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Wood rotting polyporales from the biodiversity reserves within the Indian subtropical habitat

Zohmangaiha Chawngthu[1,*], Lalnun Tluanga[1], John Zothanzama[2], V L Thachunglura[1], Benjamin Lalbiakmawia[1], Laltanpuia Renthlei[1]

[1]*Dept. of Environmental Science, Mizoram University, Aizawl, Mizoram, India*
[2]*Dept. of Biotechnology, Mizoram University, Aizawl, Mizoram, India*

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ABSTRACT

Background: Identification of fungi to species level is paramount in both basic and applied applications in scientific research. A diverse group of fungi play a crucial role in ecosystem functioning and significantly contribute to the biodiversity of various ecosystems. In the northeastern state of Mizoram, India, the study of wood-inhabiting fungi holds immense potential for uncovering the region's fungal diversity and ecological interactions. The present study investigated the phylogenetic relationships and taxonomic identities of different fungal species of polypores isolated from diverse parts of Mizoram, India.
Materials and Methods: Fungal species were collected and identified using a macro and micro-morphological characteristics and molecular approaches.
Results: Nuclear ribosomal DNA sequences, along with the internal transcribed spacers (ITS-1 and ITS-2), and the 5.8S gene area, were used to identified the collected samples. 23 fungal isolates of polypores were selected for molecular phylogenetic analysis. The 23 species were identified to the species level based on the fungal sequences with known identities in GenBank.
Conclusion: The combined approach both morphological and molecular techniques proposed in the study holds the potential to address these challenges and provide a more efficient and accurate way of understanding fungal distribution.

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1. Introduction

Polyporaceae are the most important families of Basidiomycota and despite this, they are the most diverse orders of Basidiomycota, with over 1800 known species in 216 genera and 13 families.[1] Polypores are Basidiomycetes producing the basidia, typically, on the inside of the tube lining the undersurface of the fructifications.

In the past decades, many new genera in polyporales have been recognized such as *Datroniella*, *Fragifomes*, *Megasporia*, *Pseudofibroporia* and *Pseudomegasporoporia*.[2–6] The fungal taxonomy is usually based on their comparative macro and micro-morphological features.[7] Although, cautious observations should be taken when dealing with closely related or morphologically similar fungi are identified, because the morphological traits of certain fungi are influenced by cultural and medium-dependent conditions that can significantly affect both vegetative and sexual compatibility.[8,9] Moreover, the traditional identification methods are inadequate for distinguishing fungal isolates that do not produce spores in culture, which are categorized as mycelia sterilia.[10] In contradistinction to this, modern molecular techniques showcase high specificity and sensitivity for identifying microorganisms and can be used for classifying microbial

* *Corresponding author.*
E-mail address: xohmaa1990@gmail.com (Z. Chawngthu).

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strains across various level of taxonomic hierarchy.[11]

Molecular methods are progressively being used to identify various kind of fungi[12–14] and provide a quick and precise way of characterizing and identifying organisms because they rely on objective information (molecules) derived from the target organism rather than the substantial subjectivity of classical methods.[14,15] The majority of molecular fungal species identification relies on amplification and sequencing of the fungal genome's internal transcribed spacer (ITS) region, which is extremely variable among species or even populations of the same species.[16,17] This region has two non-coding spacer sections (ITS-A and ITS-B) separated by the 5.8S rRNA gene and is located between the 18S small subunit (SSU) and the 28S large subunit (LSU) ribosomal RNA (rRNA) genes.[18] The ITS region in fungi is typically 650900 bp in length, containing the 5.8S gene, and is generally amplified by the universal primer pair ITS-1 and ITS-4.[19] Once the region has been amplified, it can be sequenced and can be compared to that of known species sequences.

Many investigations have used polymerase chain reaction (PCR) based methods for identifying wood decaying fungi.[13,20–22] Since the ITS regions of ribosomal DNA (rDNA) are extremely varied between species but highly conserved within the majority of species (with intraspecific similarities >99%) and they can be used for taxonomy.[23]

On DNA from individual species, the specific primer ITS1 and the fungal universal primer ITS419 work well. However, DNA extracts including both host plant and microbial DNA do not work as well. The primers ITS1F and ITS4B were developed to be specific for fungi and basidiomycetes, respectively excluding plants.[19,24] In different regions of India, a number of investigations have been done using a molecular approach to study of the wood rotting fungi.[25,26]

2. Materials and Methods

2.1. Study area

Mizoram, an ecologically relevant state of North East India, spans an area of 21,081 sq. km and positioned between the geographical coordinates of 21° 58' and 24° 35' N Latitude, and 92° 15' and 93° 20' E Longitude and traversed by the Tropic of Cancer specifically at the latitude of 23° 30' N. Mizoram is also considered as one of the world's biodiversity hotspots due to its diverse flora and fauna. Although a lot of research has been done on higher animals however data on wood rotting fungi in Mizoram are still very limited.

2.1.1. Dampa Tiger Reserve (No.1-1/92-PT dt. 20.10.1994)

Dampa Tiger Reserve is situated in the western region of Mizoram, adjacent to the Bangladesh border and located within the boundaries of Mamit District in Mizoram. Dampa Tiger Reserve covers an area of 500 sq. km and it is the largest designated protected area within Mizoram.

2.1.2. Murlen National Park (No. B. 11011/13/84-FST. dt. 8.7.1991)

Murlen National Park is located in the northeastern region of Mizoram within Champhai District and close to the Chin Hills of Indo-Myanmar bordering area. The demarcation area is 100 sq. km. The forest is confined to Sub-tropical broadleaved hill forest.

2.1.3. Tawi wildlife Sanctuary

Tawi Wildlife Sanctuary is located within the Aizawl District of Mizoram, encompassing an area of around 35 sq. kilometers, the sanctuary's elevation reaches up to 1700 meters above the msl. Its geographical coordinates are 23°33' N latitude and 92°56' E longitude respectively. Tropical Evergreen Forest, Scrub Forests, and Semi-Evergreen Forest make up the majority of the sanctuary's vegetation. The protected area's woodlands are quite rich and still mostly unexplored, supporting a distinctive and varied range of flora and fauna.

2.1.4. Reiek forest

Reiek forest is located in the north-western part of Mamit District, Mizoram. It occupies an area of 10 sq. km and lies between 92°37' and 93°28'E and latitude 20°45' and 22°46'N. The highest peak of Reiek Mountain stands at an elevation of 1485 meters above sea level and characterized by its tropical essence. The forest predominantly features an occurrence of evergreen tree species, thus may be classified as evergreen or semi-evergreen forest types.[27]

2.1.5. Hmuifang forest

Hmuifang is situated in the southern part of Aizawl District as a well-known tourist spot. The mountains remain cloaked in untouched forest and the vegetation falls under tropical semi-evergreen forest.

2.1.6. MZU campus

The campus of Mizoram University is located in the south western part of Aizawl city and the vast expanse of the campus is adorned with numerous trees that provide a broad variety of fungi.

2.2. Sample collection and preservation

The samples were isolated from their substrates or hosts, and in the case of soft samples, they were sometimes simply

