanda *et al*, 2016). Adequate knowledge of mushroom diversity and distribution are imperative for successful conservation, management and optimum exploitation of the ecosystem for innumerable benefits to mankind (Nwordu *et al,*. 2013). As they are important bio resource with nutritional, medicinal and ecological benefits (Odeyemi *et al,*. 2014). In the natural environment, mushrooms grow on variety of substrates, especially those containing lignin and cellulose, are abundant during the rainy and wet seasons (Gbolagade, 2005). Soil debris and dead woods due to high content of degraded nutrients and capacity to retain moisture are probably the most favourable environments for mushroom (Ayodele *et al, 2011*).

Modern systematics, based on morphological characters and analysis of rDNA sequences, divides the kingdom Fungi into four major phyla or divisions: Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota. The two fungal phyla that produce large, visible fruit bodies are the Ascomycota and Basidiomycota. The Ascomycota contains at least 40,000 different species worldwide, many of them rather inconspicuous, but including such familiar groups as the morels and truffles, the cup fungi, and most of the lichens, as well as many microscopic molds and yeasts. They all produce their spores within macroscopic cells called asci, which typically open under pressure when mature, shooting the spores out into the air currents (Roberts P, 2013). Diversity of mushrooms varies greatly ranging from the typical Agaricus mushrooms with a stalk and umbrella-shaped top to the polypores, Earth Stars (Geastrum), the Stink Horns (phalloides), and Puff Balls (Lycoperdon). (Rahi DK, *et al,*)

It is estimated that around 140,000 species of mushroom exist, with only about one-tenth taxonomically identified (Wasser, 2002). Worldwide assessment of fungi diversity revealed that fungi distribution is mainly influenced by environment and certain types of habitat (Tedersoo *et al,* 2014); greatly influence by precipitation and temperature being the most important climatic elements (Straatsma *et al,*. 2001).). Mushrooms in the tropics are more diverse and are distributed over a smaller geographical area, in contrast to what is obtainable in the temperate regions, (Tedersoo *et al,*. 2014).

In India, about 10% had been investigated (Gurudevan *et al,*. 2011In Mizoram, a number of study on fungal diversity has been done (Bisht, 2011; Zothanzama, 2011, 2013, 2016, 2017; Lalrinawmi *et al,* 2017, 2018, Vabeikhokhei *et al,*, 2017, 2019, Zohmangaiha *et al,* 2019). Recently, based on classical taxonomy and molecular characterization a new species *Ganoderma mizoramense* from Mizoram have also been identified (Zothanzama *et al* 2017).

2. Review of Literature:

Taxonomy, which is probably the oldest of sciences, was defined by Stace (1989) as 'the study and description of the variation of organisms, the investigation of the causes and consequences of this variation, and the manipulation of the data obtained to produce a system of classification'. In 2014, Rouhan and Gaudeul defined taxonomy as `the science that explores, describes, names, and classifies all organisms`. Taxonomy makes communicating biological information much easier because it facilitates categorizing organisms (Shipman, 2012). Fungi are one of the largest groups of eukaryotes that play key roles in nutrient and carbon cycling in terrestrial ecosystems as mutualists, pathogens and free-living saprotrophs (McLaughlin and Spatafora 2014). Because many fungi are unculturable and seldom produce visible sexual structures, molecular techniques have become widely used for taxonomic detection of species to understand shifts in their richness and composition along environmental gradients (Persˇoh, 2015). Accurate taxonomic identification to species, genera and higher taxonomic levels is a key for reliable assignment of ecological and functional traits to taxa for further eco physiological and biodiversity analyses (Koˇljalg et al,. 2013). Furthermore, molecular methods have revolutionized our understanding concerning phylogenetic relationships among the Fungi and have substantially altered the morphology-based classification system (Hibbett et al,, 2007). Availability of full-length rRNA gene and protein-encoding marker gene sequences (James et al,. 2006a) and evolution of high-resolution genomics tools (Spatafora et al,. 2016,2017) has further refined the order of divergence and classification of the major fungal groups (Zhao et al,. 2017). The study of taxonomy of mushrooms started with Species *Plantarum* (1753), in which Linnaeus recognized one mushroom of the genus, Agaricus among 10 fungal genera, which include all the gilled bearing fungi. Persoon (1796, 1797) established the first classification of mushrooms which stands as a starting point for fungal nomenclature and was considered as the founder of modern mycology. Fries (1821-1832) further elaborated the classification of fungi, including various microscopic characteristics and color of the spore color, the methods which are still in use by taxonomists today. Leveille (1846) and Berkeley (1856) were considered as the first to recognize that the basidia

and basidiospores are different from asci and asco-spores. Saccardo (1882-1931) also laid emphasis on the importance of spore color in the mushroom taxonomy and thus recognized four sub–divisions of agarics based on their spore color. Singer (1986) also emphasized the spore color in the taxonomy of mushroom in his monograph and recognized 17 families, 230 genera and 5658 species under order Agaricales.

The morphological species concept is where the characters (phenotypes) of individual organisms are compared, and similar individuals are designated as a species. Inherent in this construction is an assumed genetic hiatus between dissimilar organisms. Decisions about similarity and dissimilarity of characters, of course, are left to the taxonomist. Traditionally, characters used to identify mushrooms and their relatives have been taken from the macro- and micromorphology of the basidioma (i.e., the fruiting body or mushroom). It is little wonder, then, that mushroom systematics has been informed by the morphological species concept (Smith 1968, Clemençon, 1977). The morphological parameters used for the identification of mushroom specimens such as- cap colour, cap surface, cap margin, cap diameter, stipe length, gill attachment, gill spacing and spore dimension. Microscopic features were carried out using standard microscopic methods (Roy, 1998).

Taxonomy based on external features, i.e. phenotype, is still considered to be the mainstay of this science, but there are problems too. Phenotypic characters are highly variable in respect to climatic conditions and often create major problems in proper identification. Recent advances in molecular techniques have come up with solutions. Basic molecules of life like DNA, RNA and proteins can be used as much more reliable identification markers as they are very stable in nature. So based on these molecules one can identify a particular organism or can assess relationships between different organisms. This new approach of taxonomy has been named as "Molecular Taxonomy" and molecules based on which classification is done are called Molecular Markers. Molecular markers can also be defined as signs especially along the DNA that pin-point the location of desirable genetic traits or specific genetic differences. A particular fragment of DNA can be used as a marker when differences can be detected in that fragment's DNA sequence among multiple plants

or plant lines. These sequence variations, called polymorphisms, can be associated with different forms (alleles) of nearby genes involved with particular traits. The polymorphism, or difference, is the clue to find the gene of interest. Molecular markers are versatile tools in various fields other than taxonomy like physiology, embryology, genetic fingerprinting etc. Molecular phylogenetic and systematics have been found to be greatly promising in recent years, due to the development of new and diverse methods for analysis of molecular markers. Molecular taxonomic approaches permit an exact and rapid method of distinguishing specimens based on their interspecific variations. These methods allow estimation of the genetic variability of the biota carrying to super-estimation on the global biodiversity besides the relationships among taxa. (De at el., 2009).

The CTAB extraction method originally developed by Doyle and Doyle in 1987, and later, it was modified to remove polysaccharide, polyphenols, and other secondary metabolites. The superfluous quantities of cellular proteins were eliminated by triple extended treatment with chloroform-isoamyl alcohol. In addition to the removal of proteins, this treatment also helps to remove different colouring substances. Importantly, CTAB is probably the only compound that can separate partial nucleic acids from polyphenols. The polyphenol compounds may severely inhibit downstream DNA/RNA reactions. Chloroform-isoamyl alcohol is a type of liquid detergent disrupts the bonds that hold the cell membranes by dissolving proteins, lipids, and then form complexes to precipitate out of the solution.Nuclear large subunit ribosomal DNA is widely used in fungal phylogenetic and to an increasing extent also amplification based environmental sequencing. The relatively short reads produced by next-generation sequencing, however, makes primer choice and sequence error important variables for obtaining accurate taxonomic classifications. (Porter et al,, 2012).

DNA barcoding is a molecular methodology that identifies species using short genetic markers. It was first developed by Paul Hebert in 2003 for butterflies, and in 2008, a consortium of institutions joined forces under the name iBOL to start the ambitious task of building reference libraries for barcodes of all life on earth (Hebert et al., 2003; Savolainen et al., 2005). To identify mushrooms, DNA

barcoding is seen as one of the most powerful tools since identification based on morphology is not always sufficient (Xu J, 2016). To discriminate species, the nuclear internal transcribed spacer (nrITS) and the 28S nuclear ribosomal large subunit (LSU) rRNA marker sequences are generally used.(Vilgalys et al., 1990; Asemaninejad et al., 2016). The nrITS region of the rRNA gene cluster is the most commonly used target to identify fungi, which comprises a region of 600 bp. The major advantage of nrITS barcoding is the use of well-validated primer sequences, detectability due to the large number of copies of the rRNA clusters and appropriate sequence variation in the nrITS genes between related organisms (Schoch et al., 2012).

Biodiversity is defined as variety of organisms in a space. In other words, biodiversity covers the genes in a region, the species carrying these genes, the ecosystems that contain these species, and the events that link them together. This definition draws attention to many dimensions of biodiversity such as genetic, taxonomic, ecosystem and events diversity (Erten, 2004; GülsoyandÖzkan, 2008). Diversity indices should be calculated so that the diversity level is expressed as a numerical value and the diversity ratings of the different systems can be statistically compared (Odumve Barrett 2005). The ability to calculate diversity with this mathematical measure is an especial tool for biologists to understand community structure. Though diversity indices provide important information about diversity, dominancy, richness and evenness of species in a community, there is no single index sufficiently calculating biodiversity concept such as rarity and commonness of species in a community (Hurlbert, 1971; Purvis and Hector,2000). The diversity of species in a particular area depends on not only the number of species, but also in their numbers that is relative abundance. While experts determine species richness as the number of species in an area, they determine species evenness as the relative abundance of species in an area. Richness (S) is explained as the number of species and is the most common indication for diversity (Magurran, 2004). Margalef and Menhinicks indices are the some of the common used indexes to characterize species richness in a community. The simplest diversity index is Berger and Parker diversity indices that report the proportional abundance of only the most abundant species in a

community (Berger and Parker, 1970). Also, Pielou-R indice accounts evenness of the species present. The Shannon-Wiener diversity index (H') and the Simpson index are the most widely used diversity indices to obtain information on species diversity or dominancy in stations and distribution of individuals between species (Jorgensen et al,., 2005).

Opportunistic Sampling: It is used by many mycologist and mushroom hunters to collect as much of the macrofungi as possible. This method puts more emphasis on inventory; diversity becomes the main purpose. This method also does not require transect plot or boundary, which is determined only the location of sampling or sampling-site. Mushroom foraging and opportunistic sampling mechanism are almost the same, mushroom foraging is usually done by a group of people or families to collect as much as possible with the purpose of recreation; edible macrofungi becomes an option to hunt based on season and predetermined location. Taxonomy and evolution studies are well suited to use this method. The method also called as “walk-through”, if the purposes are to locate specific rare macro fungi (Pilz et al,., 1996).Plot-based sampling: This method has differences with other sampling methods. If the previous two methods only specify representative sampling-sites, adaptive-plotbased sampling uses plot and transect design used for sampling. Replicative and consistent plots assist investigators to focus sampling on their plots and ignore sampling of organisms outside the plot. In many studies the plots are designed with different sizes and shapes. (Kinge et al,., 2017). Studies in which terrestrial macrofungi are surveyed usually use arbitrary sampling units, or plots. Plots range in size from 1 m2 to 1000 m2 and can be square, rectangular, or circular. The same plots often are scrutinized for several years. When studies involve removal of most sporocarps (e.g., to determine sporocarp productivity), however, some investigators move plots on each sam- pling occasion to avoid effects of disturbance (Luoma 1991; O’Dell et al,. 1999).

Disturbance is a common feature of many ecosystems, occurring at all levels of ecological organization and at numerous temporal and spatial scales (Zak, 1992), as stated by Lodge and Cantrell (1995) this may be cause by anthropogenic or natural, natural disturbance from seasonal changes in rainfall and tree fall, to natural

disaster, cause population shifts and changes to communities of fungi. They illustrate that, only 3 of the 20 regularly surveyed mycelia of Collybia johnstonii could be found in the litter layer during 2 years following hurricane Rugobecause of increased solar radiation and desiccation (Lodge and Cantrell, 1995).There are several comprehensive reviews on the effects of human disturbance on fungal communities (Zak, 1992; Miller and Lodge, 1997), but most were restricted to the effects of disturbance on soil or mycorrhizal communities in temperate regions. Tropical environments differ ecologically from temperate habitats in physical, chemical and biological attributes (Lacher and Goldstein, 1997). They are characterized by warmer temperature, with little or no seasonality, and heavy precipitation during at least part of the year. Although tropical habitats only occupy 25.7 % of the land area of the earth, they harbour the bulk of the world's species (Deshmukh, 1986). Raven (1988) suggested that 2/3 of the vascular plant species occur in the tropics. Biodiversity of fungi in the tropics is also very high. There is little information on the effect of disturbance on fungi in rainforests or mangroves, which are habitats unique to the tropics. Numerous new taxa have been described from the tropics in the last decade despite the fact that few mycologists are located in these regions (Hyde and Hawksworth, 1997). Many tropical environments are being heavily disturbed by human activity. This disturbance is often in the form of addition of chemicals, e.g. discharge of industrial effluents and organic fertilizers, and habitat degradation, e.g. slash and burn agriculture, selective logging, destructive logging followed by reforestation, or deforestation followed by agriculture or managed forestry, affect fauna and flora by reducing species numbers and evenness. What are the effects of these disturbances on the fungi in rainforest, mangrove and other habitats? So far very little published information is available. (Tsui et al,, 1998).

The forest canopy, tautly defined as the aggregate of all crowns in a forest stand, is an important indicator used as a measure of stand density (Gill et al. 2000) and for predicting woody plant composition, leaf area index (LAI) or vegetation area index (Fassnacht et al. 1994), tree volume and net primary production, and for the evaluation of tree crown condition or forest pest damage (O'Brien, 1989) and wildlife microhabitat (Morrison et al. 1999). The literature which emphasizes the

various purposes of measuring the forest cover is wide and just as many are the articles reporting on different techniques and instruments used for its assessment. The numerous ecological processes in forest communities are influence by this parameter (Cook et al. 1995) in forest protective function assessment models (Bebi et al.2001), under story vegetative productivity (McConnell & Smith 1970). According to Economic Commission for Europe of the United Nations (UNECE)/ Food and Agriculture Organization of the United Nations (FAO) forest/other wooded land which shows natural forest dynamics, such as natural tree composition, occurrence of dead wood, natural age structure and natural regeneration processes, the area of which is large enough to maintain its natural characteristics and where there has been no known significant human intervention or where the last significant human intervention was long enough ago to have allowed the natural species composition and processes to have become re-established. Furthermore the measure of forest cover is useful to analyse the plant development, hence determining the nature of the vegetation and it is an important ecological parameter of forest ecosystem for its relationship with species richness, wildlife habitat and behaviour (Ganey & Block 1994), watershed preservation (Crookston and Stage 1999).Moreover the canopy cover is one of the Forest Resources Assessment (FRA 2000) parameters used in order to define 'forest' and 'other wooded land' (FAO2001). In this context it is considered as 'forest' the land with tree crown cover of >10% in an area of >0.5 ha and the trees should have (or should be able to reach) a minimum height of 5 m; while to 'other wooded land' belongs the land with either a crown cover of 5–10% of trees able to reach a height of 5 m (at maturity in situ) or a crown cover of >10% of trees not able to reach a height of 5 m or with shrub or bush cover of >10%. These definitions have been used and adapted in many European National Forest Inventories (Winter et al. 2008). The main in situ methods, further than the visual (ocular) estimation, com-prise crown mapping, hemispherical canopy photography, densitometers (tube or spherical), included the Finnish 'Caj-anus tube', and other similar devices such as the so-called 'moosehorn' and the 'gimbal sight'. Other methods are used to estimate the crown diameter, e.g., using logger' stapes, where two tape measurements are averaged to obtain mean crown diameter or adopting other alternative field techniques (Bechtold & Zarnoch, 2002). Light is an important

factor in plants growth, where its intensity is so low that it reduces the intensity of photosynthesis, i.e. below the light saturation point. According to Daniels (1956) this refers to the energy of light of less than 50 000 erg/cm^{2}s^{-1}, i.e. approx. 50 W/m^{-2}.Particular attentions is paid to the intensity of photo synthetic active radiation (PAR), in the range of 400-700 nm, whose measure is the photosynthetic photon flux density (PPFD), expressed in mmol/m^{-2}s^{-1}. Quantum sensors are used in direct PPFD measurements (Comeau, 2000, Lieffers et al. 1999).The intensity of light is limited by forest canopy. As a rule, the relative values of PPFD,i.e. the transmittance of PAR by the stand canopy (% PPFD), are determined in the forest understory.

3. Materials and Method

3.1. Study Sites

Study was conducted in Pualreng Wildlife Sanctuary located in 24° 6’35” - 24o 14’16’21”’ North Latitude and 92° 50’ 17.6” - 92o54’2.64” East longitude in the district of Kolasib.

Hlimen Village Jhum Sites

It is located in the 24°13'47.96"N 92°48'18.04"E in the Northern District of Kolasib.

3.2. Taxonomic identification of Mushroom

3.2.1. Collection of Specimen

The samples were collected from the selected sites and kept in air-tight container or plastics bags which are labeled after collection. Photograph of each sample collected are taken in the field and in the laboratory (Prasher, 2015).

3.2.2. Morphological analysis

Macroscopic and Microscopic Examination of Isolated Fungi the fungal morphology was studied macroscopically by observing the spores, colony features (color, shape, size and hyphae), and microscopically by a compound microscope with a digital camera using a stained slide mounted with a small portion of the mycelium (Gaddeyya *et al*, 2012). Collected specimens were identified according to standard macroscopic and microscopic characteristics through consultation with appropriate literatures (Bas, 1969; Singer, 1986; Arora, 1986; Surcek, 1988; Ainsworth *et. al*, 2001; Mohanan, 2013).

3.2.3. Molecular analysis

DNA extraction PCR, Sequencing

DNA was extracted from fruiting bodies using a CTAB extraction procedure.

PCR was amplifies at ITS region of ribosomal RNA. Primers used was-

ITS1-F (5'-CTT GGT CAT TTA GAG GAA GTA A-3')

ITS4-R (5'-TCC TCC GCT TAT TGA TAT GC-3'), White et al, (2001).

The PCR products were examined by agarose gel electrophoresis after staining and sequenced using Sanger sequencing (Sanger & Coulson, 1975).

Sequence alignment:

ITS region of each species (or till genus level) was obtained. The sequences were aligned using NCBI Blast (Altschul et al., 1990).

Nucleotide sequence retrieval:

From the BLAST result, nucleotide sequences were retrieved from NCBI nucleotide database and re-aligned using Clustal-W in MEGA X (Kumar S et al., 2018). For each tree construction a trial and error with bootstrap value was considered for the preparation of the final dataset.

Nucleotide Analysis and Phylogeny construction:

Nucleotide composition; Best tree model with minimum log likelihood and Pairwise distance matrix was computed All analysis was done in MEGA X. The result of the minimum log likelihood model was selected as the main parameter for tree construction with a bootstrap value of 1000. All other parameters were set in default. (Kimura M. 1980).

3.3. Diversity of Mushroom & Effect of disturbance on mushroom diversity:

To study the diversity and effect of disturbance on mushroom diversity species richness and diversity was conducted by selecting a disturbed area in the adjacent / nearby forest of the selected Wild Life Sanctuary. Determination of disturbed sites and undisturbed sites was done by estimation of forest canopy cover become it is an important part of forest inventories. First, canopy cover has been shown to be a multipurpose ecological indicator, which is useful for distinguishing different plant and animal habitats, assessing estimating functional variables like the leaf area index (LAI) that quantifies the photosynthesizing leaf area per unit ground area (Jennings et al. 1999, Lowman and Rinker 2004) forest floor microclimate and light conditions, and Line transects of equal length and breadth. Diversity of mushroom from the two sites was studied using quadrat method sampling method with a quadrat size of $10m^2$ having 5 replicates on each site (Luoma 1991). It was then analyse using diversity indices – Shannons (Shannon et al., 1949), Simpsons ((1949), Margalef (1958), Menhinick's (Menhinick, 1964) and using statistical analysis software MS EXCEL.

3.3.1. Diversity indices

Shannon's diversity Index (Hs) (Shanon & Weaver, 1949)

The Shannon Diversity Index (sometimes called the Shannon-Wiener Index) is a way to measure the diversity of species in a community. Denoted as H, this index is calculated as given below: The higher the value of H, the higher the diversity of species in a particular community and the lower the value of H, the lower the diversity. If value of H is equal to 0 it indicates that the community only has only one species.

The index assumes that individuals are randomly sampled from an infinitely large community (Pielou, 1975) and that all species are represented in the sample. The Shannon Index is calculated from the equation-

$$Hs = -\sum pi \ln pi$$

Where, pi = the proportion of individuals found in the i^{th} species

Or $pi = ni/N$

Where, ni =the abundance of the individual in the i^{th} species.

N = the abundance of all the species

Simpson index (Simpson, 1949)

Simpson's Diversity Index is a measure of diversity which takes into account the number of species present, as well as the relative abundance of each species. As species richness and evenness increase, so diversity increases.

Simpson index is diversity index proposed by Simpson (1949), to describe the probability that a second individual drawn from a population should be of the same species as the first.

Simpson Index (D) = $\sum$ 1-[$\sum$n(n-1)/N(n-1)]

Where, n = the total number of organisms of a particular species

N = the total number of organisms of all species

Margalef's index (Margalef, 1958).

Margalef's index was used as a simple measure of species richness

Margalef's index D= (S – 1) / In N

Where, S = total number of species

N = total number of individuals in the sample

In = natural logarithm

Menhinick's index (Menhinick, 1964)

Menhinick's index D=S/ $\sqrt{N}$

Where, S= total number of species

N= total number of individual

Evenness Index (Pielou, 1966).

$e = H / \ln S$

Where, H = Shannon – Wiener diversity index

S = total number of species in the sample

4. RESULTS AND DISCUSSION:

4.1. Taxonomic Description

4.1.1. Morphological Description:

1. Amanita vaginata Photo Plate 1.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Agaricales
Family - Amanitaceae
Genus - *Amanita*
Species - *vaginata*
Synonymous:
Morphological Characteristics:

- Cap: Convex or flat 3-8 cm in diameter.
- Hymenium: Gills on hymenium, adnate.
- Stipe: 6-15 cm long, 5-1.5 cm wide, tapering slightly to the apex.
- Spore Size: Spores 8-9µm, spherical.
- Habitat: Mycorrhizal, growing solitary, in group or gregarious on ground broad-leaved forest.
- Edibility: Not recommended.

- Season: Monsoon.

Specimen Examined: JZT/2020/PL21 INDIA, Mizoram, Kolasib, Pualreng WLS, October 2020.

2. ***Amauroderma rugosum*.** (Blume et Nees ex Fr.) Torrend, 1920
   Photo Plate 2.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Polyporales
Family - Ganodermataceae
Genus - *Amauroderma*
Species - *rugosum*
Synonymous: Fomes rugosus (Blume & T. Nees) Cooke, (1885),
Ganoderma rugosum (Blume & T. Nees) Pat., (1889), Polyporus rugosus Blume & T.
Nees, (1826), Scindalma rugosum (Blume & T. Nees) Kuntze, (1898)

Morphological Characteristics:

- Cap: - Brown to black, up to 10cm, off centre or central position of stipe, flat or convex with wavy structure.
- Hymenium: Pores.
- Stipe: 15cm long 1-2cm thick.
- Spore Size: Globose 8-10 μm.
- Habitat: Saprophytic, generally found in wood buried inside soil, tree lump or other wood substrate.
- Edibility: Unknown.

- Season: Can be found throughout the year.

Specimen Examined: JZT/2020/PL16, Mizoram, Kolasib, Pualreng WLS, August 2020.

3. ***Auricularia delicate*** (Mont.) Henn., (1893)
   Photo Plate 3.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Auriculariales
Family - Auriculariaceae
Genus - *Auricularia*
Species - *delicate*
Synonyms - Laschia delicata Fr.(1830), Auricula delicata (Fr.) Kuntze (1898), Auricularia auricula-judae var. delicata (Mont.) Rick (1958).

Morphological Characteristics:

- Cap: brown to deep brown, wavy and irregular; form ear shape,.5–15 cm across, 0.5 cm in thickness, attach laterally, gelatinous or slimy on touch.
- Hymenium: Smooth.
- Stipe: Absent.
- Spore Size: Allantoid 10-12 μm.
- Habitat: Habitat: Saprophytic, can be found in moist area, common in monsoon on wood substrate such as decaying branches and bamboos especially in Jhum sites and other disturbed areas.
- Edibility: Edible.

- Season: Can be found throughout the year.

Specimen Examined: JZT/2021/PL14, Mizoram, Kolasib, Pualreng WLS, August 2021.

4. ***Cantharellus tropicalis***

   Photo Plate 4.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Cantharellales
Family - Cantharellaceae
Genus - Cantharellus
Species - tropicalis

Morphological Characteristics:

- Cap: Up to 12 cm across; broadly convex before maturity, shallowly depressed on maturity, off white to yellowish in colour.
- Hymenium: adnate.
- Stipe: 6-10 cm long; 1 cm wide, slender, equal or tapering slightly to base, hollow.
- Spore Size: 7–11 x 4–6 µm, ellipsoid; smooth.
- Habitat: Mycorrhizal, are generally found growing solitary, in group or gregarious on ground bamboo forest.
- Edibility: Edible.
- Season: Monsoon.

Specimen Examined: JZT/2021/PL05, Mizoram, Kolasib, Pualreng WLS, August 2021.

5. ***Clavulinopsis laeticolor*** (Berk. & M.A.Curtis) R.H.Petersen (1965) Photo Plate.5

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Russulales
Family - Clavariaceae
Genus - *Clavulinopsis*
Species - *laeticolor*

Synonyms - Clavaria laeticolor Berk. & M.A. Curtis (1869), Ramariopsis laeticolor (Berk. & M.A. Curtis) R.H. Petersen (1978), Donkella laeticolor (Berk. & M.A. Curtis) Malysheva & Zmitr. (2006), Donkella laeticolor (Berk. & M.A. Curtis) Malysheva (2008), Clavaria pulchra Peck (1876).

Morphological Characteristics:

- Cap: flatten at the tip of the stipe.
- Hymenium: smooth.
- Stipe: Form cylindrical, thin and fragile structure, golden yellow to orange in color, are generally found in group.
- Spore Size: 4–8 x 3–6 µm, irregularly shape.
- Habitat: Saprophytic, growing alone, scattered, gregariously on ground, occasionally appearing on well-rotted, moss-covered stumps.
- Edibility: Unknown.

- Season: Monsoon.

Specimen Examined: JZT/2020/PL09, Mizoram, Kolasib, Pualreng WLS, August 2020.

6. ***Coprinellus disseminatus*** (Persoon) Gray (1938)
   Photo Plate.6

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Agaricales
Family - Psathyrellaceae
Genus - *Coprinellus*
Species - *disseminates*

Synonyms - Agaricus pallescens Schaeff.(1774), Agaricus disseminatus Pers., Comm. Schaeff. (1800), Agaricus disseminatus var. digitaliformis (Bull.) Pers.(1801), Coprinus disseminatus (Pers.) Gray,(1821), Agaricus disseminatus f. digitaliformis (Bull.) Fr.(1821), Coprinus petasiformis Corda (1837), Agaricus gyroflexus Fr.(1838), Coprinarius disseminatus (Pers.) P. Kumm.(1871), Psathyra gyroflexa (Fr.) P. Kumm.(1871), Coprinus digitaliformis (Bull.) P. Kumm.(1871), Psathyrella disseminata (Pers.) Quél.(1872), Drosophila gyroflexa (Fr.) Quél.(1886), Pilosace pallescens (Schaeff.) Kuntze(1898), Pseudocoprinus disseminatus (Pers.) Kühner.(1928), Psathyrella gyroflexa (Fr.) Konrad & Maubl.(1949).

Morphological Characteristics:

- Cap: up to 2cm are white and oval in development, convex or bell shape brown colour on maturity.
- Hymenium: white Gills, adnate.
- Stipe: Up to 6cm in length, thin slender, often curved, fragile, white in colour.

- Spore Size: 5-10 x 5-6 µm; elliptical.
- Habitat: Saprophytic, growing in clusters on decaying wood, especially on tree stump. A single tree stump often contains hundreds of sporocarps.
- Edibility: Not recommended.
- \Season: Monsoon may also on rare occasion be found during dry season in wet tree stump.

Specimen Examined: JZT/2020/PL15, Mizoram, Kolasib, Pualreng WLS, October 2020

7. ***Coriolopsis sp.***

Photo Plate 7.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Polyporales
Family - Polyporaceae
Genus - *Coriolopsis*
Species -
Synonyms-
Morphological Characteristics:

- Cap: 6 cm in radius, 12 cm wide, with alternating colors, orange to yellow and turn dark and pale with time.
- Hymenium: Pores.
- Stipe: Absent.
- Spore Size: Spores 5-8 x 2-3.5 µm .
- Habitat: Saprophytic

- Edibility: unknown.
- Season: Can be found throughout the year

Specimen Examined: JZT/2021/PL08, Mizoram, Kolasib, Pualreng WLS, October 2021.

8. ***Dacryopinax spathularia*** (Schwein.), (1948).
   Photo Plate 8.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Dacrymycetes
Order - Dacrymycetales
Family - Dacrymycetaceae
Genus - *Dacryopinax*
Species - *spathularia*

Synonyms - Merulius spathularius Schwein., (1822), Guepinia spathularia (Schwein.) Fr., (1828), Cantharellus spathularius Schwein., (1832), Guepiniopsis spathularia (Schwein.) Pat., (1900).

Morphological Characteristics:

- Cap: - Gelatinous and slimy, up to 1 cm, fan shape, yellow or orange in colour
- Hymenium: Smooth.
- Stipe: Small up to 2cm, cylindrical.
- Spore Size:- 8–10 × 4–7 µm curve.
- Habitat: Saprophytic, are commonly found in rotting bamboos.

- Edibility: Edible.
- Season: Monsoon.

Specimen Examined: JZT/2020/PL04, Mizoram, Kolasib, Pualreng WLS, August 2020.

9. ***Daedalea confrogosa*** (Bolton) Pers., (1801)).
   Photo Plate 9.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Polypore's
Family - Polyporaceae
Genus - *Daedalea*
Species - *confrogosa*

Synonyms - Daedaleopsis confragosa (Bolton) J. Schröt., (1888), Boletus confragosus Bolton, (1791), Trametes confragosa (Bolton) Rabenh., (1844), Polyporus confragosus (Bolton) P. Kumm., (1871),Striglia confragosa (Bolton) Kuntze, (1891), Lenzites confragosa (Bolton) Pat., (1900),Agaricus confragosus (Bolton) Murrill, (1905),Daedalea confragosa f. bulliardii (Fr.) Domanski, Orlos & Skirg., (1967), Ischnoderma confragosum (Bolton) Zmitr., (2001), Ischnoderma confragosa(Bolton) Zmitr. (2001), Daedaleopsis confragosa var. confragosa , Boletus suaveolens Bull., (1787), Boletus angustatus Sowerby, (1799),Daedalea rubescens Alb. & Schwein., (1805), Daedalea corrugata Klotzsch, (1833), Daedalea discolor Klotzsch, (1833), Lenzites crataegi Berk., (1847), Lenzites ungulaeformis Berk. & M.A. Curtis, (1849), Lenzites unguliformis Berk.& M.A. Curtis, (1849), Daedalea pruinata Secr.,
(1855),Lenzites atropurpurea Sacc., (1873), Lenzites cookei Berk., (1876), Lenzites proxima Berk. (1876), Trametes purpurascens Berk.& Broome, (1879)>
Morphological Characteristics:

- Cap: Fan shaped 5-15 cm, broadly convex brown or reddish brown; circular zones of colour
- Hymenium: Pores
- Stipe: Absent
- Spore Size: 8-12 x 2-3 µm smooth, cylindrical to elliptical
- Habitat: Saprophytic
- Edibility: unknown
- Season: Can be found throughout the year.

Specimen Examined: JZT/2020/PL25, Mizoram, Kolasib, Pualreng WLS, November 2020.

10. ***Daldinia cincentrica***

Photo Plate 10.

Domain - Eukaryota
Kingdom - Fungi
Division - Ascomycota
Class - Sordariomycetes
Order - Xylariales
Family - Hypoxylaceae
Genus - *Daldinia*
Species - *cincentrica*

Synonyms – Hemisphaeria concentrica (Bolton) Klotzsch , Sphaeria concentrica Bolton,(1792), Peripherostoma concentricum (Bolton) Gray,(1821), Peripherostomavar. concentricum (Bolton) Gray,(1821), Hypoxylon concentricum (Bolton) Grev.,(1828), Stromatosphaeria concentrica (Bolton) Grev.,(1828), Hemisphaeria concentrica (Bolton) Klotzsch,(1843).

Morphological Characteristics:

- Cap: spherical like a ball, upto 5 cm across; surface hard, smooth at first, brown to reddish brown
- Hymenium: absent
- Stipe: Absent
- Spore Size:10–68 x 6–8 µm, ellipsoid
- Habitat: Saprophytic
- Edibility: unknown
- Season: Can be found throughout the year.

Specimen Examined: JZT/2020/PL17, Mizoram, Kolasib, Pualreng WLS, October 2020.

11. ***Ganoderma applanatum*** (Fries) Patouillard (1889)
    Photo Plate 11.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order -Polyporales
Family - Ganodermataceae
Genus - *Ganoderma*
Species -*aapplanatum*

Synonyms – Fomes annularis Lloyd,(1912), Ganoderma annulare (Lloyd) Boedijn,(1940),Fomes konigsbergii Lloyd, (1915), Fomes polyzonus Lloyd, (1915) Fomes pseudoaustralis Lloyd, (1915), Polyporus scansilis Berk., (1877), Fomes scansilis (Berk.) Cooke, (1885), Scindalma scansile (Berk.) Kuntze, (1898),

Polyporus tornatus Pers., (1827), Ganoderma tornatum (Pers.) Bres., (1912), Elfvingia tornata (Pers.) Murrill, (1903), Scindalma tornatum (Pers.) Kuntze, (1898), Ganoderma applanatum var. tornatum (Pers.) (1931), Ganoderma tornatum var. tornatum (Pers.) (1912), Fomes undatus Lázaro Ibiza, (1916), Fomes koningsbergii Lloyd, (1915), Ganoderma koningsbergii (Lloyd) Teng, (1963).

Morphological Characteristics:

- Cap: - up to30 cm, zoned with curvy corner, inner curve white to off-white, with outer curve brown in colour
- Hymenium: Pores
- Stipe: Absent
- Spore Size: Spores 6–9 x 4–5 µm
- Habitat: Saprophytic
- Edibility: unknown
- Season: Can be found throughout the year.

Specimen Examined: JZT/2020/PL03, Mizoram, Kolasib, Pualreng WLS, July 2020.

12. ***Heimiomyces tenuipes*** (Schwein.) Singer (1943).

Photo Plate 12.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Agaricales
Family - Mycenaceae
Genus - *Heimiomyces*
Species - *tenuipes*

Synonyms - Collybia tenuipes (Schwein.) (1887), Gymnopus tenuipes (Schwein.) (1916), Xeromphalina tenuipes (Schwein.) (1953)

Morphological Characteristics:

- Cap: 2.5–4.5 cm across; convex to broadly convex or flat.
- Hymenium: whitish to yellowish; short-gills frequent.
- Stipe: 3–7.5 cm long; 2–4 mm thick; more or less equal
- Spore Size: Spores 6–8 x 3.5–4.5 µm; ellipsoid;
- Habitat: Saprophytic
- Edibility: unknown
- Season: Monsoon

Specimen Examined: JZT/2021/PL10, Mizoram, Kolasib, Pualreng WLS, August 2020.

13. Hymenopellis sp. (Berk.)R.H.Petersen (2010).

Photo Plate 13.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class -Agaricomycetes
Order - Agaricales
Family - Physalacriaceae
Genus - *Hymenopellis*
Species -

Synonyms –

Morphological Characteristics:

- Cap: 1-5 cm wide, convex
- Hymenium: Gills on Hymenium
- Stipe: 25 cm high with long roots
- Spore Size: : 2.5-3.5 x 1-1.5 µm, elliptical or oblong
- Habitat: Saprophytic
- Edibility: unknown
- Season: Monsoon

Specimen Examined: JZT/2021/PL11, Mizoram, Kolasib, Pualreng WLS, August 2020.

14. ***Lentinus squarrosulus*** Mont (1842).
    Photo Plate 14.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Dacrymycetes
Order - Dacrymycetales
Family - Dacrymycetaceae
Genus - *Dacryopinax*
Species - *spathularia*
Synonyms -
Pleurotus squarrosulus (Mont.) (1962), Pleurotus squarrosulus (Mont.) (1969 Pocillaria squarrosula (Mont.) (1891) Lentinus tigrinus f. squarrosulus (Mont.) Mycologici (1936).

Morphological Characteristics:

- Cap: 12 cm convex with deeply umblicate center.
- Gills: Gills on hymenium
- Hymenium: white to yellowish white
- Stipe: Upto 5 cm, central to eccentric, solid, white, smooth, equal, somewhat flattened.
- Spore Size:: 5-7 x 2-3 µm ellipisoid
- Habitat: Saprophytic
- Edibility: Edible
- Season: Monsoon

Specimen Examined: JZT/2020/PL19, Mizoram, Kolasib, Pualreng WLS, July 2020.

15. Microporus xanthopus (Fr.) Kuntze, (1898)

Photo Plate 15.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Polyporales
Family - Polyporaceae
Genus - *Microporus*
Species - *xanthopus*

Synonyms - Polyporus xanthopus Fr., (1818), Polystictus xanthopusFr., (1851),Coriolus xanthopus (Fr.) G. Cunn., (1950), Trametes xanthopus (Fr.) Corner, 1989), Polyporus saccatus Pers., (1827), Polyporus pterygodes Fr., (1838), Polyporus florideus Berk., (1854), Polyporus cupreonitens Kalchbr., (1881).

Morphological Characteristics:

- Cap: Flat to broadly funnel shaped, 2- 6 cm diameter
- Hymenium: Pores
- Stipe: Stipe central to slightly eccentric, upto 5 cm.
- Spore Size:: Ellipsoid; 3.5–4 × 2– 2.5µm, smooth.
- Habitat: Saprophytic
- Edibility: unknown
- Season: Can be found throughout the year.

Specimen Examined: JZT/2020/PL02, Mizoram, Kolasib, Pualreng WLS, August 2020.

16. ***Mycena acicula*** (Schaeff.) P.Kumm. (1871)
    Photo Plate 16.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Agaricales
Family - Mycenaceae
Genus - *Mycena*
Species - *acicula*

Synonyms - Trogia acicula (Schaeff.) Corner (1966), Marasmiellus acicula (Schaeff.) Singer, (1951), Hemimycena acicula (Schaeff.) Singer (1891), Agaricus miniatus Batsch, (1783).

Morphological Characteristics:

- Cap: convex and bell shape upto 0.5 cm
- Hymenium: Gills are adnate
- Stipe: Red or Orange upto 2 cm
- Spore Size: 9–11 by 3.5–4.5 um
- Habitat: Saprophytic
- Edibility: unknown
- Season: Monsoon

Specimen Examined: JZT/2020/PL13, Mizoram, Kolasib, Pualreng WLS, August 2020.

17. Panus sp.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Polyporales
Family - Polyporaceae
Genus - Panus
Species -

Morphological Characteristics:

- Cap: Convex funnel shape, up to 10 cm in diameter.
- Hymenium: Gillis are decurrent
- Stipe: Cylindrical, 2-5 cm, hairy brown.
- Spore Size: Clavate, 15-25 x 5-10 um.

- Habitat: Saprophytic
- Edibility: unknown
- Season: Monsoon

Specimen Examined: JZT/2021/PL13, Mizoram, Kolasib, Pualreng WLS, August 2020.

18. ***Phallus indusiatus*** Vent. (1798)
19. Photo Plate 18.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Phallales
Family - Phallaceae
Genus - *Phallus*
Species - *indusiatus*

Synonyms - Dictyophora indusiata (Vent.) Desv. (1809), Hymenophallus indusiatus (Vent.) Nees (1817).

Morphological Characteristics:

- Cap: Spike-like forming a net like structure
- Stipe: up 25 cm with a volva.
- Spore Size: s 2.5-3.5 x 1-1.5 µ; long-elliptical to nearly cylindric.
- Habitat: Saprophytic
- Edibility: unknown
- Season: Monsoon

Specimen Examined: JZT/2020/PL27, Mizoram, Kolasib, Pualreng WLS, August 2020.

20. ***Schizophyllum commune*** Fr., (1821)

Photo Plate 19.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Agaricales
Family - Schizophylaceae
Genus - *Schizophylum*
Species - *commune*

Synonyms - Schizonia vulgaris Pers., (1828), Daedalea commune (Fr.) P. Kumm., (1871), Merulius communis (Fr.) Spirin & Zmitr., (2004), Agaricus alneusL., (1753), Scaphophoeum agaricoides Ehrenb. (1820), Schizophyllum alneus (L.) Kuntze, (1898)

Morphological Characteristics:

- Cap: 1–4 cm across; fan-shaped
- Hymenium: Greyish, folded
- Stipe: Up to 4 cm
- Spore Size: 4–6.5 x 1.5–2 µm
- Habitat: Saprophytic
- Edibility: Edible
- Season: Can be found throughout the year

Specimen Examined: JZT/2020/PL28, Mizoram, Kolasib, Pualreng WLS, August 2020.

21. ***Termitomyces heimii*** Natarajan (1979).
    Photo Plate 20.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Agaricales
Family - Lyophyllaceae
Genus - *Termitomyces*
Species - *heimii*

Synonyms –
Morphological Characteristics:

- Cap: Upton 10 com Convex to flat on maturity.
- Hymenium: Free gills
- Stipe: Upto 15 cm, cylindrical with a thick annulus
- Spore Size: Spores 7-8.5 x 4.2-5.6 µm.
- Habitat: mycorrhizal
- Edibility: Edible
- Season: Monsoon

Specimen Examined: JZT/2020/PL20, Mizoram, Kolasib, Pualreng WLS, August 2020.

22. ***Trametes coccineus*** Murrill (1904)

Photo Plate 21.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Polyporales
Family - Polyporaceae
Genus - *Trametes*
Species - *coccineus*

Synonyms - Boletus sanguineus L., (1763), Boletus nitens Batsch, (1783), Polyporus sanguineus (L.) G. Mey., (1818),Polystictus sanguineus (L.) Fr., (1851), Microporus sanguineus (L.) Kuntze, 1898), Trametes sanguinea (L.) Lloyd, (1924), Trametes cinnabarina var. sanguinea (L.) Pilát, (1940), Trametes sanguinea(L.) Imazeki, (1943), Coriolus sanguineus (L.) G. Cunn., (1949), Fabisporus sanguineus (L.) Zmitr., (2001), Boletus ruber Lam., (1783), Polyporus cristula Klotzsch ex Berk., (1839).

Morphological Characteristics:

- Cap: Semicircular to kidney-shaped; upto 10 cm across
- Hymenium: Pores
- Stipe: absent
- Spore Size: Spores 5-8 x 2.5-3 µ; smooth;.
- Habitat: Saprophytic
- Edibility: unknown
- Season: Can be found throughout the year.

Specimen Examined: JZT/2020/PL18, Mizoram, Kolasib, Pualreng WLS, August 2020.

23. Trametes elegans (Spreng.) Fr.,(1838),
Photo Plate 22.

Domain - Eukaryota
Kingdom - Fungi
Division - Basidiomycota
Class - Agaricomycetes
Order - Polyporales
Family - Polyporaceae
Genus - *Trametes*
Species - *elegans*

Synonyms - Daedalea elegans Spreng., (1820), Lenzites elegans(Spreng.) Pat., (1900), Whitfordia elegans (Spreng.) Singer,(1951), Daedaleopsis elegans (Spreng.) (1974), Artolenzites elegans (Spreng.) (1986), Daedalea amanitoides P. Beauv., (1806), Daedalea levis Hook., (1822), Boletus aesculi-flavae Schwein., (1822), Daedalea repanda Pers., (1827), Daedalea deplanata Link ex Fr., (1830), Daedalea polita Fr., (1830).

Morphological Characteristics:

- Cap: Up to 35 cm across and 3 cm thick; semicircular, irregularly bracket-shaped, or kidney-shaped
- Hymenium: Pores
- Stipe: Absent
- Spore Size: Spores 5-7 x 2-3 µ; smooth; cylindrical to long-elliptic
- Habitat: Saprophytic

- Edibility: unknown
- Season: Can be found throughout the year

Specimen Examined: JZT/2020/PL30, Mizoram, Kolasib, Pualreng WLS, August 2020.

24. ***Xylaria bambusicola*** Y.M. Ju & J.D. Rogers(1999).
Photo Plate 23.

Domain - Eukaryota
Kingdom - Fungi
Division - Ascomycota
Class - Sordariomycetes
Order - Xylariales
Family - Xylariaceae
Genus - *Xylaria*
Species - *bambusicola*

Synonyms –
Morphological Characteristics:

- Cap: 2.5–6.5 cm tall; 0.5–1.5 cm thick; shaped more or less like a club, with a pointy;
- Hymenium: Absent
- Stipe: often proportionally long, but also frequently short or nearly absent, upto 5 cm
- Spore Size: 12–16 x 5–6 µm
- Habitat: Saprophytic
- Edibility: unknown
- Season: Can be found throughout the year

Specimen Examined: JZT/2020/PL23, Mizoram, Kolasib, Pualreng WLS, August 2020.

Disucussion: The morphological characteristics were used to identify 20 species of mushroom up to species level while 3 species were identified up to genus level. The morphological parameters used for the identification of mushroom specimens such as- cap colour, cap surface, cap margin, cap diameter, stipe length, gill attachment, gill spacing and spore dimension. Microscopic features were carried out using standard microscopic methods (Roy, 1998). Due to the lack of appropriate literature for references 3 species were identified up to genus level only. According to Smith (1968) and Clemençon, (1977) morphological species concept is where the characters (phenotypes) of individual organisms are compared, and similar individuals are designated as a species. And due to the polymorphism nature (Cooke, 1871) of mushrooms, 9 species were confirmed by molecular analysis.

4.1.3. Molecular Anlaysis

1. Auricularia delicata

Sequence alignment

The ITS PCR amplicon generated 581 base pair (GenBank Acc. No. OL839322). The sequence was aligned through BLAST (Results shown in Table 1). The query coverage, total score and percentile identity was approx. 91 - 98%, 953 – 968 and 96.7 – 99.25 respectively.

Table 1: NCBI BLAST result of *Auricularia delicate* specimen

Sl No.	Scientific Name	Max Score	Total Score	Query Cover	E value	% Identity	Acc. Len	Accession
1	*Auricularia sp.*	968	968	95%	0	98.03	617	MN832910.1
2	*Auricularia delicata*	968	968	95%	0	98.03	576	KX022017.1
3	*Auricularia delicata*	968	968	95%	0	98.03	567	MW363490.1
4	*Auricularia delicata*	965	965	98%	0	97.2	569	MT252524.1
5	*Auricularia delicata*	965	965	91%	0	99.25	533	KX621149.1
6	*Auricularia delicata*	965	965	91%	0	99.25	533	KX621147.1
7	*Auricularia delicata*	963	963	95%	0	97.85	586	KX022020.1
8	*Auricularia delicata*	961	961	97%	0	97.19	567	MT252526.1
9	*Auricularia*	961	961	91%	0	99.06	533	KX6211

	delicata							56.1
10	*Auricularia delicata*	953	953	98%	0	96.7	604	KF297965.1

Nucleotide sequence retrieval

From the BLAST result, query coverage of 98% was selected for the final dataset. Additional nucleotide sequences were retrieved from NCBI GenBank (Table 2) for sequence analysis and phylogeny construction. These sequences were also included in the final dataset.

Table 2: Nucleotide sequence retrieved for phylogeny construction.

Sl No.	Scientific Name	Query Cover	Percent Identity	Acession No.
1	*Auricularia delicata*			OL839322
2	*Auricularia delicata*	98%	97.2	MT252524.1
3	*Auricularia delicata*	98%	96.7	KF297965.1
4	*Auricularia cornea*			JX065164.1
5	*Auricularia cornea*			MK610700.1
6	*Auricularia nigricans*			JX065176.1
7	*Auricularia nigricans*			JX65167.1
8	*Auricularia auricula judae*			MW830140.1
9	*Auricularia auricula judae*			MW830139.1

Nucleotide Analysis and Phylogeny construction

The final data set had 9 sequences of the genus *Auricularia*. The overall nucleotide sequences had an average of 602.89 base pair in length. The nucleotide composition of each base was Thymine (26.43 %), Cytosine (25.56 %), Adenine (24.20%) and Guanine (23.81%). 510 bases were computed identical for all 9 ITS sequences, 16

bases as transitional pair and 12 bases had undergone transversion. The rate of nucleotide substitution was computed to be 1.37.

Analyses were conducted using the Kimura 2-parameter model with gamma distribution. A total of 796 nucleotide positions were computed for phylogeny construction in MEGA X as Shown in Figure 1.

The clade of *Auricularia delicata* had a bootstrap value of 99% indicating that species clustering is more or less well supported (Figure 1). However, when each sequence distance was evaluated, the sample obtained locally was found to be more diverse than expected when compared with sequences retrieved from GenBank (MT252524 and KF297965). The distance matrix computed between our sample and GB Acc. No. MT252524 was 0.027 ± 0.007; with KF297965 it was 0.036 ± 0.008.

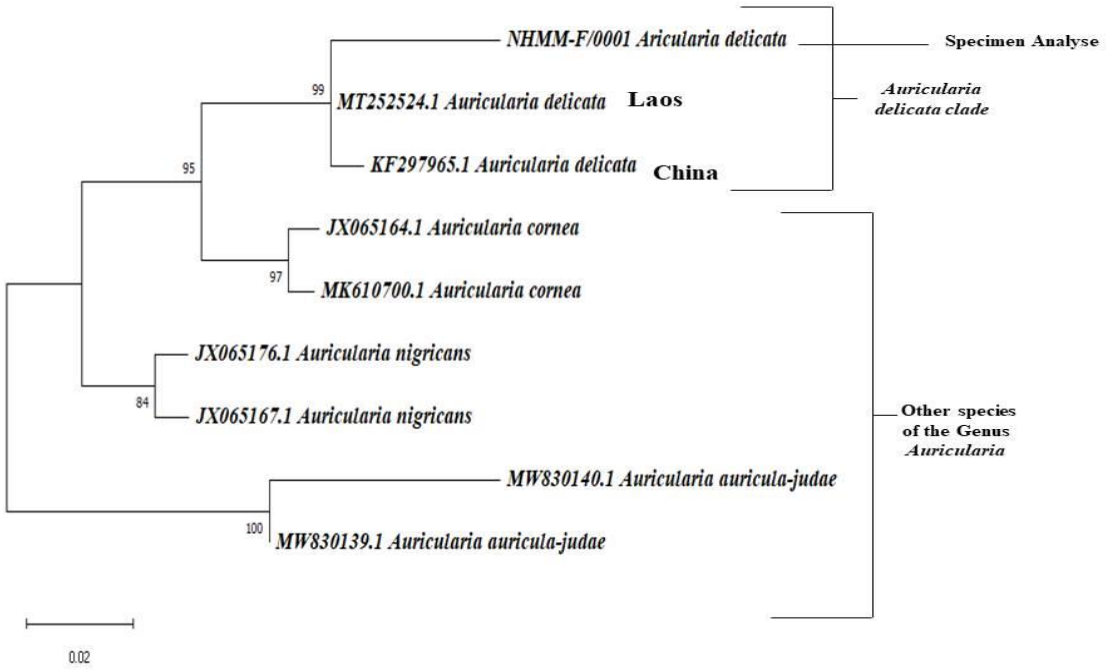


Fig 1: Phylogeny constructs of Auricularia delicata using MEGA X software.

1. ***Coriolopsis sp.***

Sequence alignment

The ITS PCR amplicon generated 633 base pair (GenBank Acc. No. OL839323). The sequence was aligned through BLAST (Results shown in Table 3). The query coverage, total score and percentile identity was approx. 89 - 100%, 1020 – 1134 and 99.01 – 99.89% respectively.

Table 3: NCBI BLAST result of *Coriolopsis sp.* specimen

Sl No.	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
1	*Coriolopsis caperata*	1134	1134	100%	0	99.05	783	MZ649036.1
2	*Coriolopsis sanguinaria*	1134	1134	100%	0	99.05	656	MW742553.1
3	*Coriolopsis sanguinaria*	1129	1129	100%	0	98.89	656	MW742555.1
4	*Coriolopsis caperata*	1127	1127	99%	0	98.89	648	MZ649021.1
5	*Coriolopsis sanguinaria*	1118	1118	100%	0	98.58	656	MW742554.1
6	*Coriolopsis caperata*	1109	1109	99%	0	98.42	654	KU535647.1
7	*Coriolopsis sanguinaria*	1101	1101	96%	0	99.01	620	MK192428.1
8	*Coriolopsis sanguinaria*	1042	1042	89%	0	99.82	567	KC867389.1
9	*Coriolopsis sanguinaria*	1026	1026	89%	0	99.29	567	KC867387.1
10	*Coriolopsis sanguinaria*	1020	1020	89%	0	99.12	567	KC867388.1

Nucleotide sequence retrieval

From the BLAST result, query coverage of 100% was selected for the final dataset. Additional nucleotide sequences were retrieved (Table 2) for sequence analysis and phylogeny construction. These sequences were also included in the final dataset.

Table 4: Nucleotide sequence retrieved for phylogeny construction.

Sl No.	Scientific Name	Query Cover	Percent Identity	Acession No.
1.	*Coriolopsis Sp.*			NHMM-F/0002
2	*Coriolopsis caperata*	100%	99.05	MZ649036.1
3	*Coriolopsis sanguinaria*	100%	98.89	MW742555.1
4	*Coriolopsis sanguinaria*	100%	99.05	MW742553.1
5	*Coriolopsis trogii*			MW335162.1
6	*Coriolopsis trogii*			OK641918.1
7	*Coriolopsis hainanensis*			KC867377.1
8	*Coriolopsis hainanensis*			KC867376.1
9	*Coriolopsis dendriformis*			KC867408.1
10	*Coriolopsis dendriformis*			KC867409.1

Nucleotide Analysis and Phylogeny construction

The final data set had 10 sequences of the genus *Coriolopsis*. The overall nucleotide sequences had an average of 637.8 base pair in length. The nucleotide composition of each base was Thymine (29.82%), Cytosine (22.26%), Adenine (23.42%) and Guanine (24.49%). 530 bases were computed identical for all 10 ITS sequences, 34 bases as transitional pair and 18 bases had undergone transversion. The rate of nucleotide substitution was computed to be 1.88.

Analyses were conducted using the Kimura 2-parameter model with gamma distribution. A total of 796 nucleotide positions were computed for phylogeny construction in MEGA X as Shown in Figure 2.

The clade of *Corioplopsis sp* had a bootstrap value of 99% indicating that species clustering is more or less well supported (Figure 2). However, when each sequence distance was evaluated, the sample obtained locally was found to be more diverse than expected when compared with sequences retrieved from GenBank (MZ649036.1, MW742555.1 and MW742553.1). The distance matrix computed between our sample and GB Acc. No. MZ649036.1 was 0.012± 0.004; with MW742555.1 it was 0.0096± 0.004 and with MW742553.1 it was 0.063± 0.003.

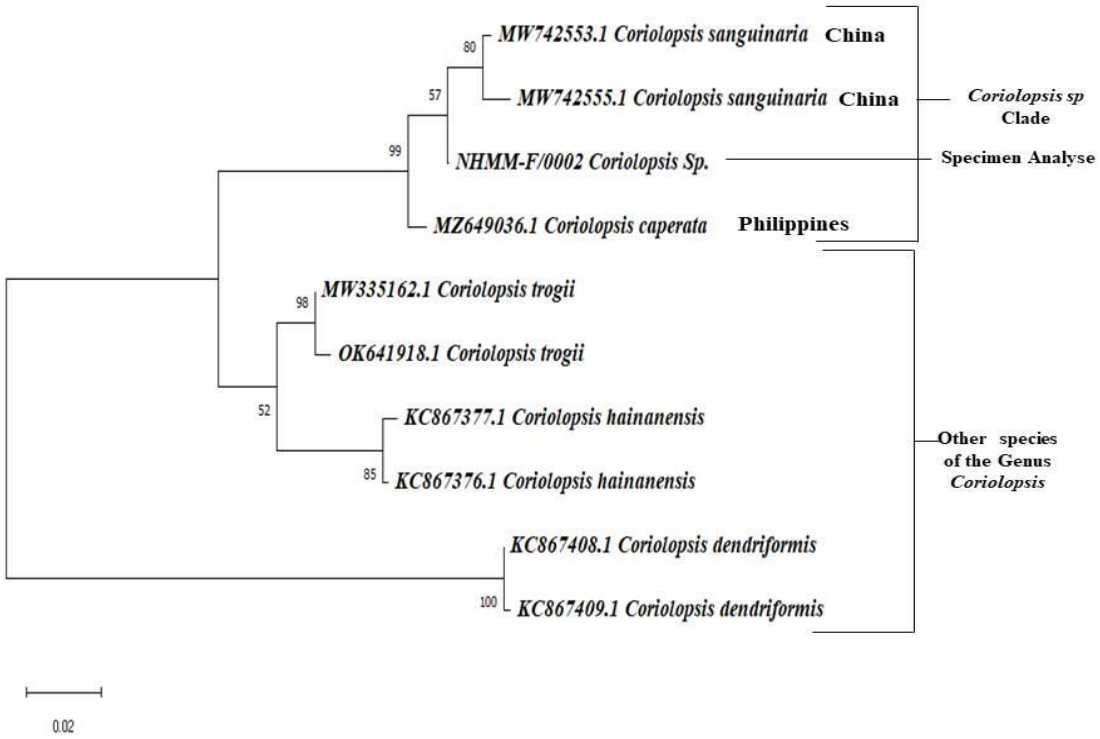


Fig 2: Phylogeny constructs of *Auricularia delicata* using MEGA X software.

2. ***Ganoderma applanatum***

Sequence alignment

The ITS PCR amplicon generated 738 base pair (GenBank Acc. No. MG448603*). The sequence was aligned through BLAST (Results shown in Table 5). The query

coverage, total score and percentile identity was approx. 92 - 100%, 1020 – 1134 and 96.51 – 98.54% respectively.

Table 5: NCBI BLAST result of *Ganoderma appalanatum* specimen

Sl No.	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
1	*Ganoderma australe*	1310	1310	100%	0	98.26	772	LC084663.1
2	*Ganoderma sp.*	1306	1306	100%	0	98.26	812	MK131240.1
3	*Ganoderma sp.*	1306	1306	100%	0	98.26	1196	KP012934.1
4	*Ganoderma sp.*	1301	1301	100%	0	98.12	824	MK131242.1
5	*Ganoderma australe*	1290	1290	100%	0	97.86	769	LC084749.1
6	*Ganoderma applanatum*	1277	1277	98%	0	98.09	757	MZ649010.1
7	*Ganoderma applanatum*	1273	1273	100%	0	97.45	777	GU213473.1
8	*Ganoderma australe*	1267	1267	100%	0	97.32	796	GU213474.1
9	*Ganoderma applanatum*	1234	1234	100%	0	96.51	810	GU213472.1
10	*Ganoderma applanatum*	1219	1219	92%	0	98.54	716	KR867655.1

Nucleotide sequence retrieval

From the BLAST result, query coverage of 98% was selected for the final dataset. Additional nucleotide sequences were retrieved (Table 6) for sequence analysis and phylogeny construction. These sequences were also included in the final dataset.

Table 6: Nucleotide sequence retrieved for phylogeny construction.

Sl No.	Scientific Name	Query Cover	Percent Identity	Acession No.
1.	*Ganoderma applanatum*			MG448603
2	*Ganoderma australe*	100%	98.26	*LC084663.1*

3	*Ganoderma applanatum*	98%	98.09	*MZ649010.1*
4	*Ganoderma applanatum*	100%	97.45	*GU213473.1*
5	*Ganoderma lingzhi*			*MH109677.1*
6	*Ganoderma lingzhi*			*MW139644.1*
7	*Ganoderma lucidum*			*KX358403.1*
8	*Ganoderma lucidum*			*KX358402.1*
9	*Ganoderma sinense*			*MK313125.1*
10	*Ganoderma sinense*			*MK313128.1*

Nucleotide Analysis and Phylogeny construction

The final data set had 10 sequences of the genus *Ganoderma*. The overall nucleotide sequences had an average of 658.3base pair in length. The nucleotide composition of each base was Thymine (28.63%), Cytosine (23.04%), Adenine (23.16%) and Guanine (25.15%). 547 bases were computed identical for all 10 ITS sequences, 24 bases as transitional pair and 13 bases had undergone transversion. The rate of nucleotide substitution was computed to be 1.84.

Analyses were conducted using the Kimura 2-parameter model. A total of 827 nucleotide positions were computed for phylogeny construction in MEGA X as Shown in Figure 3.

The clade of *Ganoderma applanatum* had a bootstrap value of 86% indicating that species clustering is more or less well supported (Figure 3). However, when each sequence distance was evaluated, the sample obtained locally was found to be more diverse than expected when compared with sequences retrieved from GenBank (*LC084663.1, MZ649010.1 and GU213473.1*). The distance matrix computed between our sample and GB Acc. No. *LC084663.1* was 0.004± 0.002; with *MZ649010.1* it was 0.008± 0.003 and with *GU213473.1 it* was 0.013± 0.004.

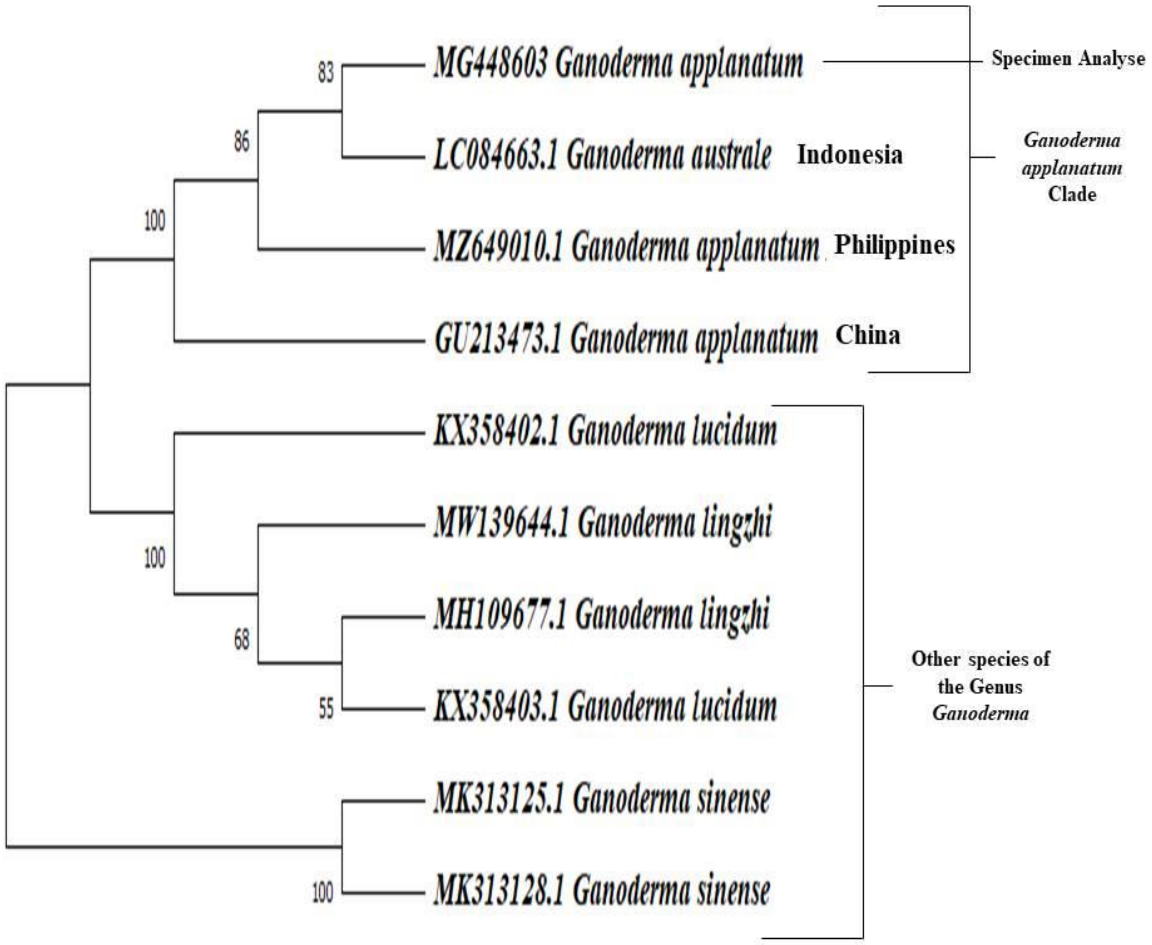


Fig 3: Phylogeny constructs of *Ganoderma appalanatum* using MEGA X software.

3. ***Heimiomyces tenuipes***

Sequence alignment

The ITS PCR amplicon generated 704 base pair (GenBank Acc. No. OL839329). The sequence was aligned through BLAST (Results shown in Table 7). The query coverage, total score and percentile identity was approx. 47 - 96%, 549 – 1267 and 84.48 – 98.09% respectively.

Table 7. NCBI BLAST result of *Hemiomyces tenupes* specimen

Sl No.	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
1	*Heimiomyces tenuipes*	1267	1267	96%	0	98.09	756	MF100953.1

2	*Heimiomyces tenuipes*	1164	1164	88%	0	97.93	676	MW445914.1
3	*Heimiomyces sp.*	1009	1009	85%	0	95.05	644	MN492640.1
4	*Xeromphalina sp*	693	693	98%	0	84.48	755	KP133251.1
5	*Heimiomyces neovelutipes*	675	675	89%	0	85.59	664	KT120056.1
6	*Heimiomyces sp.*	665	665	69%	0	90.06	699	MT755874.1
7	*Xeromphalina sp.*	664	664	47%	0	100	359	AB509965.1
8	*Heimiomyces atrofulvus*	658	833	88%	0	90.33	661	KM975407.1
9	*Heimiomyces sp.*	586	586	61%	1.00E-162	89.87	618	MT755873.1
10	*Xeromphalina enigmatica*	549	549	67%	2.00E-151	86.9	721	MK049915.1

Nucleotide sequence retrieval

From the BLAST result, query coverage of 88% was selected for the final dataset. Additional nucleotide sequences were retrieved (Table 8) for sequence analysis and phylogeny construction. These sequences were also included in the final dataset.

Table 8: Nucleotide sequence retrieved for phylogeny construction.

Sl No.	Scientific Name	Query Cover	Percent Identity	Acession No.
1.	*Heimiomyces tenuipe*			
2	*Heimiomyces tenuipes*	96%	98.09	MF100953.1
3	*Heimiomyces tenuipes*	88%	97.93	MW445914.1
4	*Xeromphalina sp*	98%	84.48	KP133251.1
5	*Amphisphaeria flava*			*NR 168782.1*
6	*Amphisphaeria flava*			*MH971224*
7	*Panellus stipticus*			*MH855557*
8	*Panellus stipticus*			*MH855556.1*
9	*Mycena acicula*			*MW540677*

10	*Mycena acicula*			*MW448625.1*

Nucleotide Analysis and Phylogeny construction

The final data set had 10 sequences of the Family *Mycenaceae*. The overall nucleotide sequences had an average of 704 base pair in length. The nucleotide composition of each base was Thymine (31.5%), Cytosine (22.7%), Adenine (23.3%) and Guanine (22.5%). 411 bases were computed identical for all 10 ITS sequences, 90 bases as transitional pair and 99 bases had undergone transversion. The rate of nucleotide substitution was computed to be 0.9.

Analyses were conducted using the hasegawa-kishino-yano model with invariant sites. A total of 720 nucleotide positions were computed for phylogeny construction in MEGA X as Shown in Figure 4.

The clade of *Hemiomyces tenupes* had a bootstrap value of 100% indicating that species clustering is more or less well supported (Figure 4). However, when each sequence distance was evaluated, the sample obtained locally was found to be more diverse than expected when compared with sequences retrieved from GenBank (MF100953.1, MW445914.1 *and* KP133251.1). The distance matrix computed between our sample and GB Acc. No. MF100953.1 was 0.008± 0.003; with MW445914.1 it was 0.009± 0.003 and with KP133251.1*it* was 0.15± 0.002.

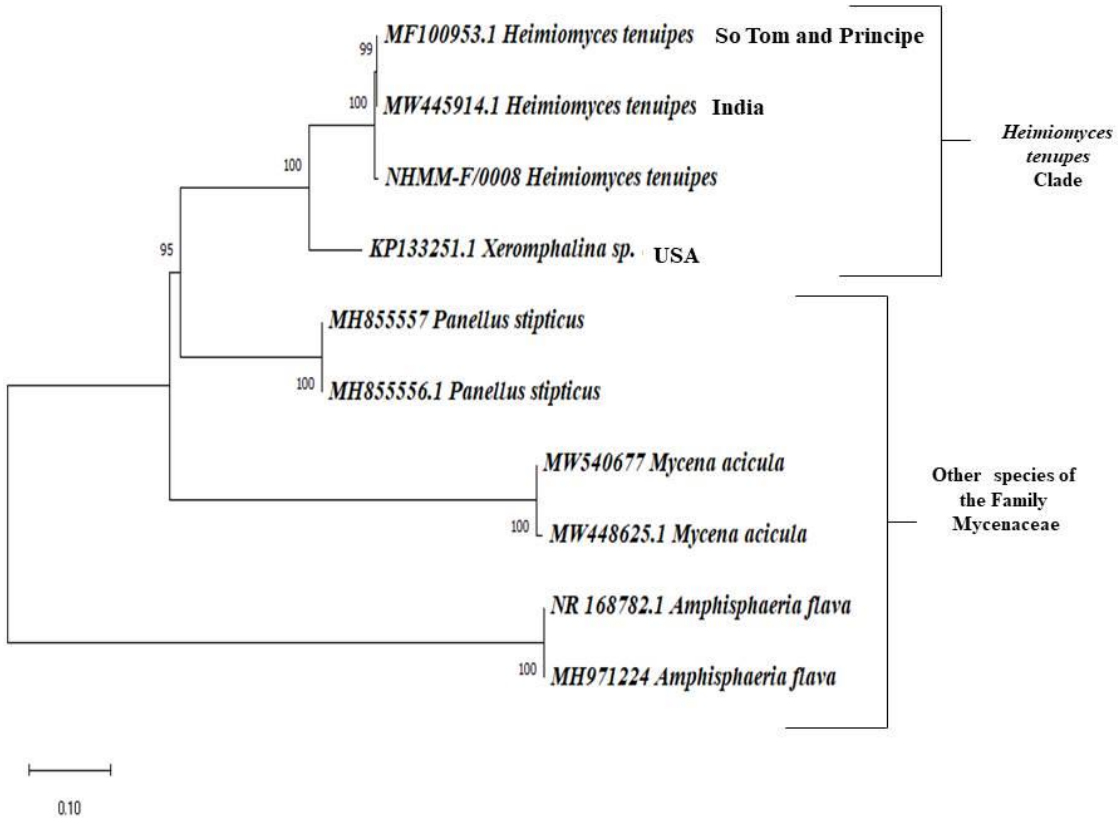


Fig 4: Phylogeny constructs of Hemiomyces tenupes using MEGA X software.

4. ***Hymenopellis sp.***

Sequence alignment

The ITS PCR amplicon generated 741 base pair (GenBank Acc. No. OL839324). The sequence was aligned through BLAST (Results shown in Table 9). The query coverage, total score and percentile identity was approx. 92 - 98%, 1325 – 1419 and 97.49 – 99.49% respectively.

Table 9. NCBI BLAST result of *Hemiomyces raphanipes* specimen

Sl. No	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
1	*Hymenopellis raphanipes*	1419	1419	98%	0	99.49	830	KX688231.1

2	*Hymenopellis raphanipes*	1404	1404	98%	0	99.11	832	KX688246.1
3	*Hymenopellis raphanipes*	1395	1395	98%	0	98.97	826	KX688233.1
4	*Hymenopellis raphanipes*	1387	1387	98%	0	98.72	831	KX688240.1
5	*Hymenopellis raphanipes*	1378	1378	98%	0	98.47	830	KX688247.1
6	*Hymenopellis raphanipes*	1352	1352	97%	0	98.44	785	LC512057.1
7	*Hymenopellis raphanipes*	1352	1352	99%	0	97.49	835	MW857135.1
8	*Hymenopellis raphanipes*	1341	1341	94%	0	99.19	780	MT822928.1
9	*Hymenopellis raphanipes*	1327	1327	92%	0	99.45	775	KX688239.1
10	*Hymenopellis raphanipes*	1325	1325	93%	0	98.92	752	GU980129.1

Nucleotide sequence retrieval

From the BLAST result, query coverage of 97% was selected for the final dataset. Additional nucleotide sequences were retrieved (Table 10) for sequence analysis and phylogeny construction. These sequences were also included in the final dataset.

Table 10: Nucleotide sequence retrieved for phylogeny construction.

Sl No.	Scientific Name	Query Cover	Percent Identity	Acession No
1	*Hymenopellis Sp.*			NHMM-F/00
2	*Hymenopellis raphanipes*	98%	98.97	KX688231
3	*Hymenopellis raphanipes*	97%	98.44	LC512057

4	*Hymenopellis raphanipes*	99%	97.49	MW857135.1
5	*Hymenopellis chiangmaiae*			GU980123
6	*Hymenopellis chiangmaiae*			GU980127
7	*Armillaria aotearoa*			KR063261
8	*Armillaria aotearoa*			KR063263.1
9	*Xerula pudens*			AF321493
10	*Xerula pudens*			AF321492.1
11	*Rhizomarasmius oreinus*			KM588679.1
12	*Rhizomarasmius oreinus*			KM588676

Nucleotide Analysis and Phylogeny construction

The final data set had 12 sequences of the Family *Physalacriaceae*. The overall nucleotide sequences had an average of 723.5 base pair in length. The nucleotide composition of each base was Thymine (33.7%), Cytosine (20.9%), Adenine (22.5%) and Guanine (22.9%). 577 bases were computed identical for all 12 ITS sequences, 58 bases as transitional pair and 51 bases had undergone transversion. The rate of nucleotide substitution was computed to be 1.1.

Analyses were conducted using the hasegawa-kishino-yano model with gamma distribution. A total of 805 nucleotide positions were computed for phylogeny construction in MEGA X as Shown in Figure 5.

The clade of *Hymenopellis sp* had a bootstrap value of 75% indicating that species clustering is more or less supported (Figure 5). However, when each sequence distance was evaluated, the sample obtained locally was found to be more diverse than expected when compared with sequences retrieved from GenBank (KX688231, LC512057 *and* MW857135.1). The distance matrix computed between our sample and GB Acc. No. KX688231 was 0.004± 0.004; with LC512057 it was 0.012± 0.004 and with MW857135.1 *it* was 0.018± 0.005.

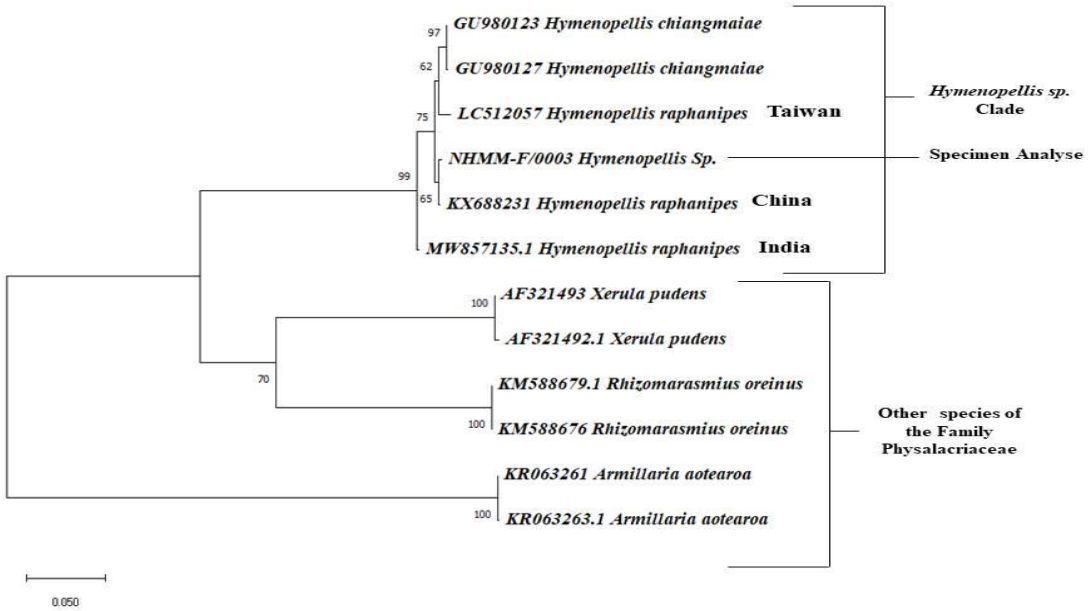


Fig 5: Phylogeny constructs of *Hymenopellis sp.* using MEGA X software.

5. ***Lentinus squarrosulus***

Sequence alignment

The ITS PCR amplicon generated 625 base pair (GenBank Acc. No. OL839327). The sequence was aligned through BLAST (Results shown in Table 11). The query coverage, total score and percentile identity was approx. 89 - 100%, 937 – 1122 and 92.62 – 97.92 % respectively.

Table 11. NCBI BLAST result of *Lentinus squarrosulus* specimen

Sl No.	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
1	*Lentinus squarrosulus*	1122	1122	100%	0	97.71	681	JQ868749.1
2	*Lentinus squarrosulus*	1122	1122	100%	0	97.71	682	JQ868748.1
3	*Lentinus squarrosulus*	1103	1103	98%	0	97.81	670	MW577318.1

4	*Lentinus squarrosulus*	1096	1096	97%	0	97.8	636	MW374186.1
5	*Lentinus squarrosulus*	1081	1081	95%	0	97.92	629	JQ868747.1
6	*Lentinus sp.*	1046	1046	97%	0	96.26	642	JQ868745.1
7	*Lentinus squarrosulus*	1024	1024	91%	0	97.65	596	KP340800.1
8	*Lentinus squarrosulus*	979	979	89%	0	96.92	593	MK851533.1
9	*Lentinus squarrosulus*	968	968	90%	0	96.43	593	MK851532.1
10	*Lentinus squarrosulus*	937	937	100%	0	92.62	683	GU001951.1

Nucleotide sequence retrieval

From the BLAST result, query coverage of 98% was selected for the final dataset. Additional nucleotide sequences were retrieved (Table 12) for sequence analysis and phylogeny construction. These sequences were also included in the final dataset.

Table 12: Nucleotide sequence retrieved for phylogeny construction.

Sl No.	Scientific Name	Query Cover	Percent Identity	Acession No.
1.	*Lentinus squarrosulus*			NHMM-F/0006
2	*Lentinus squarrosulus*	100%	97.71	JQ868749.1
3	*Lentinus squarrosulus*	98%	97.81	MW577318.1
4	*Lentinus tigrinus*			KY565250.1
5	*Lentinus tigrinus*			MT212398.1
6	*Lentinus sajor-caju*			MT249306.1
7	*Lentinus sajor-caju*			MT249305.1

8	*Lentinus roseus*			KY490136
9	*Lentinus roseus*			KY490135.1

Nucleotide Analysis and Phylogeny construction

The final data set had 9 sequences of the Genus *Lentinus.* The overall nucleotide sequences had an average of 611.4 base pair in length. The nucleotide composition of each base was Thymine (29.7%), Cytosine (23.5%), Adenine (22.5%) and Guanine (24.2%). 521 bases were computed identical for all 9 ITS sequences, 41 bases as transitional pair and 29 bases had undergone transversion. The rate of nucleotide substitution was computed to be 1.4.

Analyses were conducted using Kimura 2-parameter model with gamma distribution. A total of 652 nucleotide positions were computed for phylogeny construction in MEGA X as Shown in Figure 5.

The clade of *Lentinus squarrosulus* had a bootstrap value of 100% indicating that species clustering is more or less well supported (Figure 5). However, when each sequence distance was evaluated, the sample obtained locally was found to be more diverse than expected when compared with sequences retrieved from GenBank (JQ868749.1 and MW577318.1). The distance matrix computed between our sample and GB Acc. No. JQ868749.1 was 0.019± 0.005and with MW577318.1 it was 0.019± 0.005.

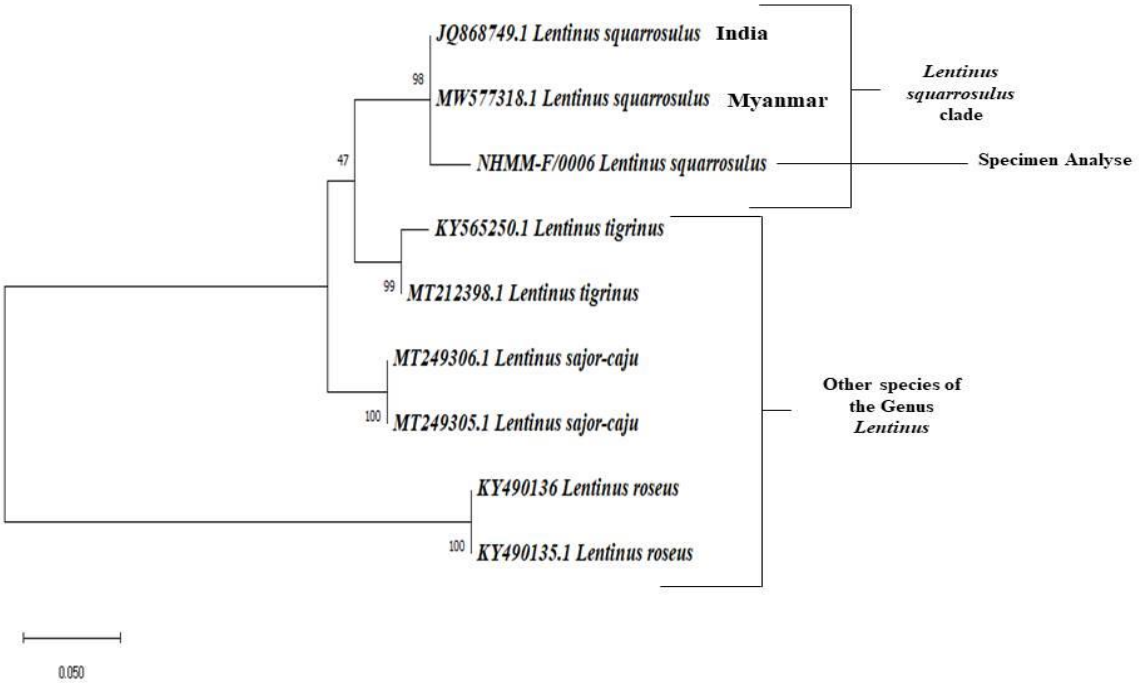


Fig 6: Phylogeny constructs of *Lentinus squarrosulus* using MEGA X software.

6. ***Microporus xanthopus***

Sequence alignment

The ITS PCR amplicon generated 618 base pair (GenBank Acc. No. MG719305*). The sequence was aligned through BLAST (Results shown in Table 13). The query coverage, total score and percentile identity was approx. 89 - 100%, 937 – 1122 and 92.62 – 97.92 % respectively.

Table 13. NCBI BLAST result of *Lentinus squarrosulus* specimen

Sl No.	Scientific Name	Max Score	Total Score	Query Cover	E valu e	Per. ident	Acc. Len	Accession
1	*Microporus xanthopus*	1232	1232	99%	0	97.38	842	LC14959 5.1
2	*Microporus xanthopus*	1210	1210	99%	0	96.71	125 3	KP01268 6.1

3	*Microporus vernicipes*	1171	1171	89%	0	99.23	649	KU863045.1
4	*Microporus affinis*	1162	1162	95%	0	96.85	1196	KP013022.1
5	*Microporus affinis*	1160	1160	96%	0	96.59	1205	KP012889.1
6	*Microporus vernicipes*	1144	1144	88%	0	99.06	637	MH221090.1
7	*Microporus vernicipes*	1138	1138	89%	0	98.31	658	MW742523.1
8	*Microporus xanthopus*	1133	1133	90%	0	98.02	657	MK811322.1
9	*Microporus vernicipes*	1133	1133	90%	0	98.02	657	MW742522.1
10	*Microporus vernicipes*	1131	1131	90%	0	98.02	655	MW742527.1

Nucleotide sequence retrieval

From the BLAST result, query coverage of 95% was selected for the final dataset. Additional nucleotide sequences were retrieved (Table 14) for sequence analysis and phylogeny construction. These sequences were also included in the final dataset.

Table 14: Nucleotide sequence retrieved for phylogeny construction.

Sl No.	Scientific Name	Query Cover	Percent Identity	Acession No
1	*Microporus xanthopus*			MG719305
2	*Microporus xanthopus Australia*	99%	96.71	KP012686
3	*Microporus affinis Australia*	96%	96.59	KP012889
4	*Microporus affinis Australia*	95%	96.85	KP013022

5	*Microporus xanthopus Nepal*	99%	97.38	LC149595
6	*Trametes elegans*			MG270573
7	*Trametes elegans*			MN888943.1
8	*Daedalea quercina*			MK607490.1
9	*Daedalea quercina*			MZ159711
10	*Coriolopsis caperata*			MZ649036
11	*Coriolopsis caperate*			OL629609.1

Nucleotide Analysis and Phylogeny construction

The final data set had 11 sequences of the Family Polyporaceae. The overall nucleotide sequences had an average of 618 base pair in length. The nucleotide composition of each base was Thymine (29.5 %), Cytosine (23.6%), Adenine (23.1%) and Guanine (23.8%). 497 bases were computed identical for all 11 ITS sequences, 40 bases as transitional pair and 37 bases had undergone transversion. The rate of nucleotide substitution was computed to be 1.1.

Analyses were conducted using Kimura 2-parameter model with gamma distribution. A total of 669 nucleotide positions were computed for phylogeny construction in MEGA X as Shown in Figure 7.

The clade of Microporus xanthopus had a bootstrap value of 95% indicating that species clustering is more or less well supported (Figure 7). However, when each sequence distance was evaluated, the sample obtained locally was found to be more diverse than expected when compared with sequences retrieved from GenBank (KP012686 and LC149595). The distance matrix computed between our sample and GB Acc. No. KP012686 was 0.027± 0.007 and with LC149595it was 0.03± 0.007.

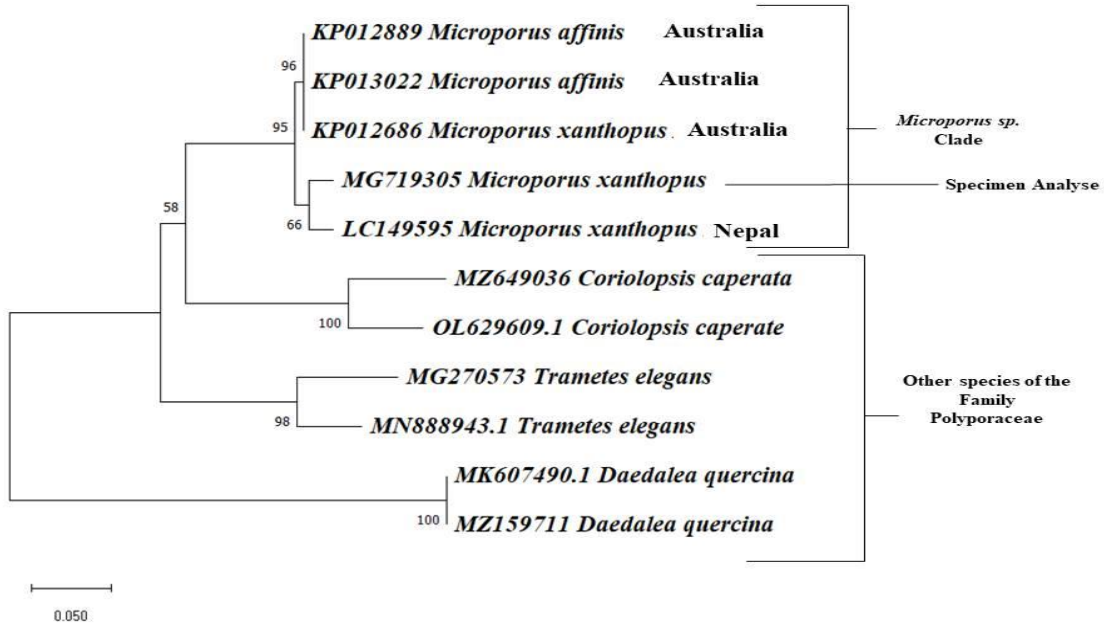


Fig 7: Phylogeny constructs of *Microporus xanthopus* using MEGA X software.

7. ***Panus sp.***

Sequence alignment

The ITS PCR amplicon generated 1209 base pair (GenBank Acc. No.OL839328). The sequence was aligned through BLAST (Results shown in Table 15). The query coverage, total score and percentile identity was approx. 88 - 100%, 905 – 1149 and 92.8 – 99.37 % respectively.

Table 15. NCBI BLAST result of *Panus sp.* specimen

Sl No.	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
1	*Panus sp.*	1149	1149	100%	0	99.37	717	KP686453.1
2	*Panus sp.*	1033	1033	88%	0	99.82	611	MG279699.1
3	*Panus sp.*	981	981	94%	0	96.23	608	JF741922.1

4	*Panus conchatus*	933	933	100%	0	93.41	678	MH857778.1
5	*Panus conchatus*	929	929	99%	0	93.51	1556	JN710579.1
6	*Panus strigellus*	924	924	100%	0	93.23	695	MT669137.1
7	*Panus conchatus*	922	922	99%	0	93.35	654	MH855431.1
8	*Panus conchatus*	909	909	99%	0	92.89	632	MG231758.1
9	*Panus conchatus*	905	905	98%	0	92.8	703	KM411463.1
10	*Panus strigellus*	905	905	97%	0	93.25	625	MW407012.1

Nucleotide sequence retrieval

From the BLAST result, query coverage of 100% was selected for the final dataset. Additional nucleotide sequences were retrieved (Table 16) for sequence analysis and phylogeny construction. These sequences were also included in the final dataset.

Table 16: Nucleotide sequence retrieved for phylogeny construction.

Sl No.	Scientific Name	Query Cover	Percent Identity	Acession No.
1.	*Panus sp.*	100%	99.37	*NHMM-F/0007*
2	*Panus sp*	100%	93.41	*KP686453*
3	*Panus conchatus*			*MH857778*
4	*Panus velutinus*			*MT669138.1*
5	*Panus velutinus*			*MW374215.1*
6	*Panus similis*			*OL839236.1*
7	*Panus similis*			*OL839257.1*

8	*Panus strigellus*			*MW407012.1*
9	*Panus strigellus*			*MT669137*

Nucleotide Analysis and Phylogeny construction

The final data set had 9 sequences of the Genus *Panus.* The overall nucleotide sequences had an average of 867.8 base pair in length. The nucleotide composition of each base was Thymine (30.5%), Cytosine (22.7%), Adenine (23.6%) and Guanine (23.3%). 639 bases were computed identical for all 9 ITS sequences, 28 bases as transitional pair and 20 bases had undergone transversion. The rate of nucleotide substitution was computed to be 1.4.

Analyses were conducted using Tamura 3-parameter model with gamma distribution. A total of 1241 nucleotide positions were computed for phylogeny construction in MEGA X as Shown in Figure 8.

The clade of *Panus sp.* had a bootstrap value of 99% indicating that species clustering is more or less well supported (Figure 8). However, when each sequence distance was evaluated, the sample obtained locally was found to be more diverse than expected when compared with sequences retrieved from GenBank (*KP686453*). The distance matrix computed between our sample and GB Acc. No. *KP686453* was 0.013±0.003.

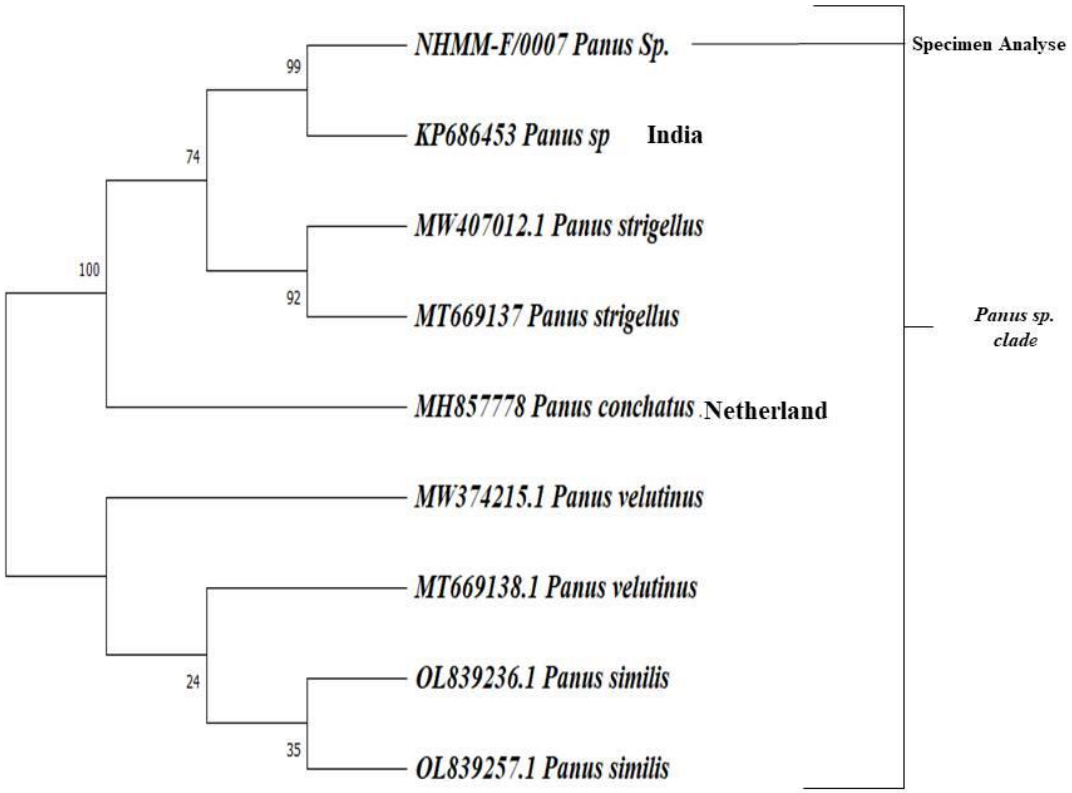


Fig 8: Phylogeny constructs of *Panus sp.*using MEGA X software.

8. ***Schizophyllum commune***

Sequence alignment

The ITS PCR amplicon generated 580 base pair (GenBank Acc. No. MG437405*). The sequence was aligned through BLAST (Results shown in Table 17). The query coverage, total score and percentile identity was approx. 89 - 100%, 937 – 1122 and 92.62 – 97.92 % respectively.

Table 17. NCBI BLAST result of *Schizophyllum commune* specimen

Sl.No	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
1	*Schizophyllu*	1295	1295	100%	0	99.8	130	MH30793

	m commune					6	6	2.1
2	*Schizophyllum commune*	1295	1295	100%	0	99.86	1601	KX958030.1
3	*Schizophyllum commune*	1295	1295	100%	0	99.86	733	OL764361.1
4	*Schizophyllum commune*	1290	1290	100%	0	99.72	729	KR706163.1
5	*Schizophyllum commune*	1290	1290	100%	0	99.72	757	MZ649042.1
6	*Schizophyllum commune*	1284	1284	100%	0	99.57	1772	KX034183.1
7	*Schizophyllum commune*	1279	1279	100%	0	99.43	1106	MN783217.1
8	*Schizophyllum commune*	1271	1271	100%	0	99.29	1767	MG569497.1
9	*Schizophyllum commune*	1271	1271	98%	0	99.86	1573	KX958047.1
10	*Schizophyllum commune*	1256	1256	97%	0	99.56	715	KX958074.1

Nucleotide sequence retrieval

From the BLAST result, query coverage of 98% was selected for the final dataset. Additional nucleotide sequences were retrieved (Table 18) for sequence analysis and phylogeny construction. These sequences were also included in the final dataset.

Table 18: Nucleotide sequence retrieved for phylogeny construction

Sl No.	Scientific Name	Query Cover	Percent Identity	Acession No.
1	*Schizophyllum commune*			MG437405
2	*Schizophyllum commune*	100%	99.86	MH307932

3	*Schizophyllum commune*	100%	99.86	KX958030
4	*Schizophyllum commune*	100%	99.86	OL764361
5	*Schizophyllum commune*	100%	99.72	KR706163
6	*Auriculariopsis ampla*			AY293169
7	*Auriculariopsis ampla*			AY570991
8	*Schizophyllum fasciatum*			L43385
9	*Schizophyllum fasciatum*			LT217559.1
10	*Panellus stipticus*			MH855557
11	*Panellus stipticus*			MH855556
12	*Mycena acicula*			MW540677
13	*Mycena acicula*			MW448625

Nucleotide Analysis and Phylogeny construction

The final data set had 13 sequences of the Order *Agaricales.* The overall nucleotide sequences had an average of 639 base pair in length. The nucleotide composition of each base was Thymine (29.8 %), Cytosine (22.7 %), Adenine (. 25.1%) and Guanine (22.4%). 425 bases were computed identical for all 13 ITS sequences, 61 bases as transitional pair and 76 bases had undergone transversion. The rate of nucleotide substitution was computed to be 0.8.

Analyses were conducted using Kimura 2-parameter model with gamma distribution. A total of 1243 nucleotide positions were computed for phylogeny construction in MEGA X as Shown in Figure 8.

The clade of Schizophyllum sp. had a bootstrap value of 99% indicating that species clustering is more or less well supported (Figure 9). However, when each sequence distance was evaluated, the sample obtained locally was found to be more diverse than expected when compared with sequences retrieved from GenBank (MH307932, KX958030, OL764361 and KR706163). The distance matrix computed between our sample and GB Acc. No. MH307932 was 0.0014± 0.003, with KX958030 it was

0.014± 0.003, with OL764361 it was 0.014±0.003 and with KR706163 it was 0.0028±0.002.

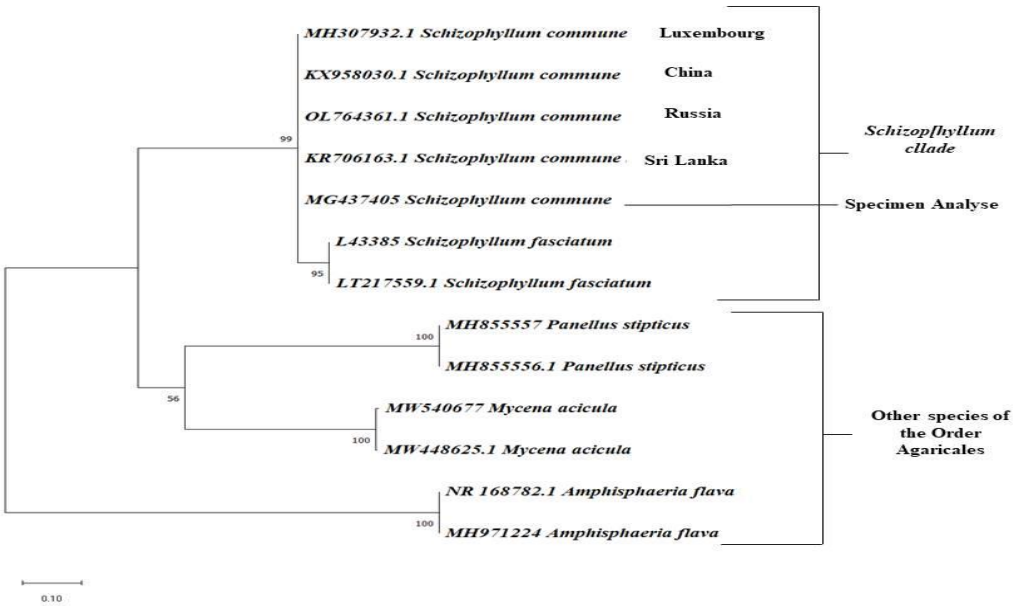


Fig 9: Phylogeny constructs of *Schizophyllum commune* using MEGA X software.

9. ***Trametes coccineus***

Sequence alignment

The ITS PCR amplicon generated 580 base pair (GenBank Acc. No. MG273728*). The sequence was aligned through BLAST (Results shown in Table 17). The query coverage, total score and percentile identity was approx. 100%, 1088 – 1094 and 99.83 – 100% respectively.

Table 19. NCBI BLAST result of *Trametes coccinea* specimen

Sl No.	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
1	*Trametes coccinea*	1094	1094	100%	0	100	630	MH142006.1
2	*Trametes sanguinea*	1088	1088	100%	0	99.83	619	MT340981.1

3	*Trametes sanguinea*	1088	1088	100%	0	99.83	624	MN416288.1
4	*Trametes sanguinea*	1088	1088	100%	0	99.83	634	MH857087.1
5	*Trametes coccinea*	1088	1088	100%	0	99.83	637	KP255835.1
6	*Trametes sanguinea*	1088	1088	100%	0	99.83	624	KJ850206.1
7	*Pycnoporus sp. (in: Fungi)*	1088	1088	100%	0	99.83	628	OK643817.1
8	*Pycnoporus sp. (in: Fungi)*	1088	1088	100%	0	99.83	644	OK586749.1
9	*Pycnoporus sp. (in: Fungi)*	1088	1088	100%	0	99.83	638	OK586736.1
10	*Trametes sanguinea*	1088	1088	100%	0	99.83	634	KC525202.1

Nucleotide sequence retrieval

From the BLAST result, query coverage of 98% was selected for the final dataset. Additional nucleotide sequences were retrieved (Table. 20) for sequence analysis and phylogeny construction. These sequences were also included in the final dataset.

Table 20: Nucleotide sequence retrieved for phylogeny construction.

Sl No.	Scientific Name	Query Cover	Percent Identity	Acession No.
1	*Pycnoporus coccineus*			MG273728.1
2	*Trametes coccinea*	100%	100	MH142006
3	*Trametes sanguinea*	100%	99.83	MT340981
4	*Pycnoporus coccineus*	100%	99.83	KP255835
5	*Trametes hirsuta*			OK271075

6	*Trametes hirsuta*			KX056103.1
7	*Trametes gibbosa*			MN096596
8	*Trametes gibbosa*			MH855141.1
9	*Trametes versicolor*			MN749366
10	*Trametes versicolor*			JF437649.1

Nucleotide Analysis and Phylogeny construction

The final data set had 10 sequences of the Genus *Trametes.* The overall nucleotide sequences had an average of 574.2 base pair in length. The nucleotide composition of each base was Thymine (28.6%), Cytosine (24.4%), Adenine (22.7%) and Guanine (22.7%). 536 bases were computed identical for all 10 ITS sequences, 20 bases as transitional pair and 13 bases had undergone transversion. The rate of nucleotide substitution was computed to be 1.6.

Analyses were conducted using Kimura 2-parameter model. A total of 592 nucleotide positions were computed for phylogeny construction in MEGA X as Shown in Figure 10.

The clade of *Trametes coccinea* had a bootstrap value of 100% indicating that species clustering is more or less well supported (Figure 10). However, when each sequence distance was evaluated, the sample obtained locally was found to be similar expected when compared with sequences retrieved from GenBank (MH142006, MT340981and KP255835). The distance matrix computed between our sample and GB Acc. No. MH142006 was 0±0, with MT340981 it was 0±0 and with KP255835 it was 0±0.

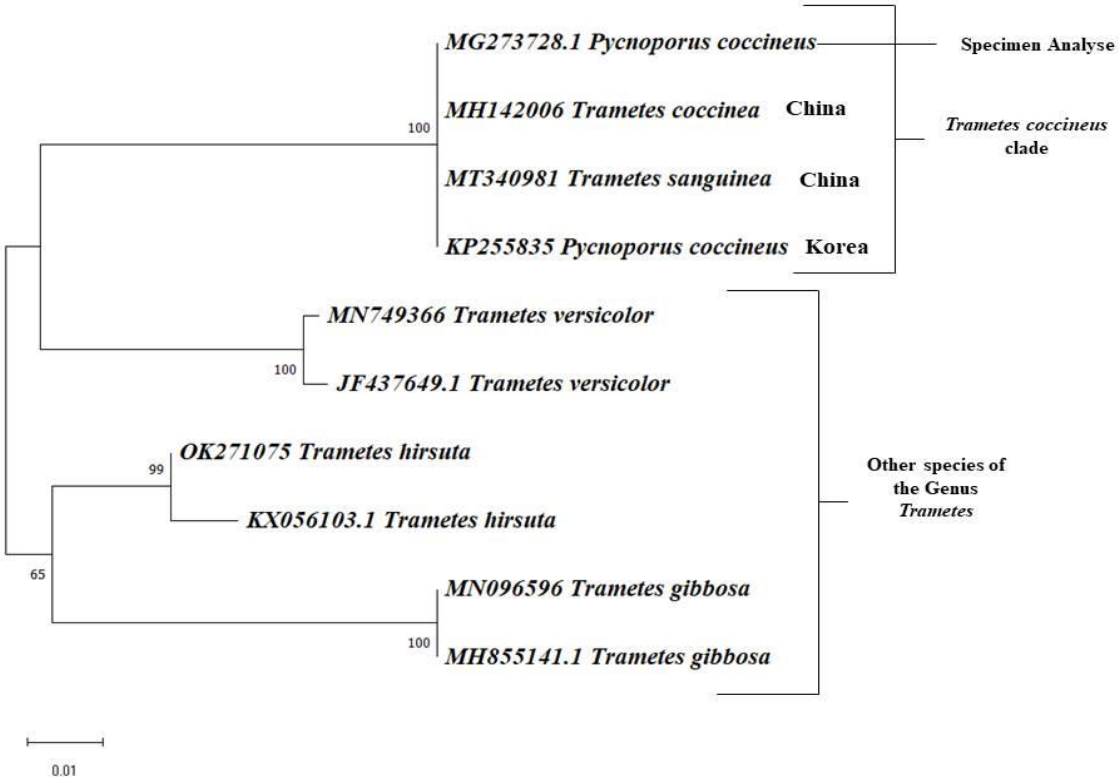


Fig 10: Phylogeny constructs of *Trametes coccinea* using MEGA X software.

10. ***Trametes elegans***

Sequence alignment

The ITS PCR amplicon generated 571 base pair (GenBank Acc. No. OL839325). The sequence was aligned through BLAST (Results shown in Table 17). The query coverage, total score and percentile identity was approx. 96-100%, 1098 – 1142 and 98.83 – 100% respectively.

Table 21. NCBI BLAST result of *Schizophyllum commune* specimen

Sl No.	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
1	*Trametes elegans*	1142	1142	100%	0	100	634	MZ452984.1
2	*Trametes elegans*	1142	1142	100%	0	100	654	MW157265.1
3	*Trametes elegans*	1142	1142	100%	0	100	643	HQ248217.1
4	*Trametes elegans*	1122	1122	98%	0	100	616	MG270573.1

5	*Trametes cubensis*	1114	1114	98%	0	99.67	624	MT672492.1
6	*Trametes cubensis*	1109	1109	98%	0	99.67	682	MT645652.1
7	*Trametes elegans*	1107	1107	97%	0	99.67	619	MT597858.1
8	*Leiotrametes lactinea*	1099	1099	96%	0	99.83	604	HM756193.1
9	*Leiotrametes lactinea*	1098	1098	100%	0	98.71	682	MH910526.1
10	*Leiotrametes lactinea*	1098	1098	100%	0	98.71	1043	KP012950.1

Nucleotide sequence retrieval

From the BLAST result, query coverage of 100% was selected for the final dataset. Additional nucleotide sequences were retrieved (Table. 22) for sequence analysis and phylogeny construction. These sequences were also included in the final dataset.

Table 22: Nucleotide sequence retrieved for phylogeny construction.

Sl No.	Scientific Name	Query Cover	Percent Identity	Acession No.
1	*Trametes elegans*			NHMM-F/0004
2	*Trametes elegans*	100%	100	MZ452984
3	*Trametes elegans*	100%	100	MW157265
4	*Lenzites elegans*	100%	100	HQ248217
5	*Trametes hirsuta*			OK271075
6	*Trametes hirsuta*			KX056103.1
7	*Trametes versicolor*			MN749366
8	*Trametes versicolor*			JF437649.1
9	*Trametes gibbosa*			MH855141.1

10	*Trametes gibbosa*			MN096596

Nucleotide Analysis and Phylogeny construction

The final data set had 10 sequences of the Genus *Trametes.* The overall nucleotide sequences had an average of 581.4 base pair in length. The nucleotide composition of each base was Thymine (28.8%), Cytosine (24.4%), Adenine (22.9%) and Guanine (24.0%). 540 bases were computed identical for all 10 ITS sequences, 18 bases as transitional pair and 12 bases had undergone transversion. The rate of nucleotide substitution was computed to be 1.5.

Analyses were conducted using Kimura 2-parameter model with gamma distribution. A total of 646 nucleotide positions were computed for phylogeny construction in MEGA X as Shown in Figure 11.

The clade of *Trametes elegans* had a bootstrap value of 100% indicating that species clustering is more or less well supported (Figure 11). However, when each sequence distance was evaluated, the sample obtained locally was found to be similar expected when compared with sequences retrieved from GenBank (MZ452984, MW157265 and HQ248217). The distance matrix computed between our sample and GB Acc. No. MZ452984 was 0±0, with MW157265 it was 0±0 and with HQ248217 it was 0±0.

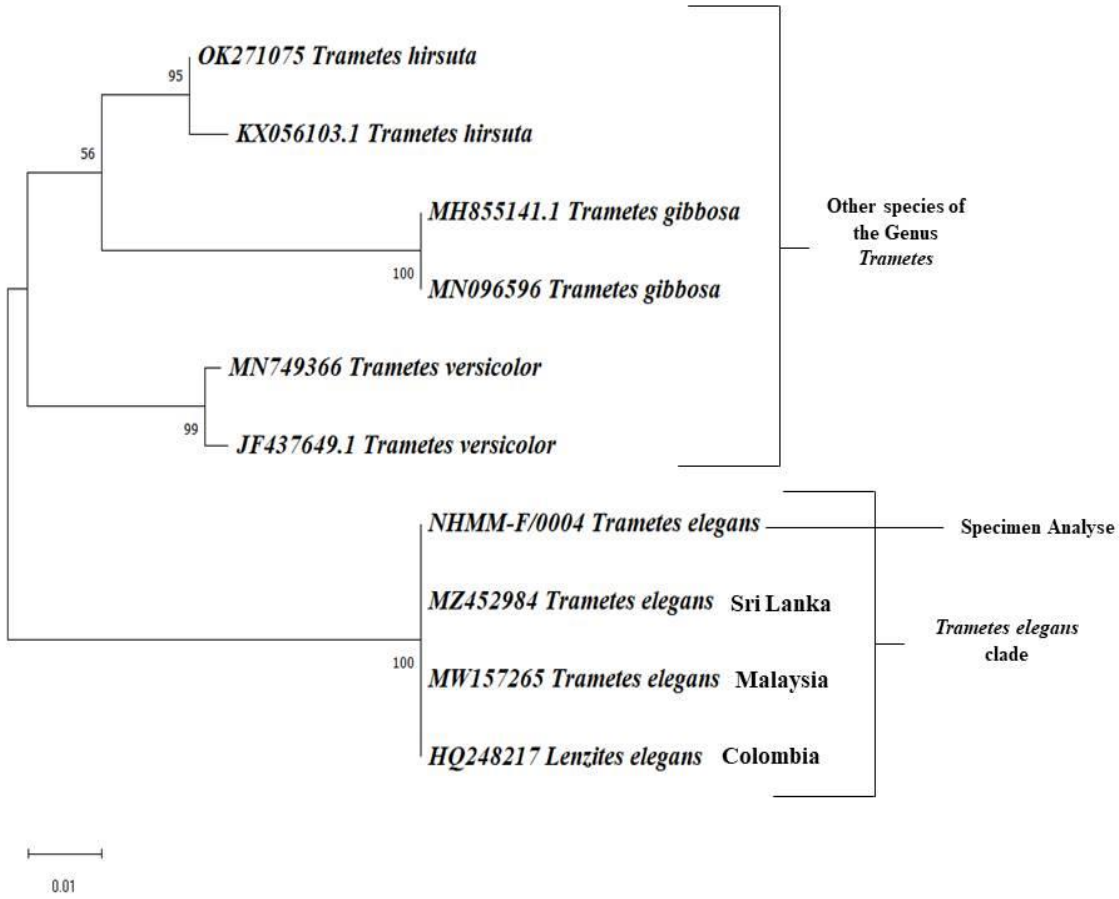


Fig 11: Phylogeny constructs of *Trametes elegans* using MEGA X software.

11. ***Xylaria bambusicola***

Sequence alignment

The ITS PCR amplicon generated 580 base pair (GenBank Acc. No. MG437400*). The sequence was aligned through BLAST (Results shown in Table 17). The query coverage, total score and percentile identity was approx. 92 - 99%, 896 – 992 and 95.14 – 97.94 % respectively.

Table 23. NCBI BLAST result of *Xylaria bambusicola* specimen

Sl No.	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
1	*Xylariaceae*	922	922	92%	0	97.94	543	MK24789

	sp.							7.1
2	*Xylaria bambusicola*	922	922	92%	0	97.94	543	MT908693.1
3	*Xylaria sp.*	911	911	99%	0	95.49	589	KF430809.1
4	*Xylaria bambusicola*	905	905	98%	0	95.46	579	JX256819.1
5	*Xylaria bambusicola*	904	904	99%	0	95.3	583	KU940160.1
6	*Xylaria bambusicola*	900	900	98%	0	95.29	581	MF379351.1
7	*Xylaria sp.*	900	900	99%	0	95.14	575	MF045812.1
8	*Xylaria bambusicola*	900	900	99%	0	95.14	585	NR_153200.1
9	*Xylaria sp.*	896	896	98%	0	95.42	576	OK643850.1
10	*Xylaria sp.*	896	896	98%	0	95.42	566	OK643845.1

Nucleotide sequence retrieval

From the BLAST result, query coverage of 99% was selected for the final dataset. Additional nucleotide sequences were retrieved (Table 22) for sequence analysis and phylogeny construction. These sequences were also included in the final dataset.

Table 24: Nucleotide sequence retrieved for phylogeny construction.

Sl No.	Scientific Name	Query Cover	Percent Identity	Acession No.
1	*Xylaria bambusicola*			MG437400
2	*Xylaria bambusicola*	99%	95.14	NR

				153200.1
3	*Xylaria bambusicola*	99%	95.3	KU940160.1
4	*Xylaria sp.*	99%	95.49	KF430809.1
5	*Lasiosphaeria ovina*			GQ922528.1
6	*Lasiosphaeria ovina*			MH863967
7	*Ophiocordyceps sinensis*			MZ701909
8	*Ophiocordyceps sinensis*			MF407089.1
9	*Tricholoma joachimii*			KY937183.1
10	*Tricholoma joachimii*			KY937184.1

Nucleotide Analysis and Phylogeny construction

The final data set had 10 sequences of the Division Ascomycota The overall nucleotide sequences had an average of 493 base pair in length. The nucleotide composition of each base was Thymine (25.7%), Cytosine (27.5%), Adenine (25.1%) and Guanine (23.8 %). 338 bases were computed identical for all 10 ITS sequences, 65 bases as transitional pair and 59 bases had undergone transversion. The rate of nucleotide substitution was computed to be 1.1.

Analyses were conducted using Kimura 2-parameter model with gamma distribution. A total of 546 nucleotide positions were computed for phylogeny construction in MEGA X as Shown in Figure 12.

The clade of Xylaria sp. had a bootstrap value of 100% indicating that species clustering is more or less well supported (Figure 12). However, when each sequence distance was evaluated, the sample obtained locally was found to be more diverse than expected when compared with sequences retrieved from GenBank (NR 153200.1, KU940160.1 and KF430809.1). The distance matrix computed between our sample and GB Acc. No. NR 153200.1 was 0.04± 0.01, with KU940160.1 it was 0.04± 0.01 and with KF430809.1 it was 0.04±0.01.

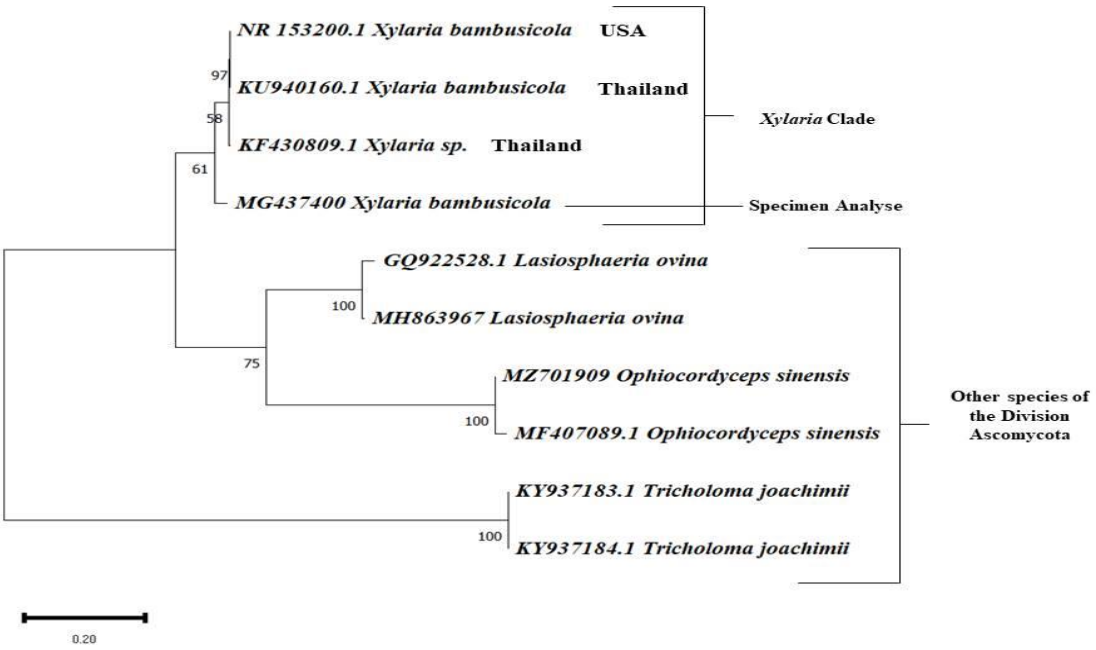
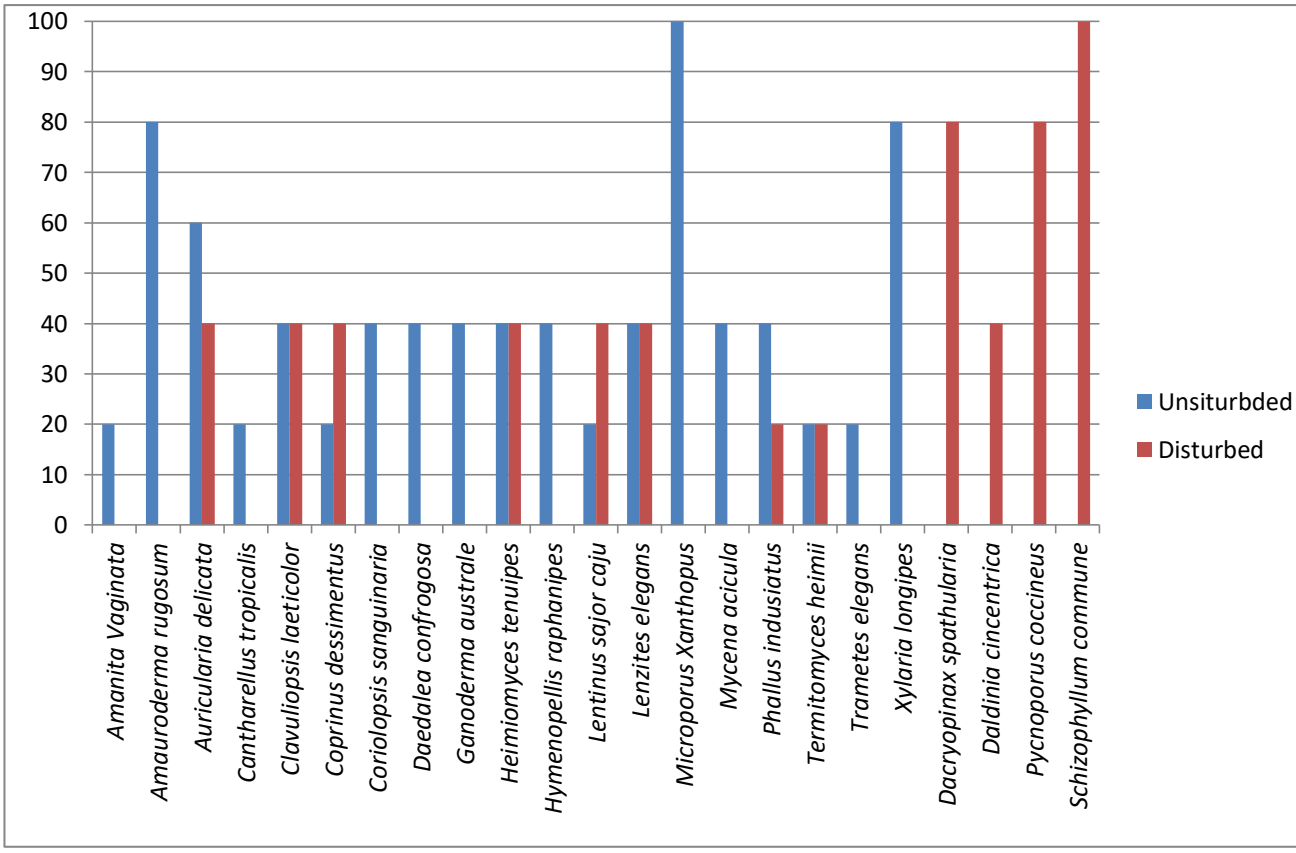


Fig 12: Phylogeny constructs of *Xylaria bambusicola* using MEGA X software.

4.2. Diversity of Mushroom & Effect of disturbance on mushroom diversity:

4.2.1. Population Study:

A total of 22 Species of mushroom were collected from two study sites undisturbed (Pualreng Wildlife Sanctuary) and disturbed sites (Jhum area around Hlimen Village) which are spread across 16 families and 22 genus. A total of 19 species were found on undisturbed site, while 12 species were found in disturbed sites and a total of 8 species are found in both ecosystems. *Mycroporus xanthopus* and *Schizophyllum commune* are the mushroom species that has the most number of frequencies for undisturbed and disturbed sites respectively and both the species are absent on alternate sites or vice versa. *Auricularia delicata* has the highest frequency among the species which are present in both the ecosystem while *Coprinus dessimentus* has the highest abundance. Also *Microporus xanthopus* has the highest density in



undisturbed sites while *Schizophyllum commune* has the highest density in disturbed sites.

Fig 13: Frequency graph representing different mushroom species in disturbed and undisturbed site.

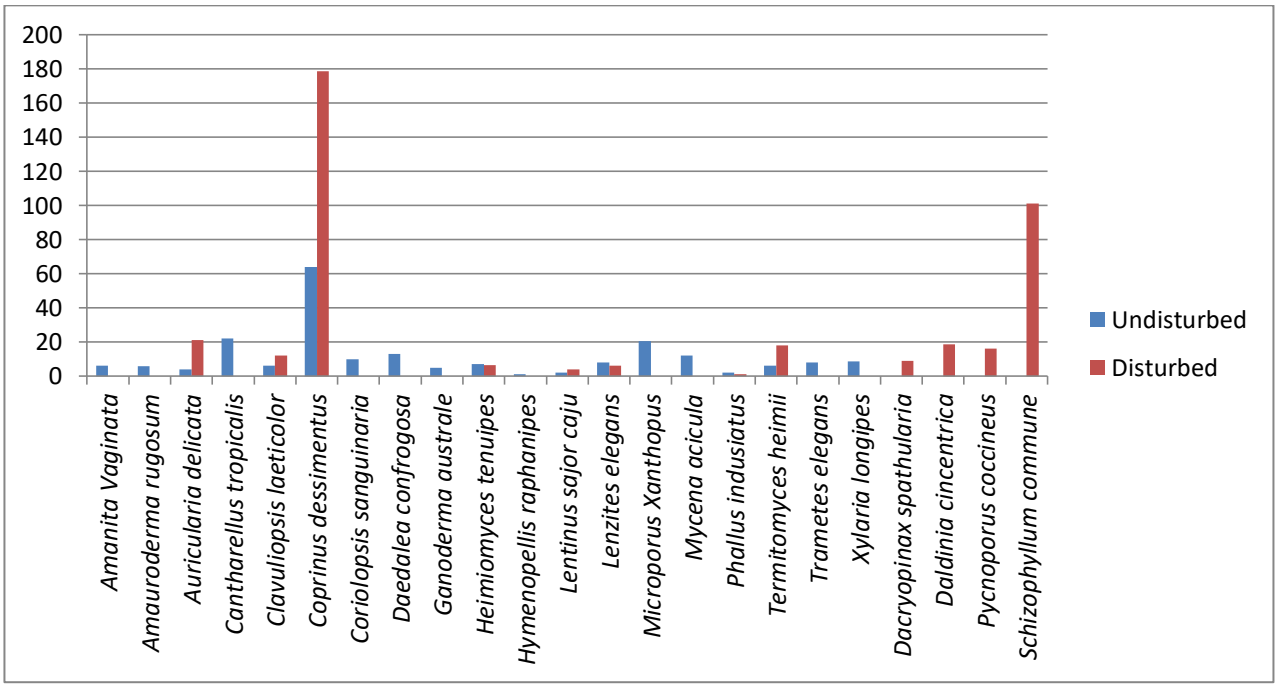


Fig 14: Abundance graph representing different mushroom species in disturbed and undisturbed site

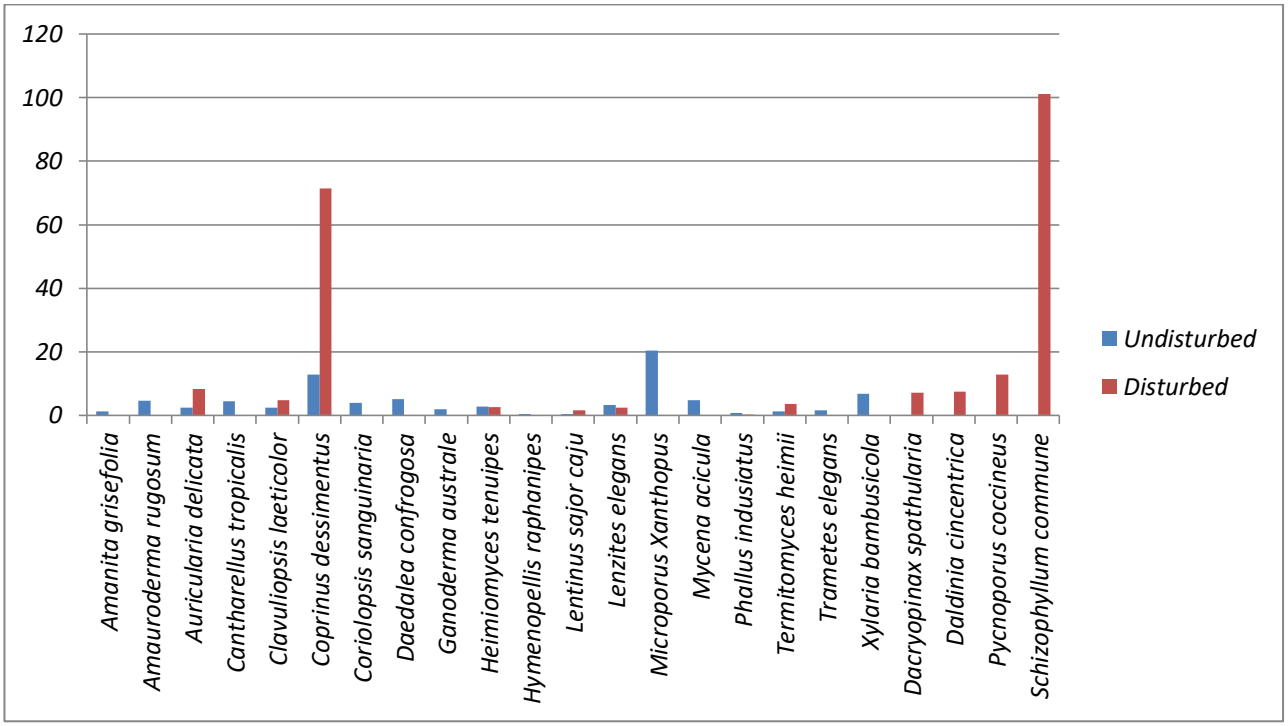


Fig 15: Density graph representing different mushroom species in disturbed and undisturbed site

4.2.2. Diversity Indices:

Species Diversity: The diversity of the two sites was compared using Shannon Diversity index and Simpson Index. The Shannon diversity index in undisturbed sites shows higher value of 2.63 than the disturbed site with a value of 1.57 (Fig-16). The Simpson Index values are 0.91 and 0.71 for undisturbed site and disturbed site respectively (Fig-17).

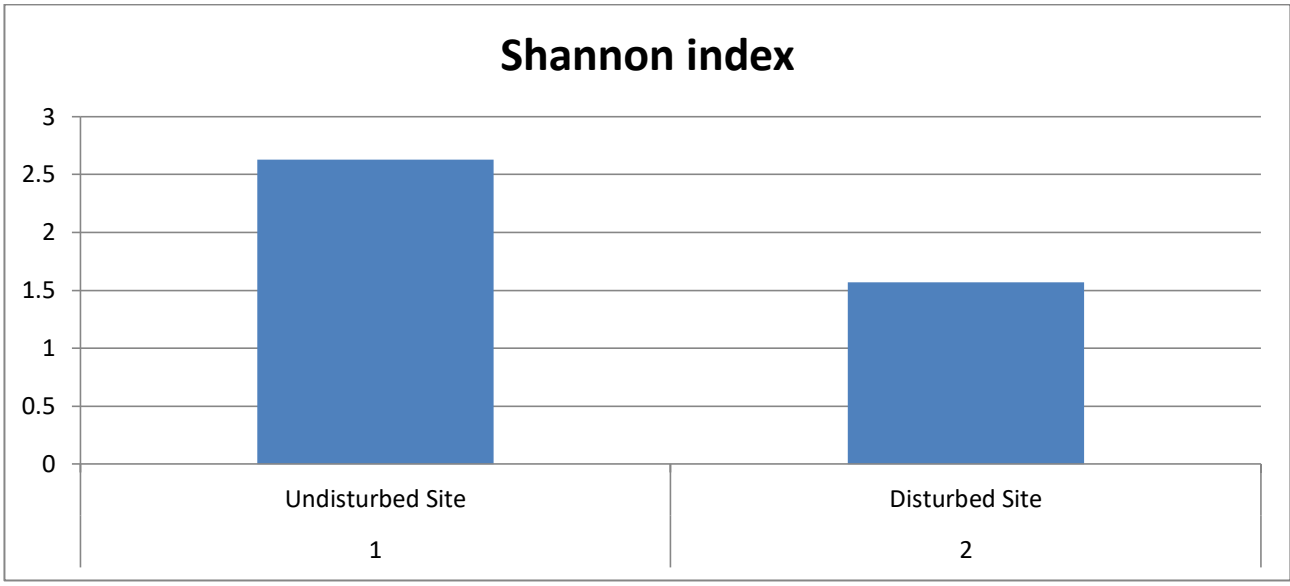


Fig 16: Shannon Diversity index graph representing mushroom diversity in undisturbed and disturbed sites.

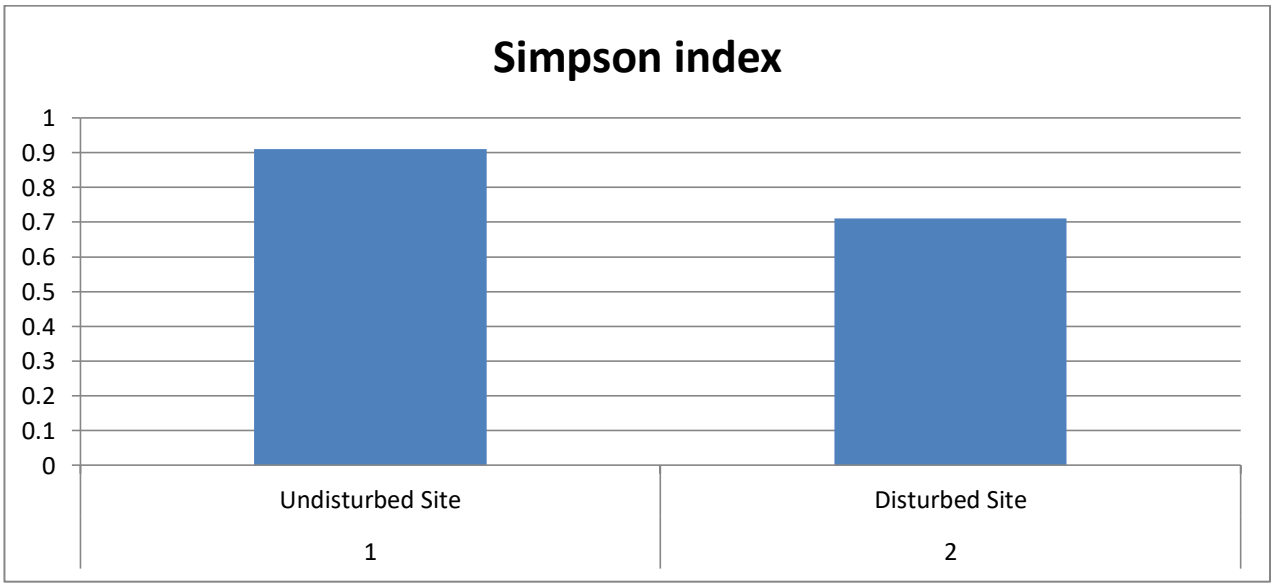


Fig 17: Simpson Diversity index graph representing mushroom diversity in undisturbed and disturbed sites.

Species Richness: Margalef's Index shows values of 2.96 in undisturbed sites while 1.57 in disturbed sites. Menhinnicks index value also gives vale 0.91 and 0.514 respectively for undisturbed and disturbed sites.

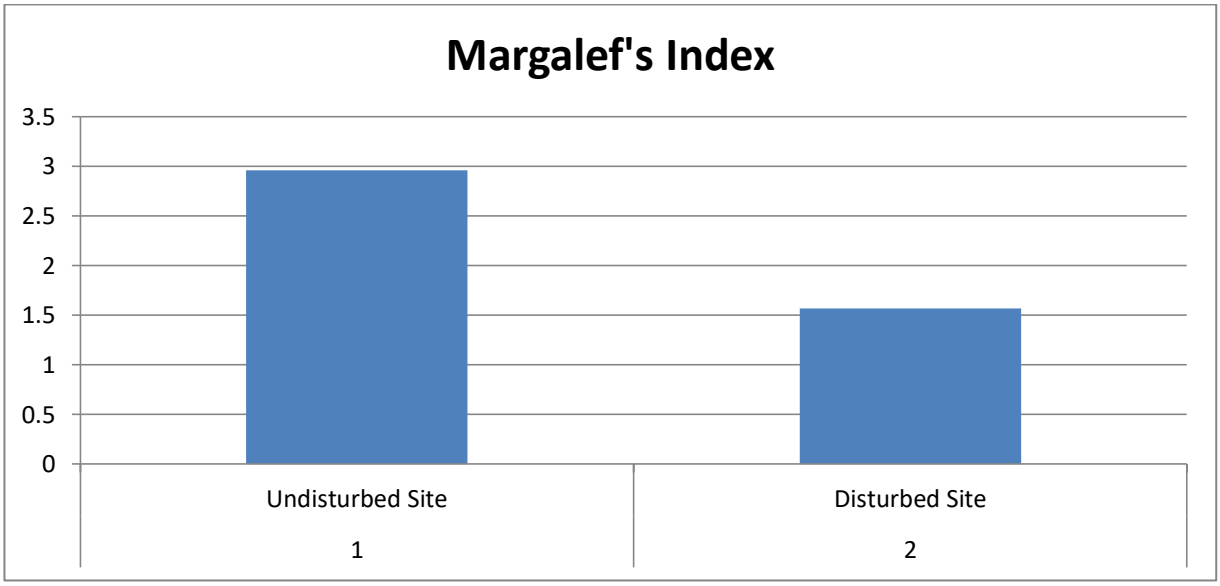


Fig 18: Species Richness graph represented by Marglef's Index.

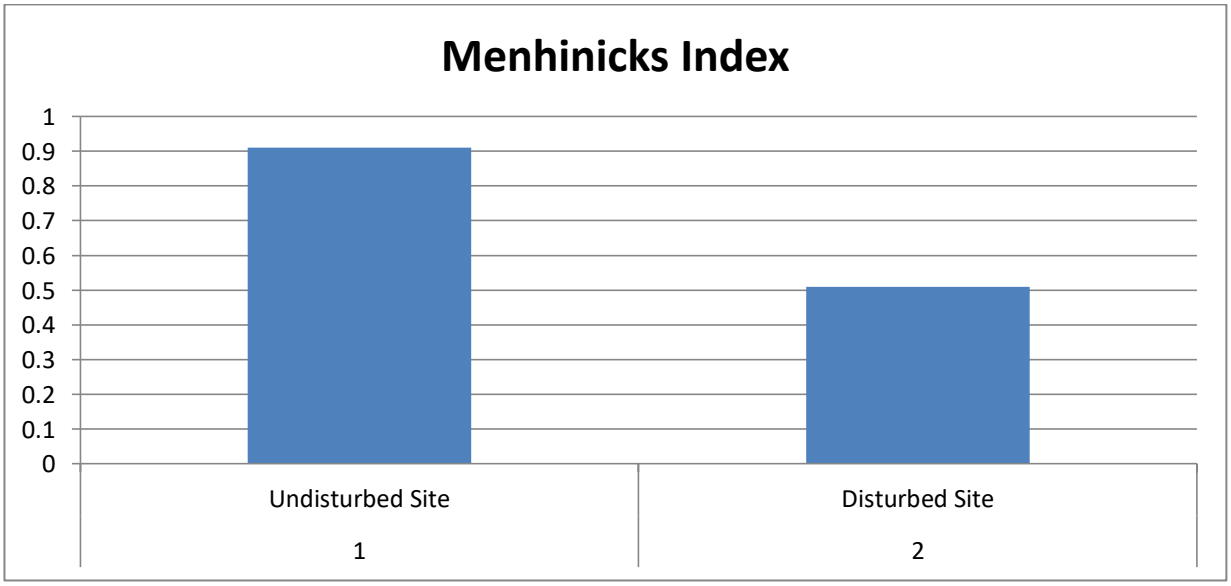


Fig 19: Species Richness graph represented by Menhinicks Index.

Species Evenness: Pilou evenness graph shows that the undisturbed sites is more homogeneous or even ecosystem closer to 1 with 0.89 as compared to 0.63 in disturbed forest.

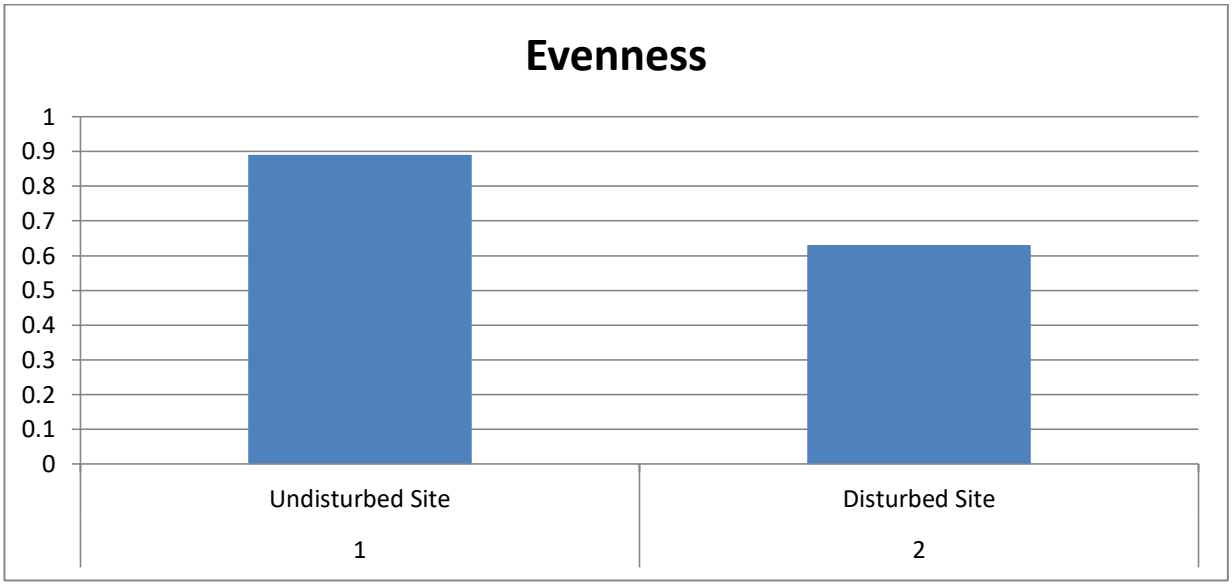
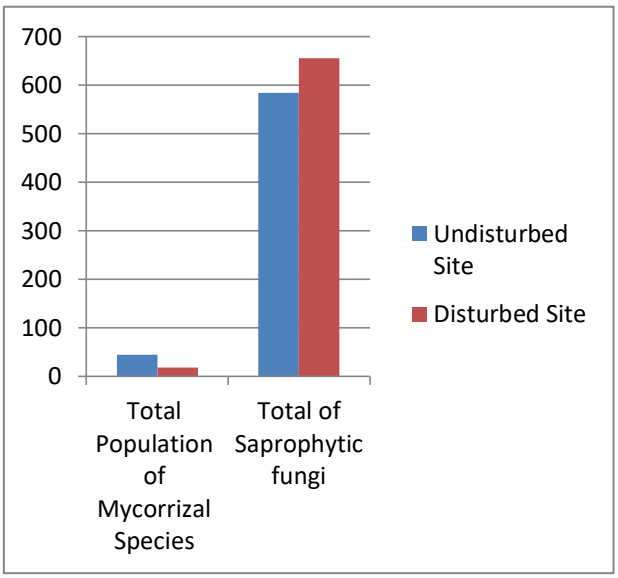


Fig 20: Pilou Evenness Graph representing disturbed and undisturbed sites.

4.2.3. Impact of Disturbance on Species:

By comparing the population of mycorrhizal species and Saprophytic species the result shows that the population of mycorrhizal species decline in disturbed ecosystem while the population of saprophytic mushrooms increases. This may be due to the decline in host trees for the mycorrhizal species while the increase in saprophytic species population may be due to the increase in substrate as a result of tree cutting and other anthropogenic activity.



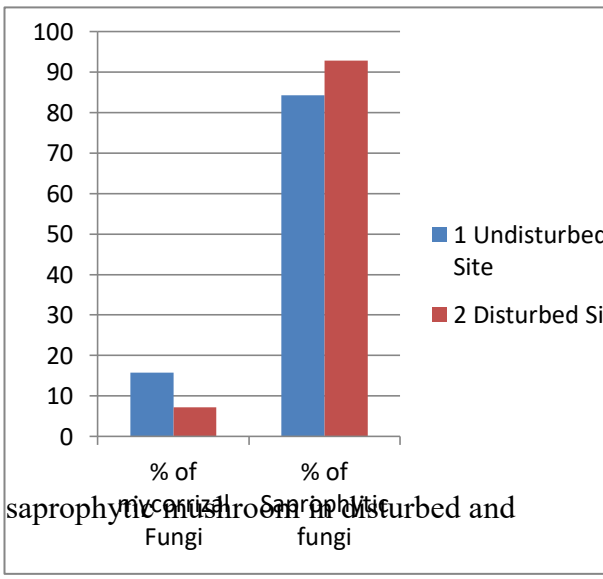


Fig 21: Total population and percentage of mycorrhizal and saprophytic mushroom in disturbed and undisturbed sites

Discussion:

The study shows that the number of mushroom species in undisturbed site is higher at 19 species as compared to 12 species in disturbed site, with 8 species common in both sites. The eight species found to be common in both disturbed and undisturbed sites are: - *Auricularia delicta, Clavuliopsis laeticolor, Coprinus dessimentus, Hemiomyces tenuipes, Hymenopellis tenuipes, Lenzites elegans, Phallus indusiatus, and Termitomyces heimiii*. The frequency, abundance and density of the mushroom greatly varied among different species in both the ecosystem. In both the sites these are impacted by a number of ecological factors such as temperature, rainfall, elevation, availability of substrate or host. Yang et al. (2012) reported that high temperature and abundant rain resulted in good productivity and mushrooms may sometimes appear later in the season by rising temperatures and reduced rain. Other report also suggests that temperature and humidity play an important role in mushroom population (Salerni et al., 2002; Jang & Hur, 2012; Holm, 2012). Vabeikhokhei (2020) also suggest that the increase in humidity increase the population and diversity of mushroom.

The diversity of mushroom from undisturbed and disturbed sites was calculated from the total population of mushroom collected from Pualreng wildlife sanctuary and Jhum area near Hlimen village respectively. Diversity index, species richness and evenness were all higher in undisturbed site as compare to disturbed site. The Shannon index was 2.63 for undisturbed site and 1.57 in disturbed site, Simpson Index values are 0.91 and 0.71 for undisturbed site and disturbed site respectively. The increase in diversity in undisturbed sites maybe link to the greater diversity of plants in undisturbed sites as recorded other workers (Bisby, 1933; Berg et al., 1994; Renvall, 1995; Hoiland & Bendiksen, 1997; Lindblad 1998; Egbe et al., 2013; Vyas et al., 2014). This condition has also been observed in other sites of Mizoram as recorded by Zothanzama (2011), Lalrinawmi (2019) and Vabeikhokhei (2020) who reported that the diversity of mushroom is higher in undisturbed ecosystem as compared to disturbed ecosystem.

In the disturbed sites wherein Margalef's Index shows values of 2.96 and 1.57 while Menhinnicks index gives vale 0.91 and 0.514 for undisturbed and disturbed sites respectively. This may be due to the increase in anthropogenic activity in disturbed sites. Zothanzama (2011), Lalrinawmi (2019) and Vabeikhokhei (2020) also reported that richness of mushroom species is higher in undisturbed forest areas, whereas fewer species are observed in disturbed forest areas. Bhattacharjee (2015) also reported that mushroom diversity and species richness decreases due to increased human activities, air pollution caused by vehicles and dumping of non-biodegradable wastes especially plastics.

Pilou's evenness value show in undisturbed forest was 0.89 while in disturbed forest the evenness value was 0.63. This indicated that the mushroom species in the undisturbed site are fairly distributed evenly as compared to the disturbed site which may be due to the homogeneity of substrate in the undisturbed site. Pushpa &Purushothama (2012) suggest that the habitats and ecosystem favours the occurrence and abundance of some mushroom species.

The study also highlight that some species of mushroom can be vulnerable to anthropogenic activity especially mycorrhizal species as the numbers of mushroom species collected from undisturbed site show high numbers of mycorrhizal species while in the disturbed site the mushroom species were mostly saprophytic. This is also observed by Pushpa & Purushothama (2012) who also concluded that occurrence of ectomycorrhizal fungal species decreased where tree species diversity decreased.

4.3. Summary and Conclusion

The study was carried out in Pualreng wildlife sanctuary (undisturbed) and Jhum site near Hlimen village (disturbed) located in 24° 6'35" - 24o 14'16'21"' N and 92° 50' 17.6" - 92o54'2.64" E and 24°13'47.96"N and 92°48'18.04"E respectively in the Northern District of Kolasib, Mizoram, India.

The study was carried out with the following objectives:

1. Taxonomic identification of Mushroom using morphological and molecular method.
2. To study the Diversity of Mushroom from selected sites.
3. To study the effect of disturbance on mushroom diversity.

For the taxonomic identification, mushroom were collected from the study sites and taxonomically identified using morphological characteristics; Macroscopic and Microscopic examination of isolated fungi the fungal morphology was studied macroscopically by observing the spores, colony features (colour, shape, size and hyphae), and microscopically by a compound microscope with a digital camera using a stained slide mounted with a small portion of the mycelium (Gaddeyya *et al*, 2012). A total of 20 species of mushroom were identified up to species level while 3 species were identified up to genus level. And due to the polymorphism nature (Cooke, 1871) of mushrooms, 9 species were confirmed and characterised by molecular analysis using ITS1 and ITS4 at species level while 3 were confirmed and characterised by molecular analysis at genus level only. The nucleotide analysis and characterisation were done for the 9 species; DNA was extracted using CTAB method, which were then amplified using PCR and sequenced by Sanger sequencing. The Sequence were then analysed using Mega X software.

For diversity study and effect of disturbance line quadrat method was used for sampling wherein 8 species were found to be common in both disturbed and undisturbed sites. It was also found that *Mycroporus xanthopus* and *Schizophyllum commune* had the maximum frequency of occurrence in both the disturbed and undisturbed sites as these species of mushroom grows on dead wood substrates which are available in good number whereas *Coprinus dessimentus* was the most abundant species. The Shannon and Simpson indices shows higher values in the undisturbed site as compare to the disturbed site indicating better diversity. The study also reveal that anthropogenic activity impact the population of mushroom especially mycorrhizal species as the numbers of mushroom species collected from undisturbed site show high numbers of mycorrhizal species while on the disturbed site the mushroom species are mostly saprophytic.

Conclusion:

Study fungal diversity had been done by several workers (Bisht, 2011; Zothanzama, 2011, 2013, 2016, 2017; Lalrinawmi *et al*, 2017, 2018, Vabeikhokhei *et al*,, 2017, 2019, Zohmangaiha *et al*, 2019) but work molecular analysis and characterisation has been limited. This study aims to bridge morphological characterisation and molecular characterisation in the identification of mushroom species.

The study shows the difference in diversity of mushroom in disturbed and undisturbed sites and highlights the impact of disturbance on mushroom diversity. It can also be assumed that the conservation of our forest resources is very crucial for the protection of mushroom diversity as some mushroom are highly dependent on specific host trees and the increase in diversity is link to a greater diversity of the plants.

It will be beneficial to continue monitoring of diversity in the study sites to understand the dynamic of mushroom diversity and continue exploration on its uses for different branches of science. The increase use of molecular tools for mushroom species identification and characterization is also recommended as it offers better diversity at the genetic level, evolutionary history and precise taxonomic position in the Fungal Kingdom.

Appendix I: Lists of presentation in conference/ symposium/ seminar

1. "Study of Microorganisms in Poultry Farm" at the International Conference on Recent Advances in Animal Sciences (ICRAAS), held at Pachhunga University College, Aizawl, Mzoram India from 6th to 8th November 2019.

2. "Morphological Identification of Mushrooms from Pulareng Wildlife Sanctuary of Mizoram" at the Mizoram Science congress 2020 (Online) held during December 3-4, 2020.

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M.sc	2019	Mizoram University	Mizoram Univeristy	68

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APPROVAL OF RESEARCH PROPOSAL	
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Head
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ABSTRACT

IDENTIFICATION AND DIVERSITY OF MUSHROOMS OF PUALRENG WILDLIFE SANCTUARY IN MIZORAM, INDIA.

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY

BENJAMIN LALBIAKMAWIA

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DEPARTMENT OF ENVIRONMENTAL SCIENCE SCHOOL OF EARTH SCIENCE AND NATURAL RESOURCE MANAGEMENT

March, 2022

ABSTRACT

Identification and Diversity of Mushrooms of Pualreng Wildlife Sanctuary in Mizoram, India.

By

BENJAMIN LALBIAKMAWIA

Department of Environmental Science

Supervisor: Dr S.T. Lalzarzovi

Jt. Supervisor: Dr. John Zothanzama

Submitted

In Partial fulfilment of the requirements for the Degree of Master of Philosophy in Environmental Science of Mizoram University, Aizawl, Mizoram.

ABSTRACT

The present study entitled “Identification and Diversity of Mushroom of Pualreng Wildlife Sanctuary in Mizoram”, was in Pualreng Wildlife Sanctuary located in 24° 6’35” - 24^{o} 14’16’21”’ North Latitude and 92° 50’ 17.6” - 92o54’2.64” East longitude in the district of Kolasib and in Jhum sites located in the 24°13'47.96"N 92°48'18.04"E in the Northern District of Kolasib.

The study was carried out with the following objectives:

1. Taxonomic identification of Mushroom using morphological and molecular method.
2. To study the Diversity of Mushroom from selected sites.
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For the taxonomic identification, mushroom were collected from the study sites and taxonomically identified using morphological characteristics; Macroscopic and Microscopic examination of isolated fungi the fungal morphology was studied macroscopically by observing the spores, colony features (colour, shape, size and hyphae), and microscopically by a compound microscope with a digital camera using a stained slide mounted with a small portion of the mycelium (Gaddeyya *et al*, 2012). A total of 20 species of mushroom were identified up to species level while 3 species were identified up to genus level. For molecular method DNA was extracted using CTAB method, which were then amplified using PCR and sequenced by Sanger sequencing, ITS region of each species (or till genus level) was obtained. The sequences were aligned using NCBI Blast (Altschul et al., 1990). From the BLAST result, nucleotide sequences were retrieved from NCBI nucleotide database and re-aligned using Clustal-W in MEGA X (Kumar S et al., 2018). For each tree construction a trial and error with bootstrap value was considered for the preparation of the final dataset. 9 species were confirmed and characterised by molecular analysis using ITS1 and ITS4 at species level while 3 were confirmed and characterised by molecular analysis at genus level only. The nucleotide analysis and characterisation were done for the 12 species; The Sequence were then analysed using Mega X software.

For diversity study and effect of disturbance line quadrat method was used for sampling and the following diversity index was used.

Shannon's diversity Index (Hs) (Shanon & Weaver, 1949)

$$Hs = -\sum pi \ln pi$$

Where, pi = the proportion of individuals found in the i^{th} species

Or $pi = ni/N$

Where, ni =the abundance of the individual in the i^{th} species.

N = the abundance of all the species

Simpson index (Simpson, 1949)

Simpson Index (D) = $\sum$ 1-[$\sum$n(n-1)/N(n-1)]

Where, n = the total number of organisms of a particular species

N = the total number of organisms of all species

Margalef's index (Margalef, 1958).

Margalef's index was used as a simple measure of species richness

Margalef's index D= (S – 1) / In N

Where, S = total number of species

N = total number of individuals in the sample

In = natural logarithm

Menhinick's index (Menhinick, 1964)

Menhinick's index D=S/ √N

Where, S= total number of species

N= total number of individual

Evenness Index (Pielou, 1966).

e = H / In S

Where, H = Shannon – Wiener diversity index

S = total number of species in the sample

8 species were found to be common in both disturbed and undisturbed sites. It was also found that *Mycroporus xanthopus* and *Schizophyllum commune* had the maximum frequency of occurrence in both the disturbed and undisturbed sites as these species of mushroom grows on dead wood substrates which are available in good number whereas *Coprinus dessimentus* was the most abundant species. Shannon and Simpson indices show higher diversity at 2.63 and 0.91 in undisturbed site respectively as compared to 1.57 and 0.71 disturbed site

respectively. Margalef's Index and Menhinnicks index also shows higher species richness at 2.96 and 0.91 in undisturbed site respectively and 1.57 and 0.514 in disturbed site respectively. : Pilou evenness also gives greater evenness for undisturbed site at 0.89 and 0.63 for disturbed sites. The study also reveal that anthropogenic activity impact the population of mushroom especially mycorrhizal species as the numbers of mushroom species collected from undisturbed site show high numbers of mycorrhizal species while on the disturbed site the mushroom species are mostly saprophytic.

Study fungal diversity had been done in Mizoram by several workers (Bisht, 2011; Zothanzama, 2011, 2013, 2016, 2017; Lalrinawmi *et al*, 2017, 2018, Vabeikhokhei *et al*,, 2017, 2019, Zohmangaiha *et al*, 2019) but work molecular analysis and characterisation has been limited. This study aims to bridge morphological characterisation and molecular characterisation in the identification of mushroom species.

The study also shows the difference in diversity of mushroom in disturbed and undisturbed sites and highlights the impact of disturbance on mushroom diversity. It can also be assumed that the conservation of our forest resources is very crucial for the protection of mushroom diversity as some mushroom are highly dependent on specific host trees and the increase in diversity is link to a greater diversity of the plants

4.1.2. Photo Plate

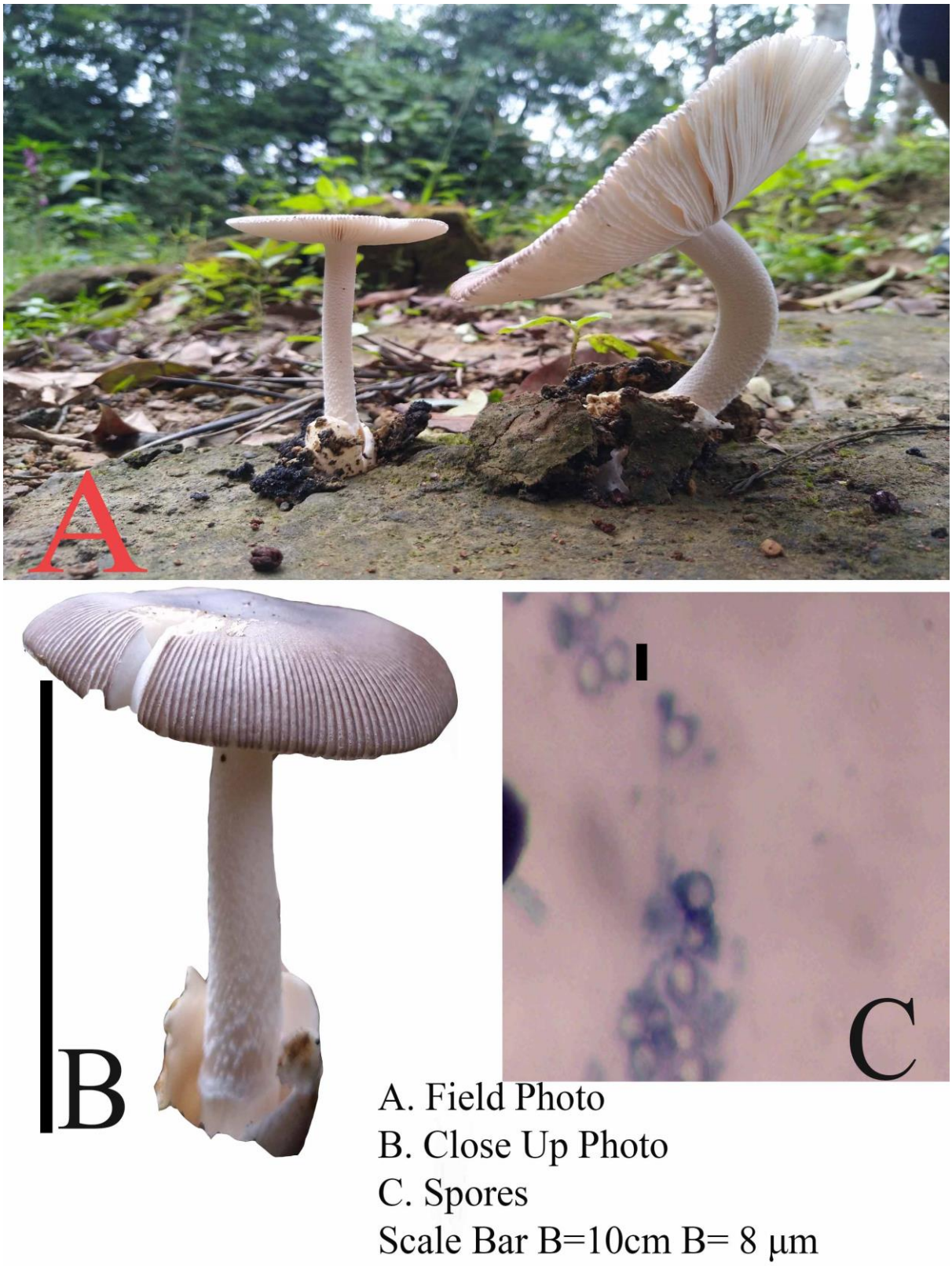


Photo plate 1: ***Amanita vaginata***

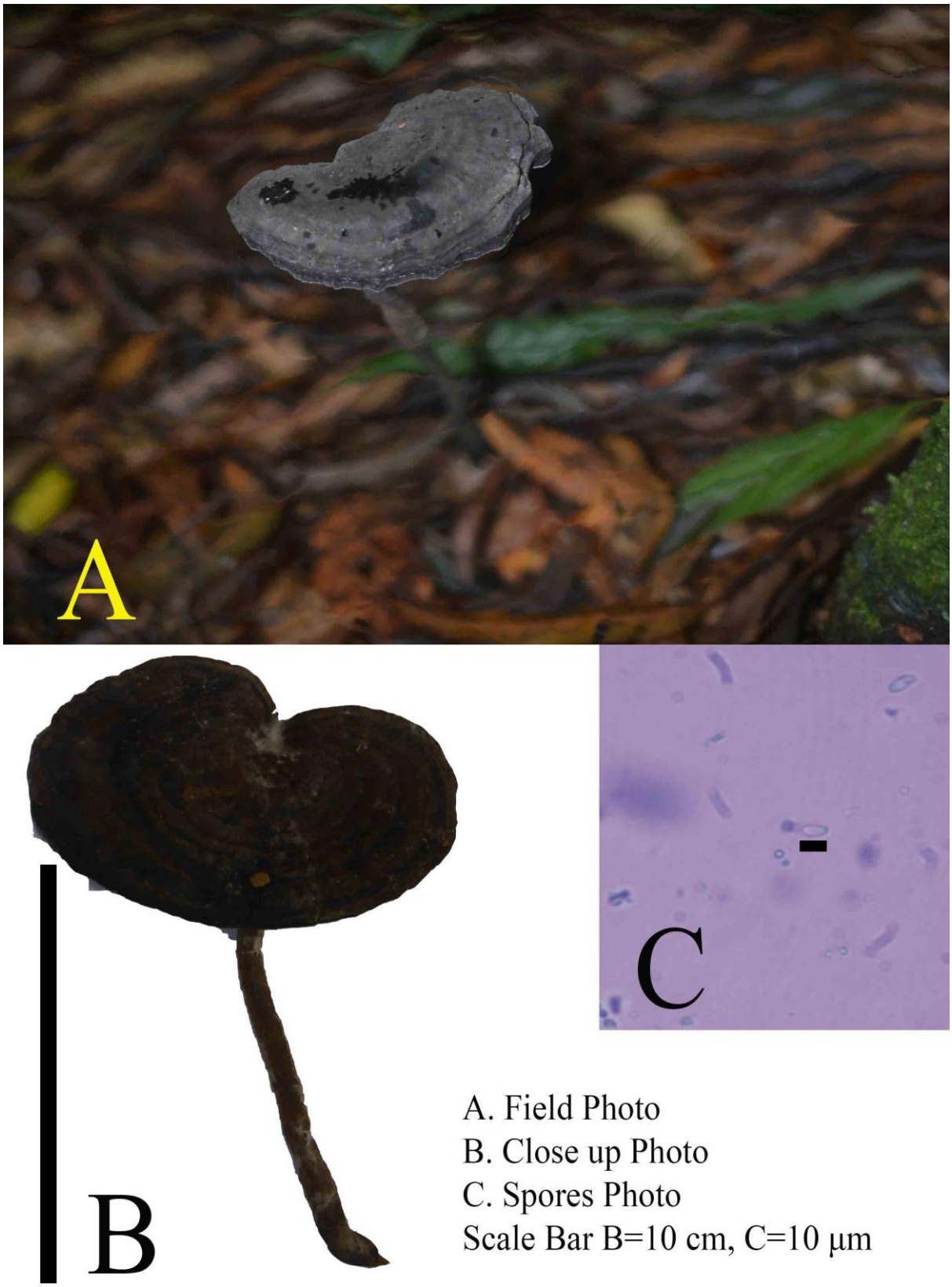


Photo plate 2: ***Amauroderma rugosum***

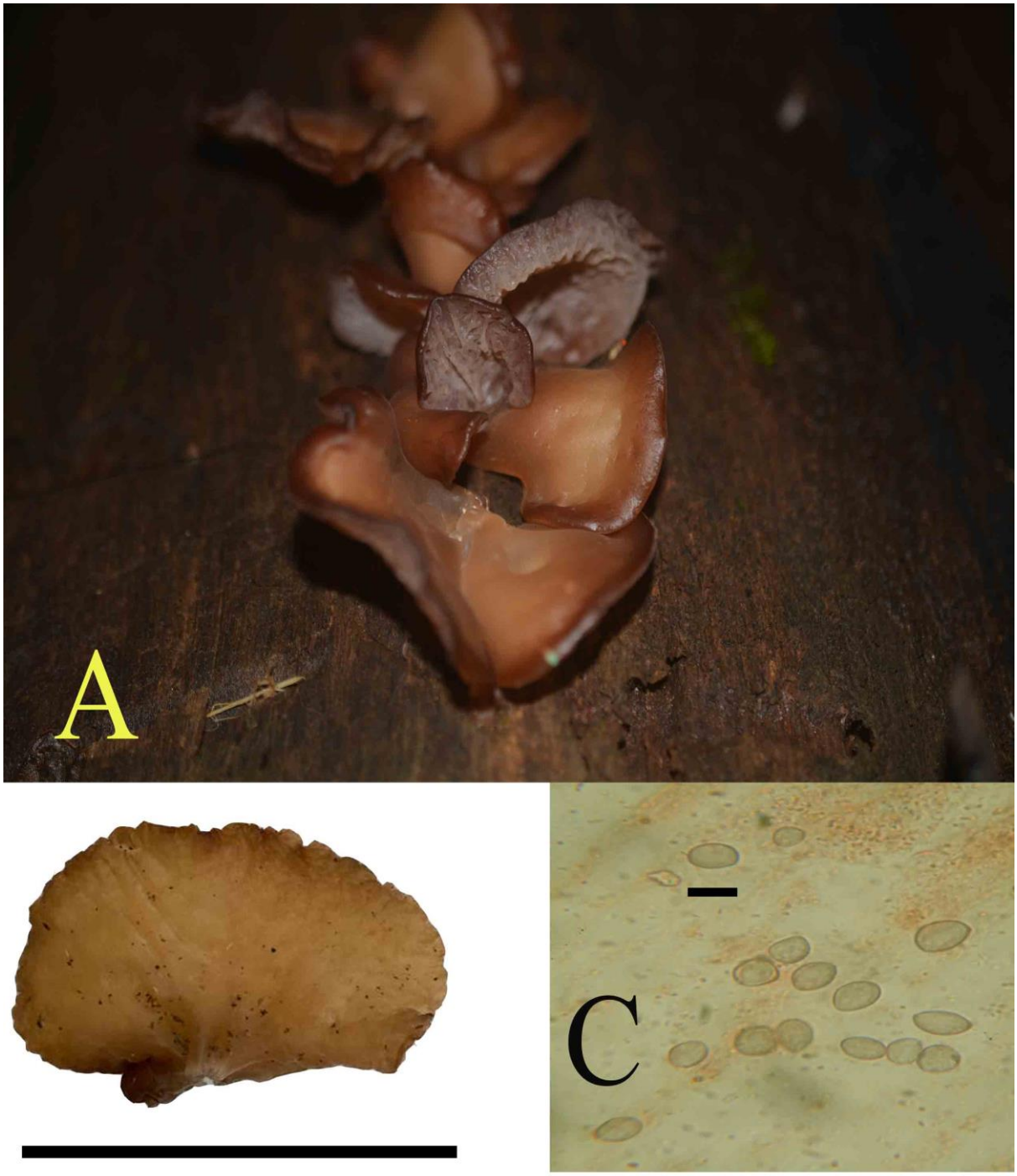


Photo plate 3: ***Auricularia delicata***

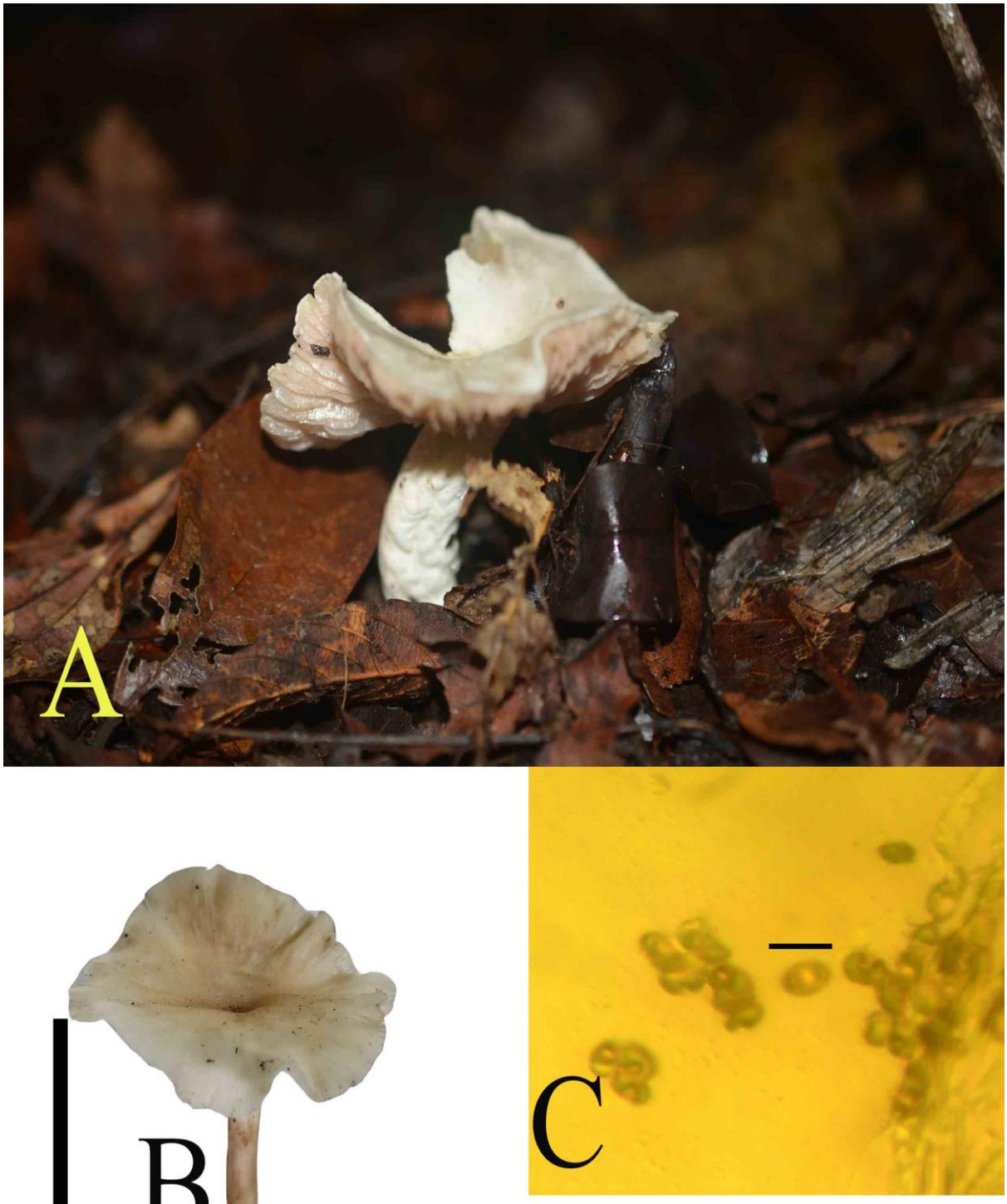


Photo plate 4: ***Cantharellus tropicalis***

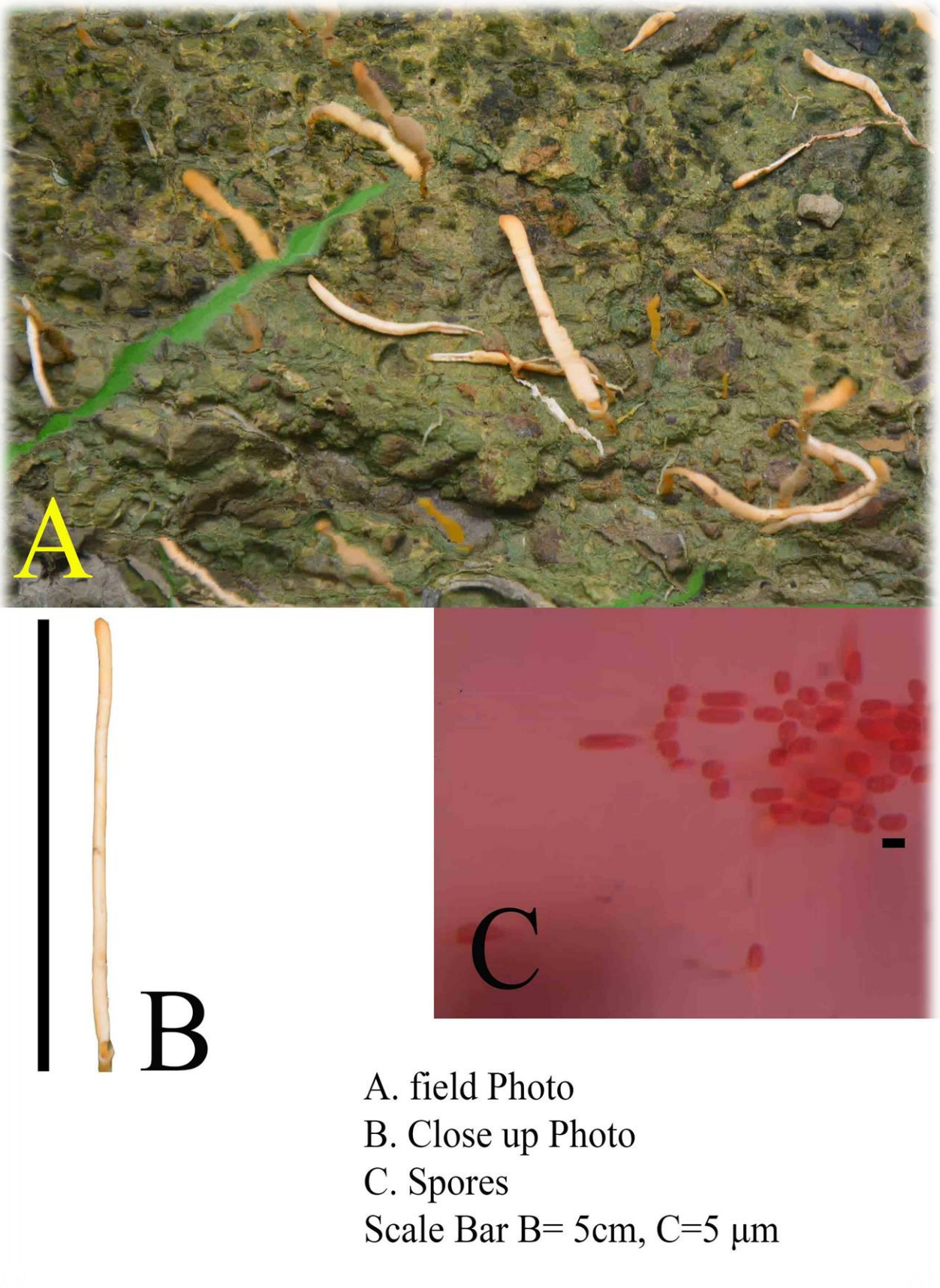


Photo plate 5: ***Clavuliopsis laeticolor***

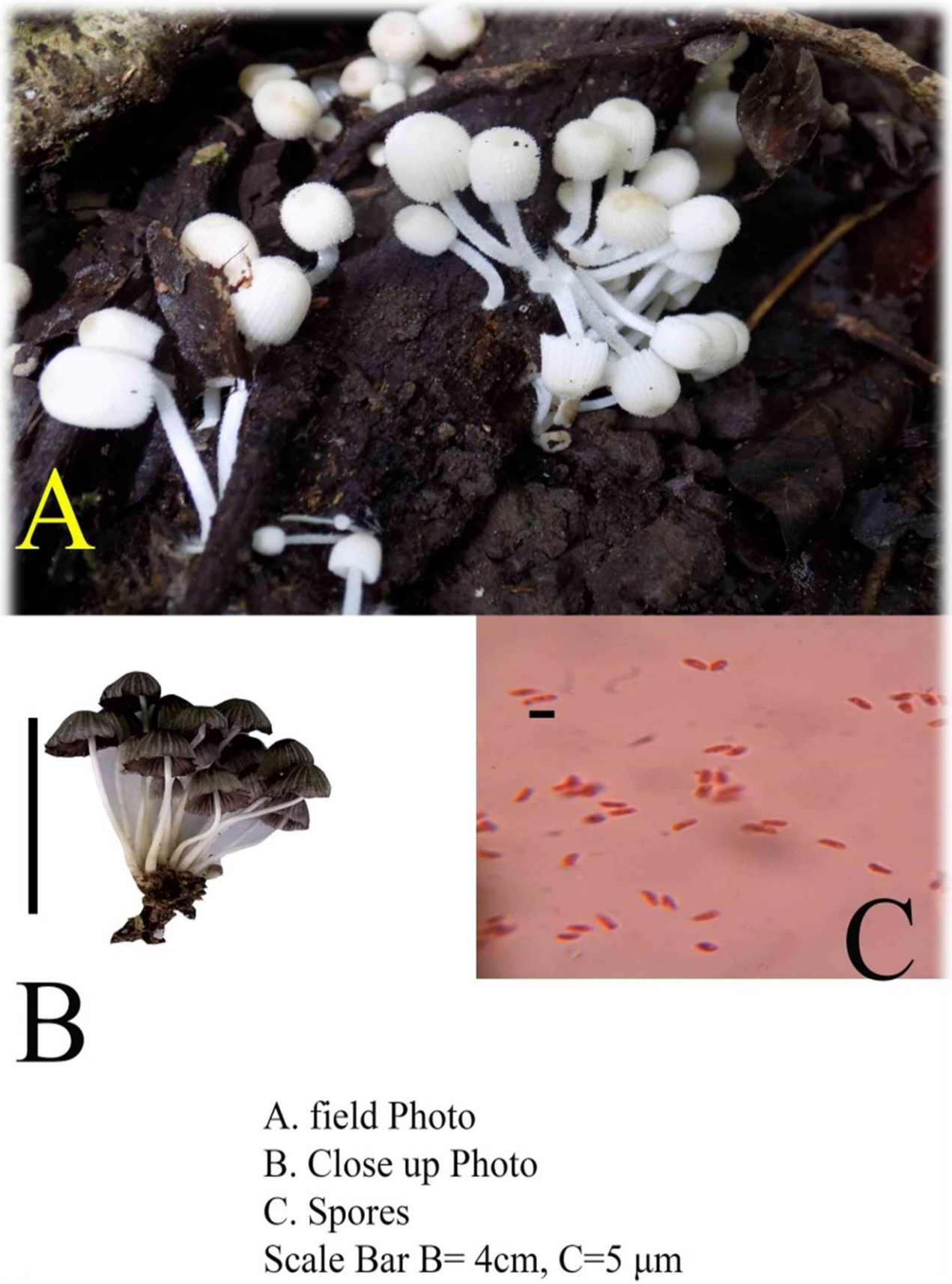


Photo plate 6: ***Coprinus dessimentus***

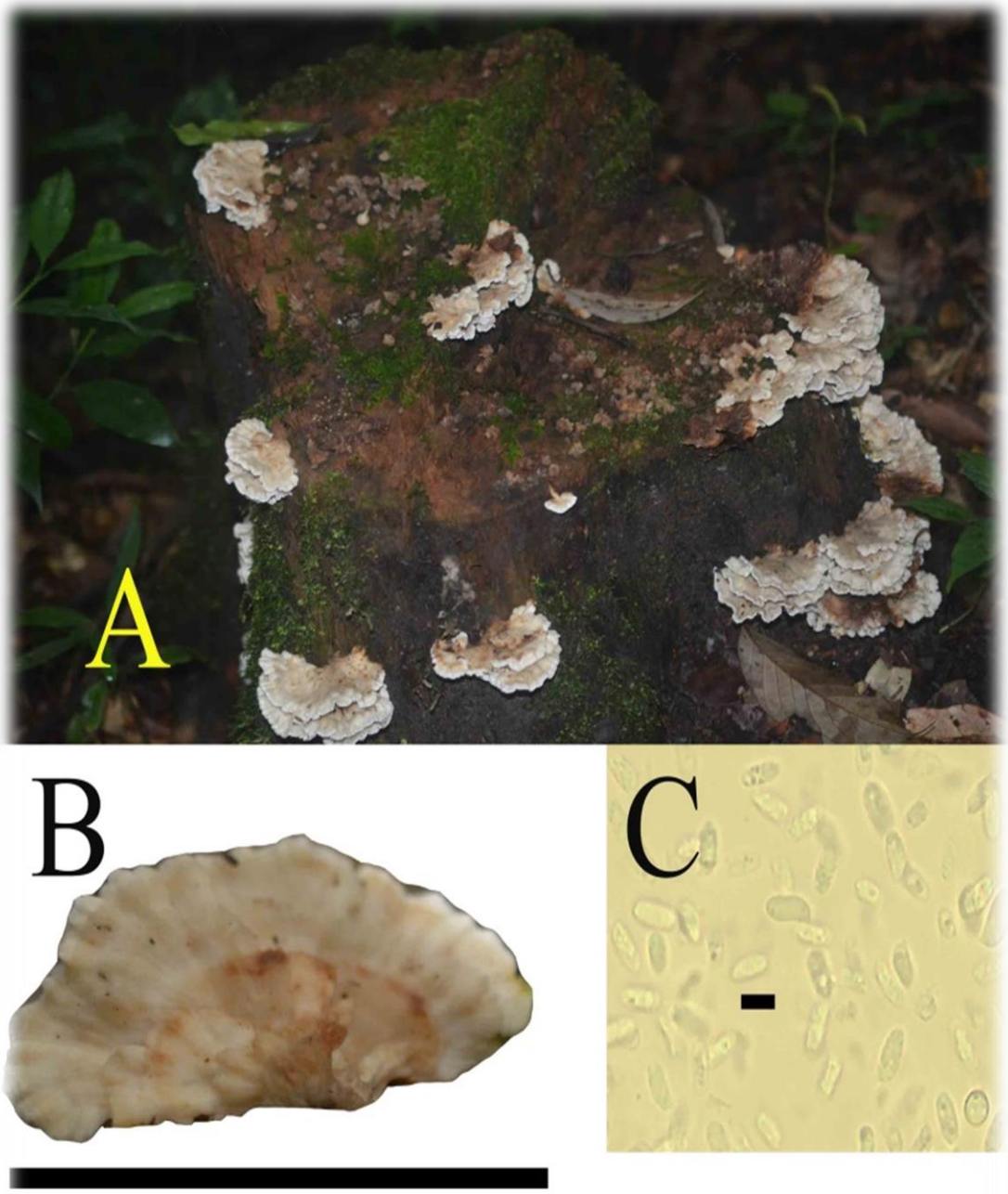
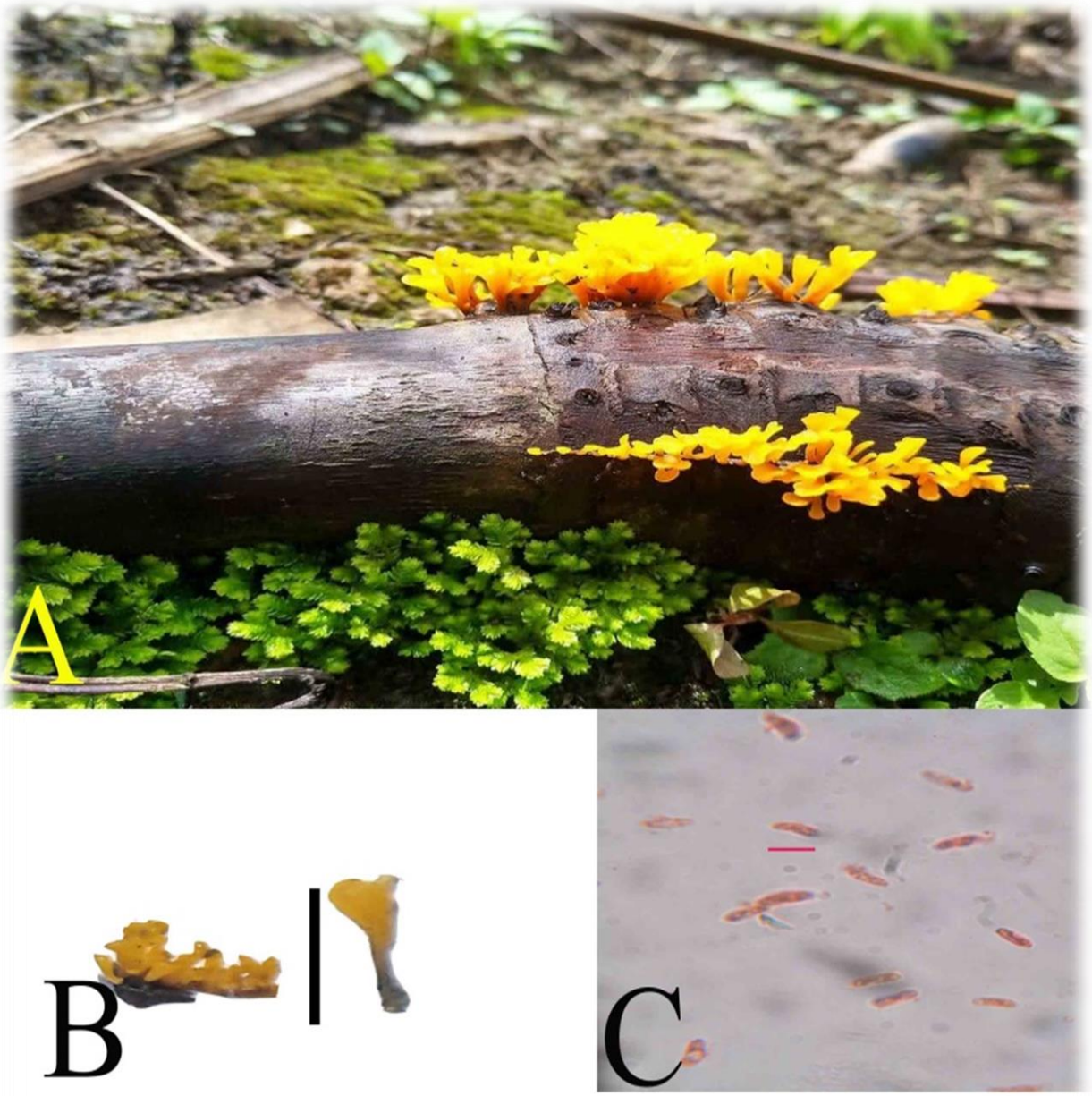
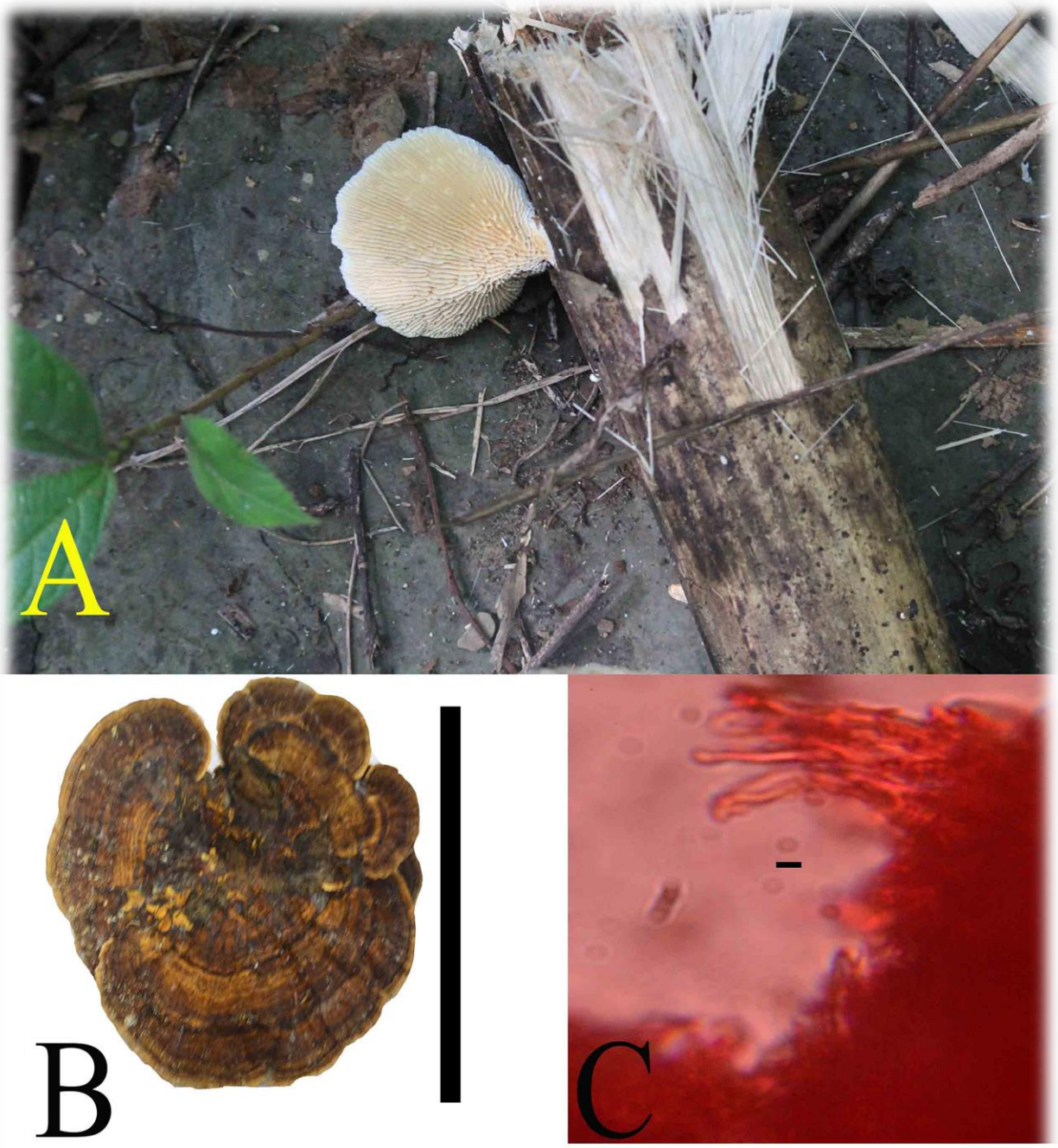


Photo plate 7: ***Coriolopsis sp.***



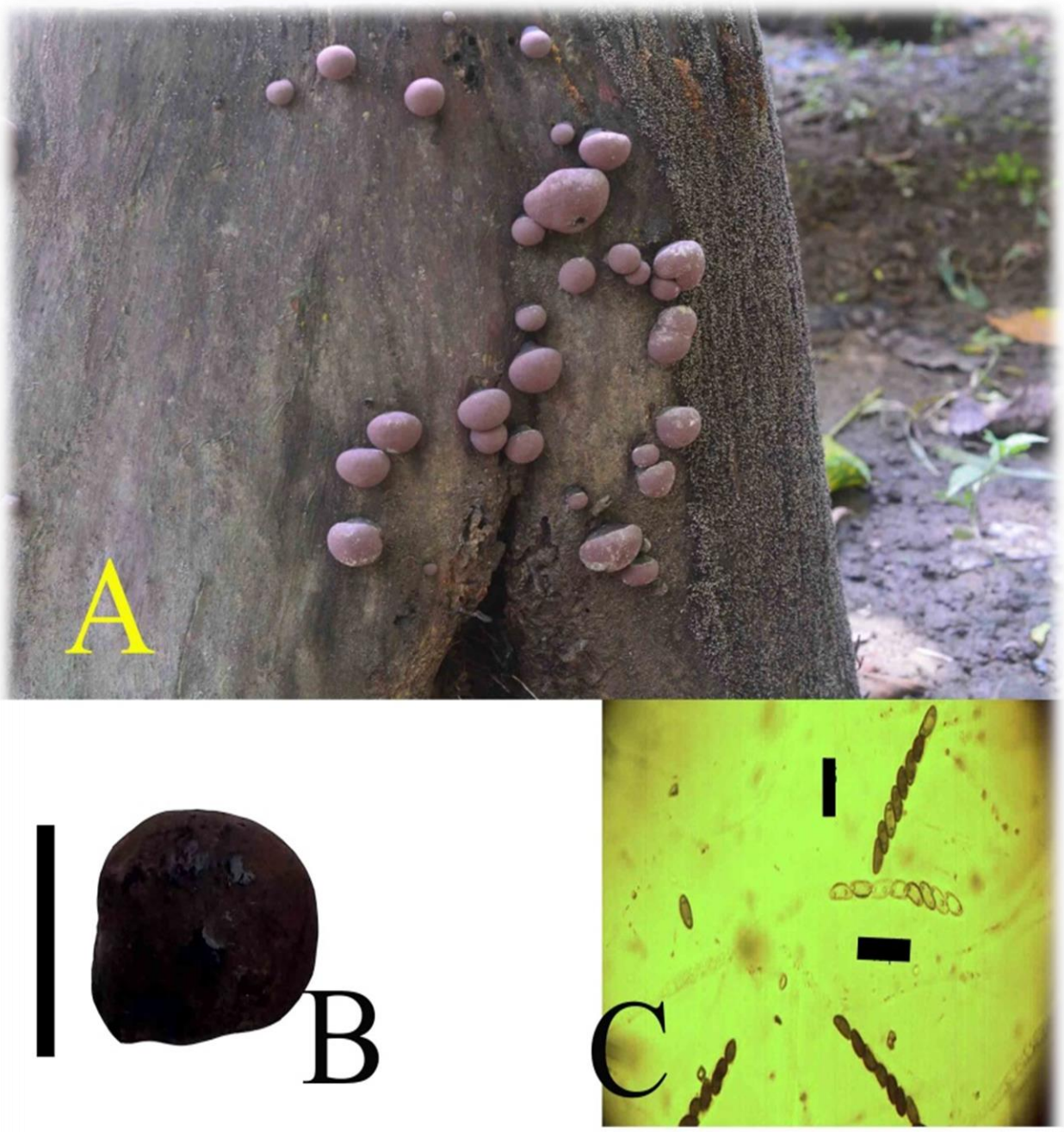
A. field Photo
B. Close up Photo
C. Spores
Scale Bar B= 2.5 cm, C=5 µm

Photo plate 8: ***Dacryopinax spathularia***



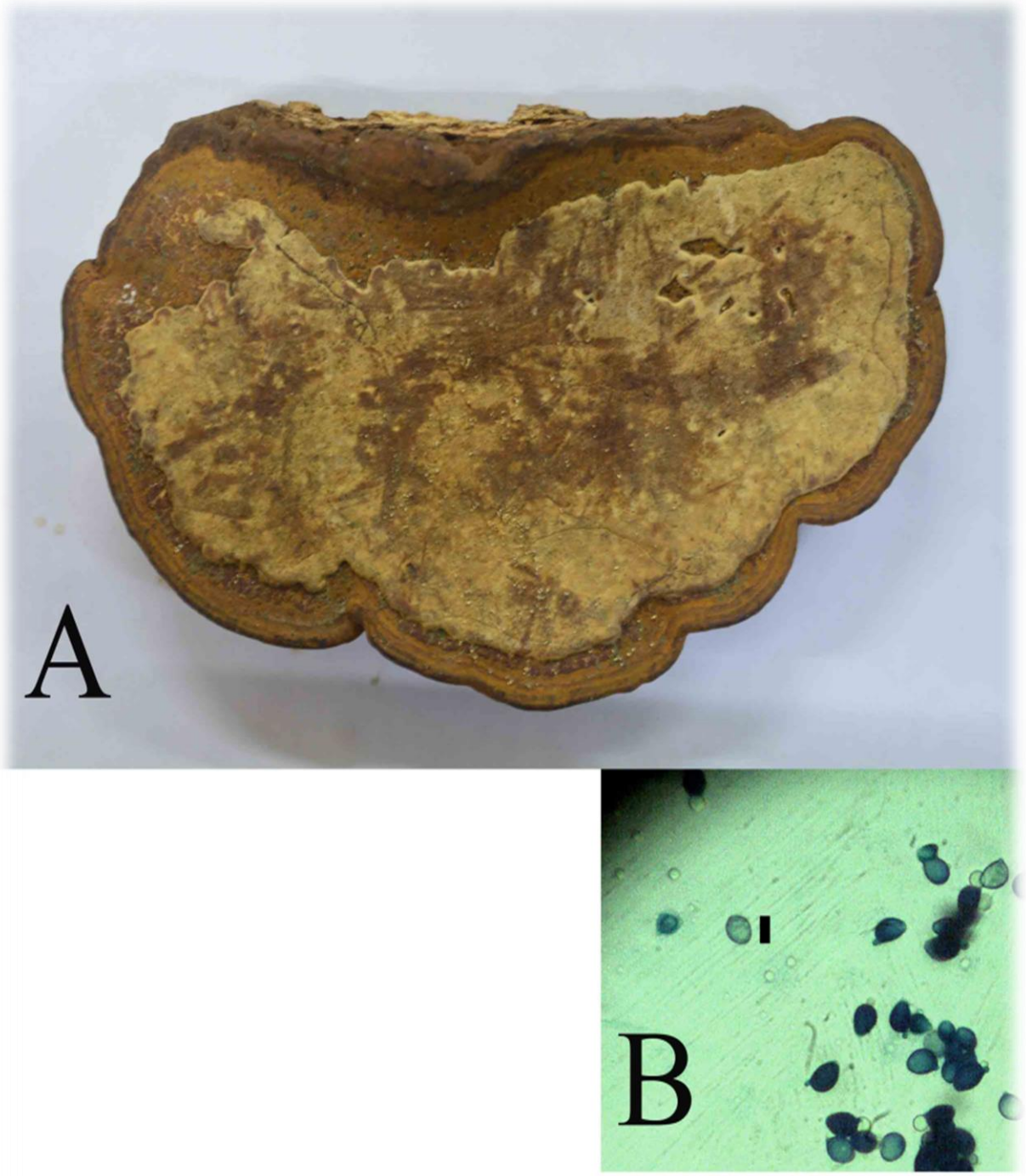
A. field Photo
B. Close up Photo
C. Spores
Scale Bar B= 12cm, C=10

Photo plate 9: Daedalea confrogosa



A. field Photo
B. Close up Photo
C. Spores
Scale Bar B= 4cm, C=10

Photo plate 10: ***Daldinia concentrica***



A. Sample Photo
B. Spores
Scale Bar B=10 µm

Photo plate 11: ***Ganoderma applanatum***

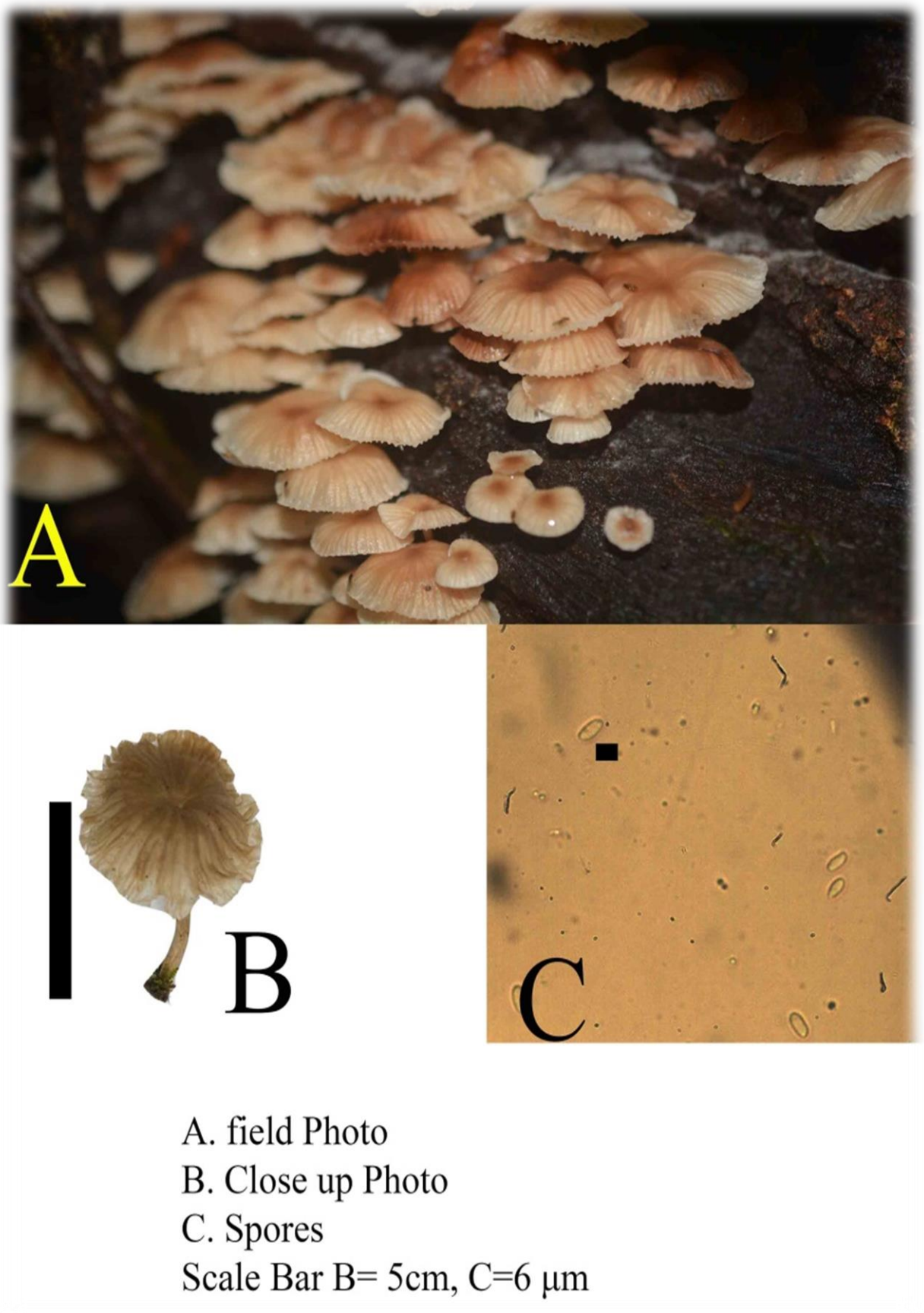


Photo plate 12: ***Heimiomyces tenuipes***

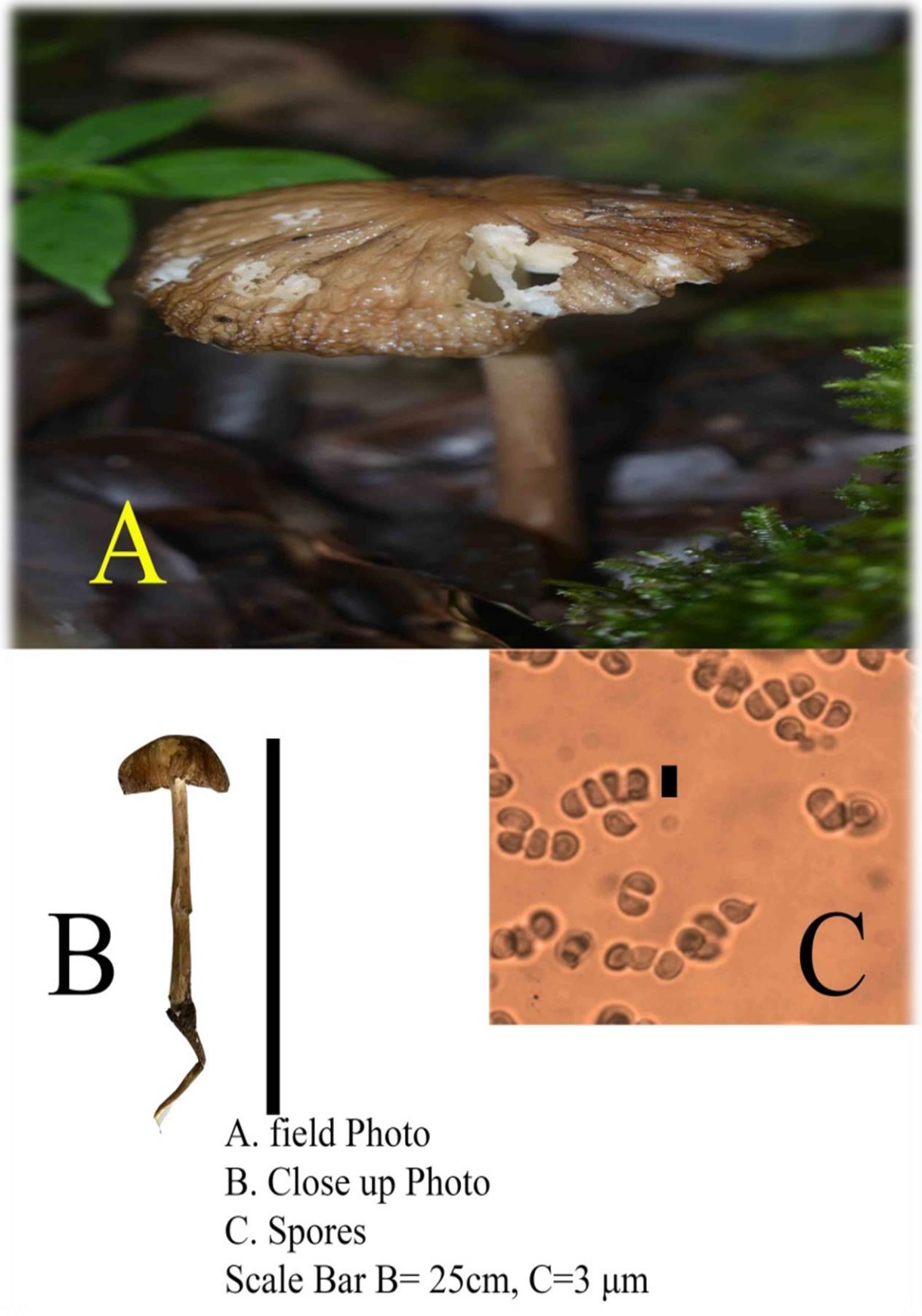
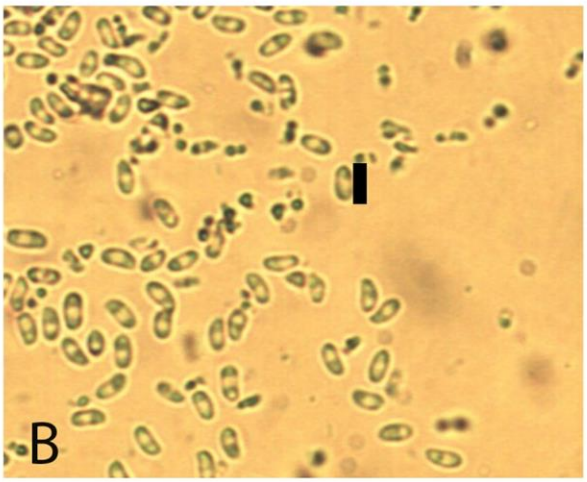
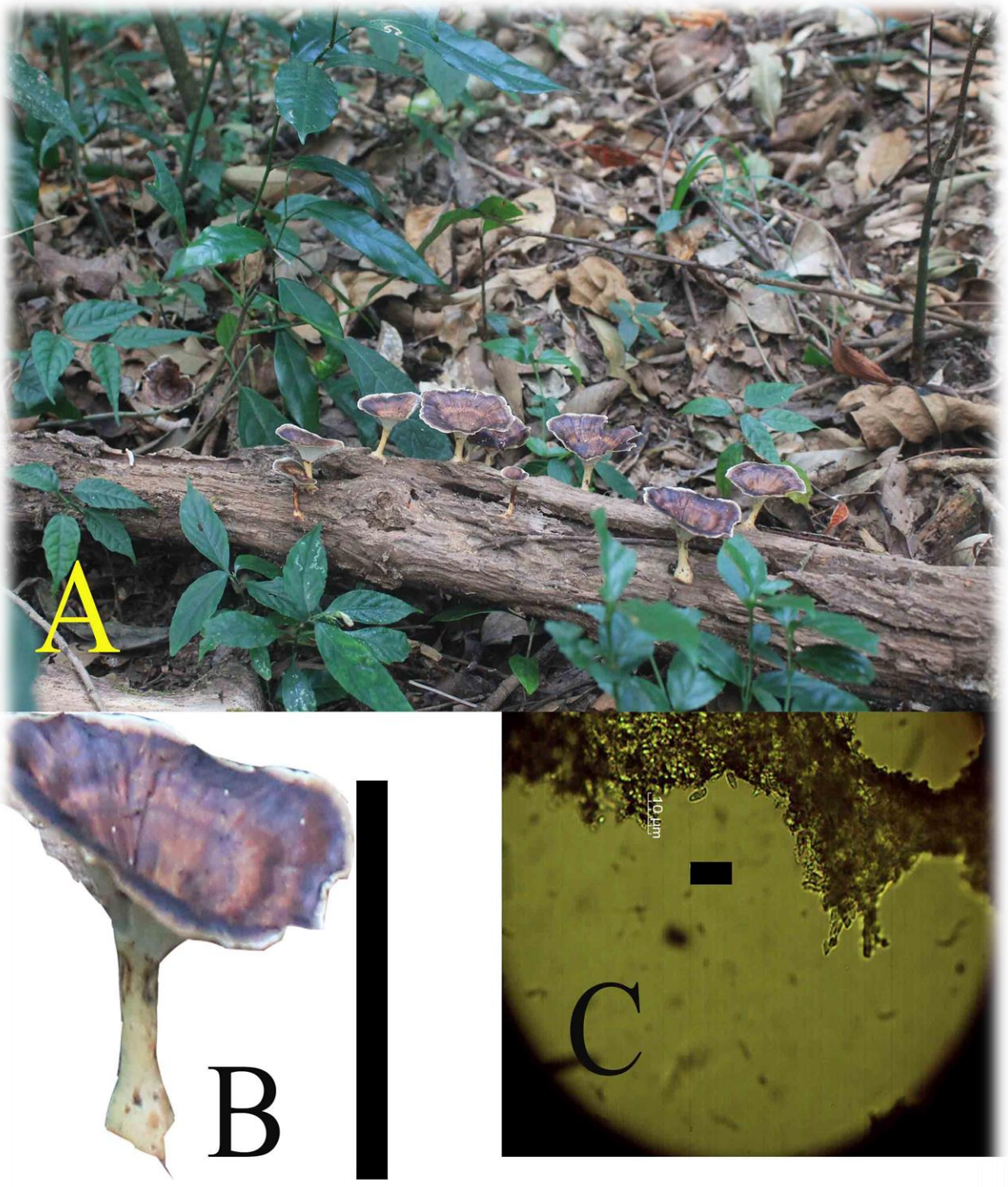


Photo plate 13: ***Hymenopellis sp.***



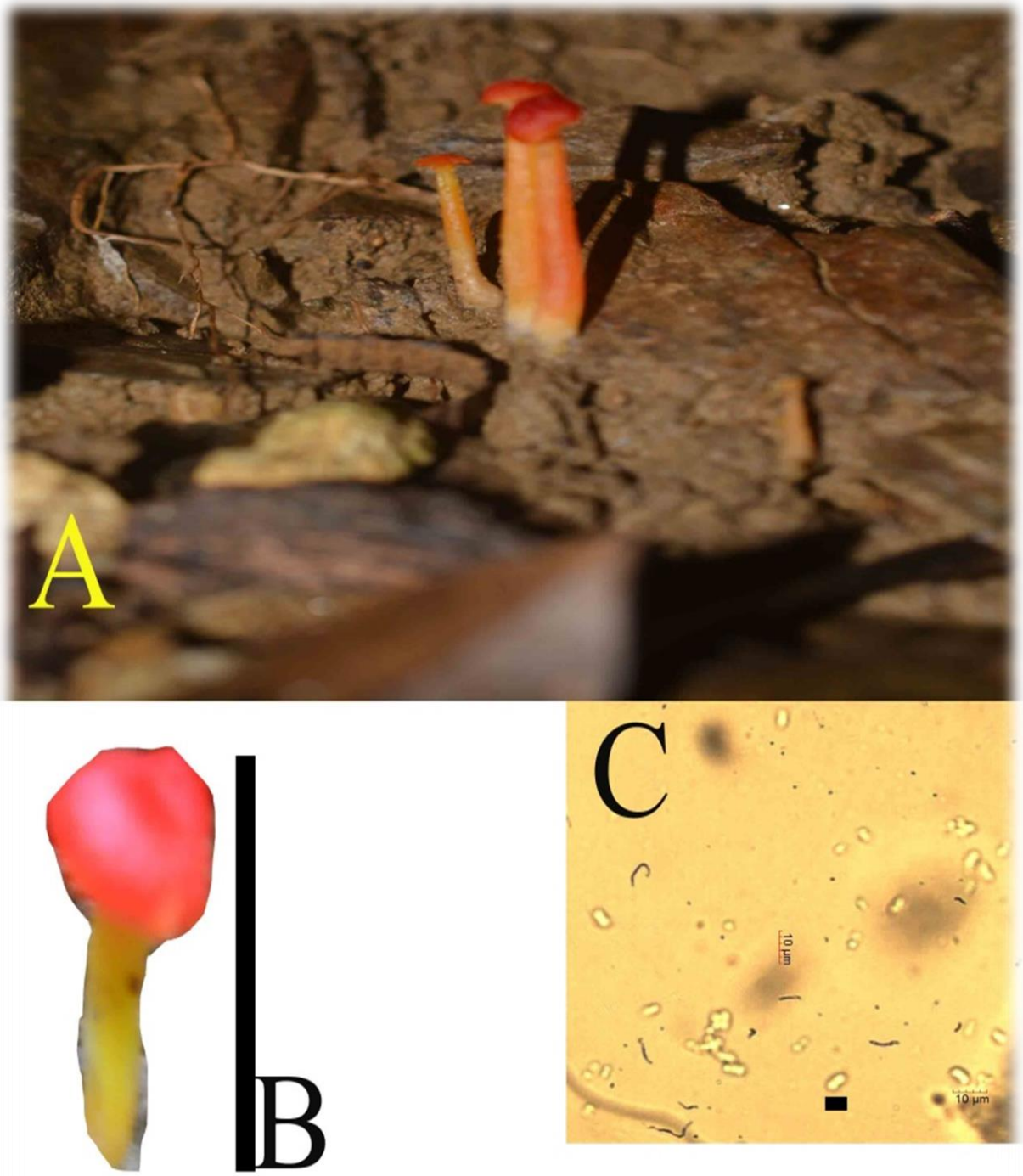
A. Field Photo
B. Spores
Scale Bar B=7µm

Photo plate 14: ***Lentinus squarrosulus***



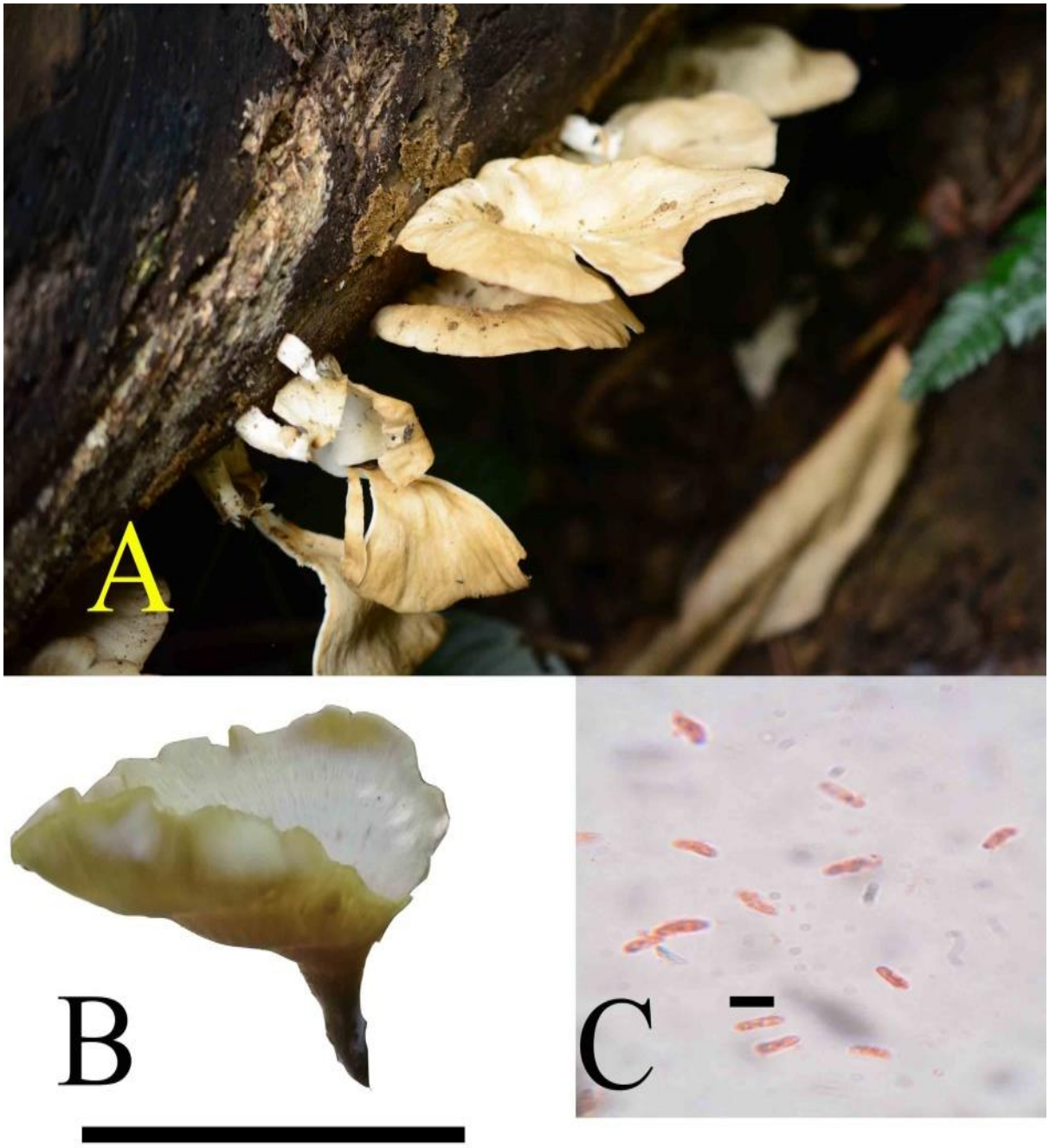
A. Field Photo
B. Close up Photo
C. Spores
Scale Bar B=4 cm, C=10 µm

Photo plate 15: ***Microporus xanthopus***



A. Field Photo
B. Close up Photo
C. Spores
Scale Bar B=1.5 cm, C=10 µm

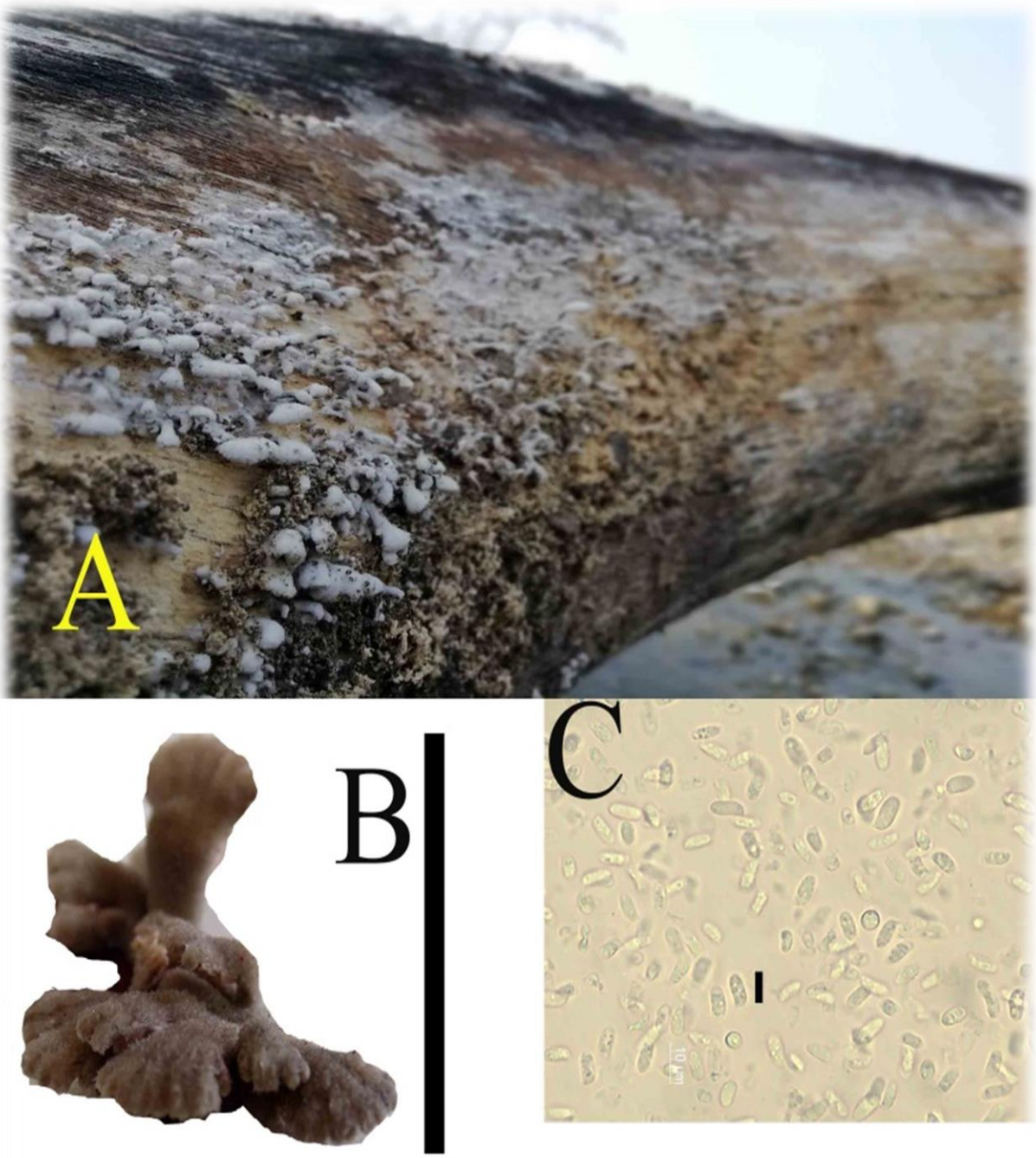
Photo plate 16*: Mycena acicula*



A. field Photo
B. Close up Photo
C. Spores
Scale Bar B= 8cm, C=5 μm

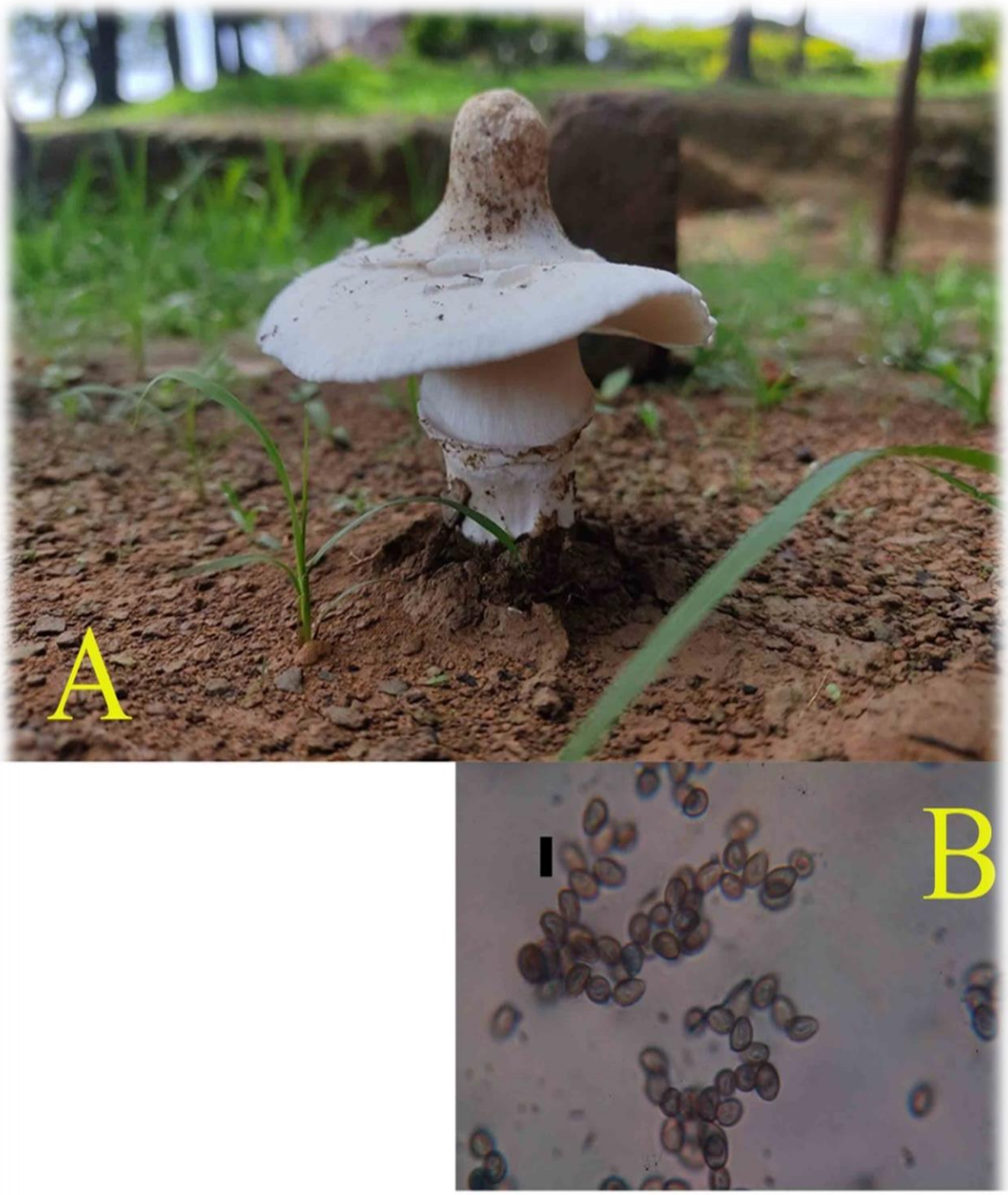
Photo plate 17*: Panus sp.*

Photo plate 18*: Phallus indusiatus*



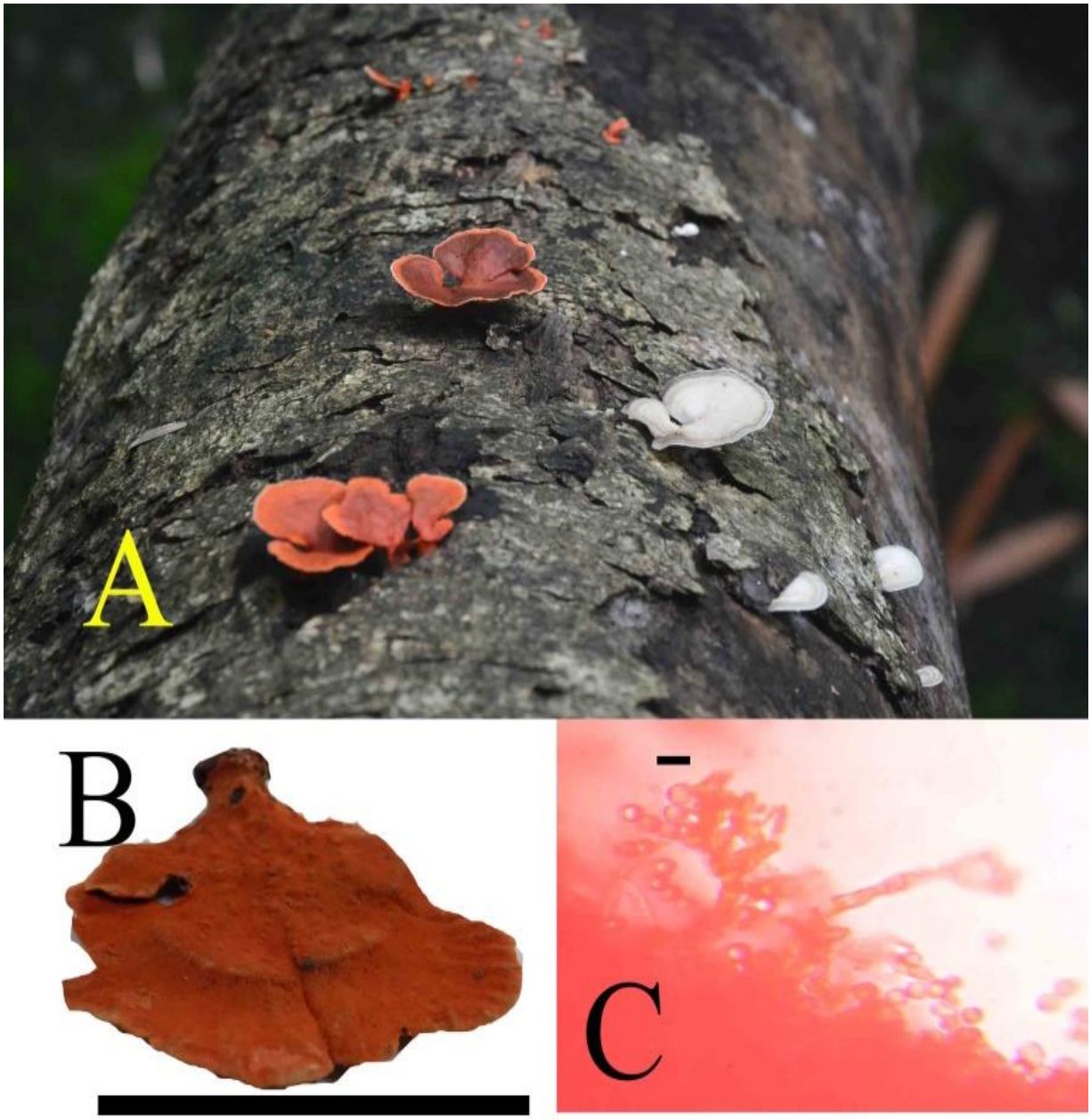
A. Field Photo
B. Close up Photo
C. Spores
Scale Bar B=2 cm, C=5

Photo plate 19*: Schizophyllum commune*



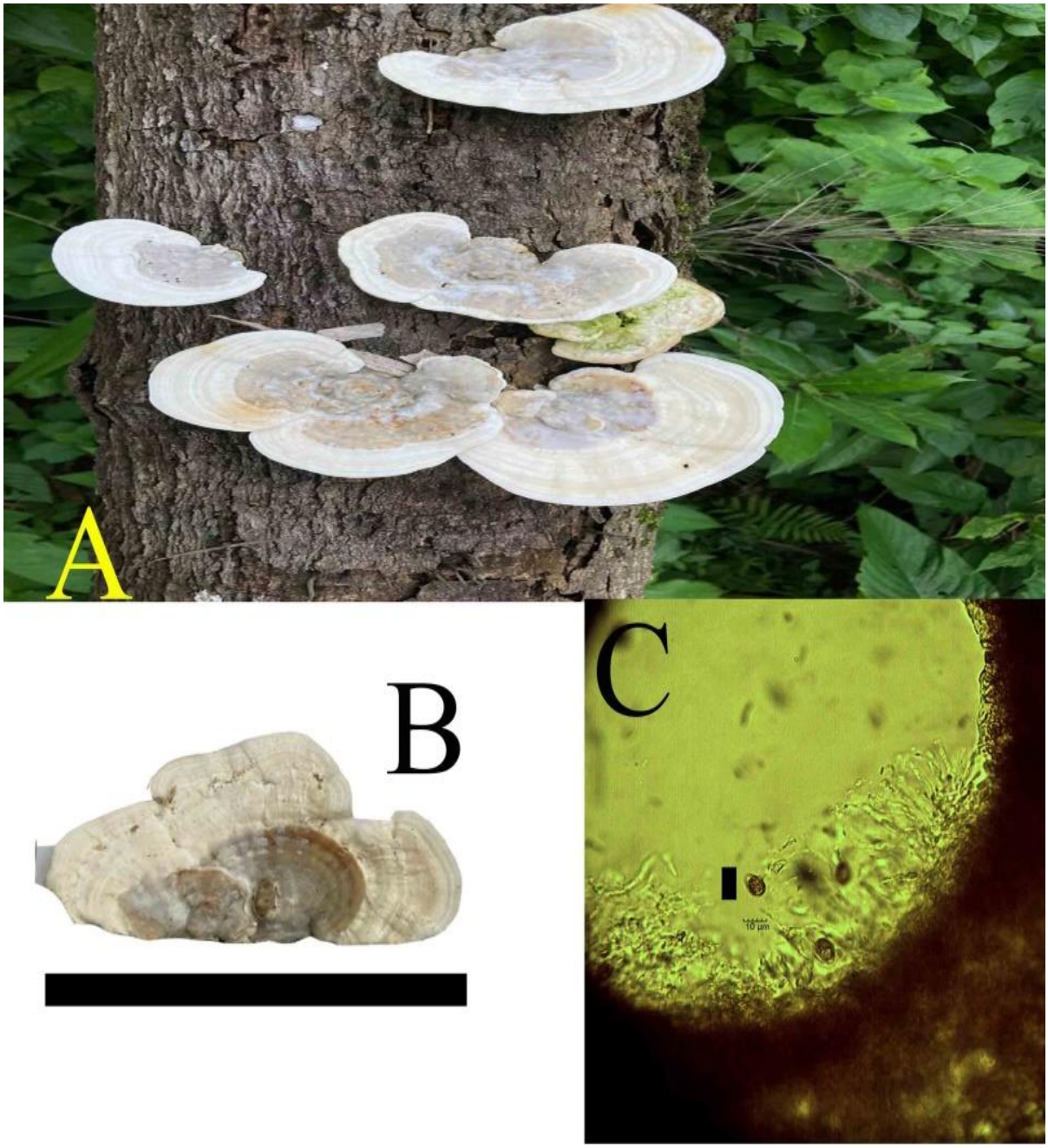
A. Field Photo
B. Spores
Scale Bar B=10 µm

Photo plate 20*: Termitomyces heimii*



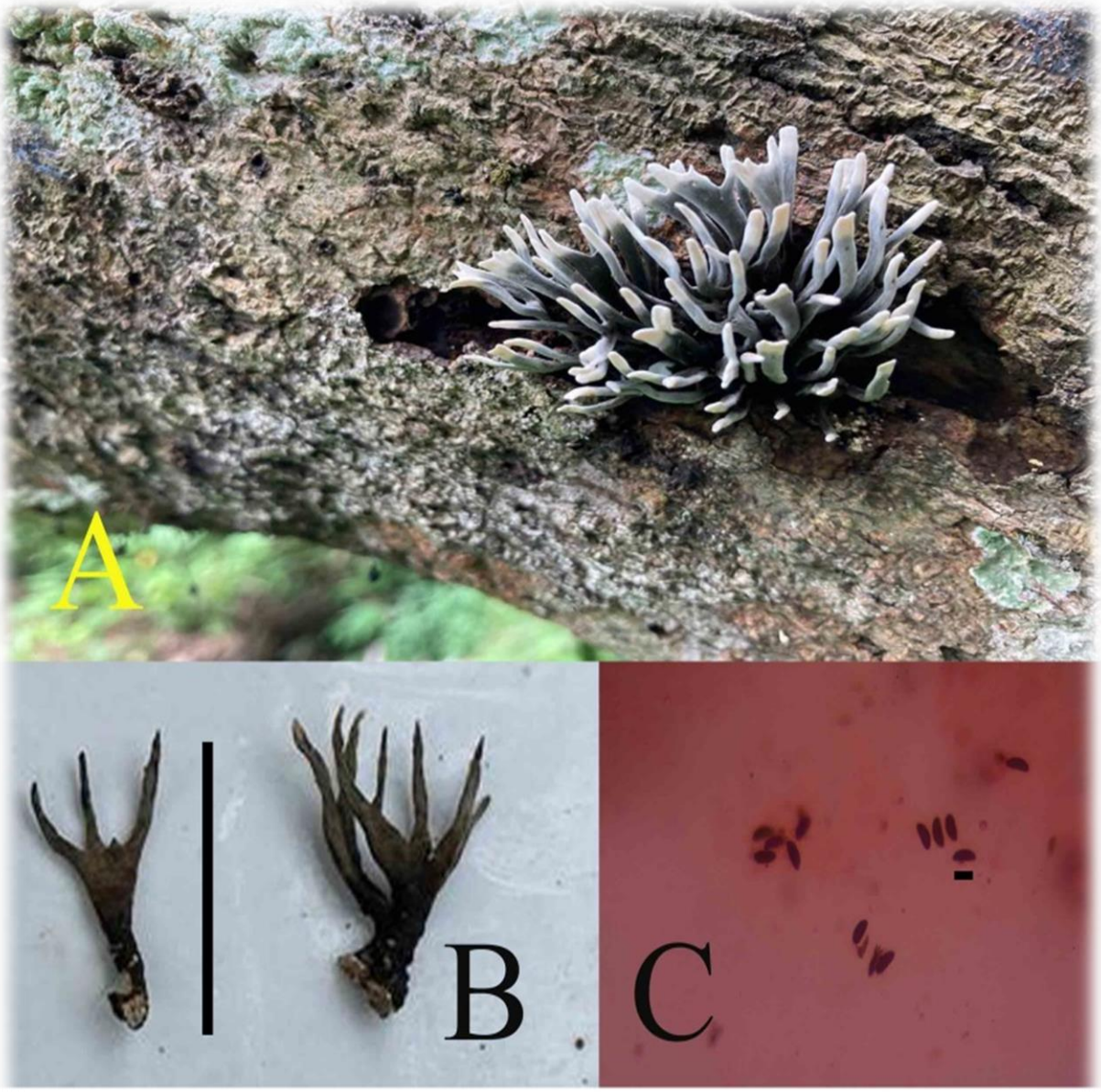
A. field Photo
B. Close up Photo
C. Spores
Scale Bar B= 8 cm, C=8 µm

Photo plate 21*: Trametes coccineus*



A. field Photo
B. Close up Photo
C. Spores
Scale Bar B= 15cm, C=6 µm

Photo plate 22*: Trametes elegans*



A. Field Photo
B. Close up Photo
C. Spores
Scale Bar B=4 cm, C=12 µm

Photo plate 23:*Xylaria bambusicola*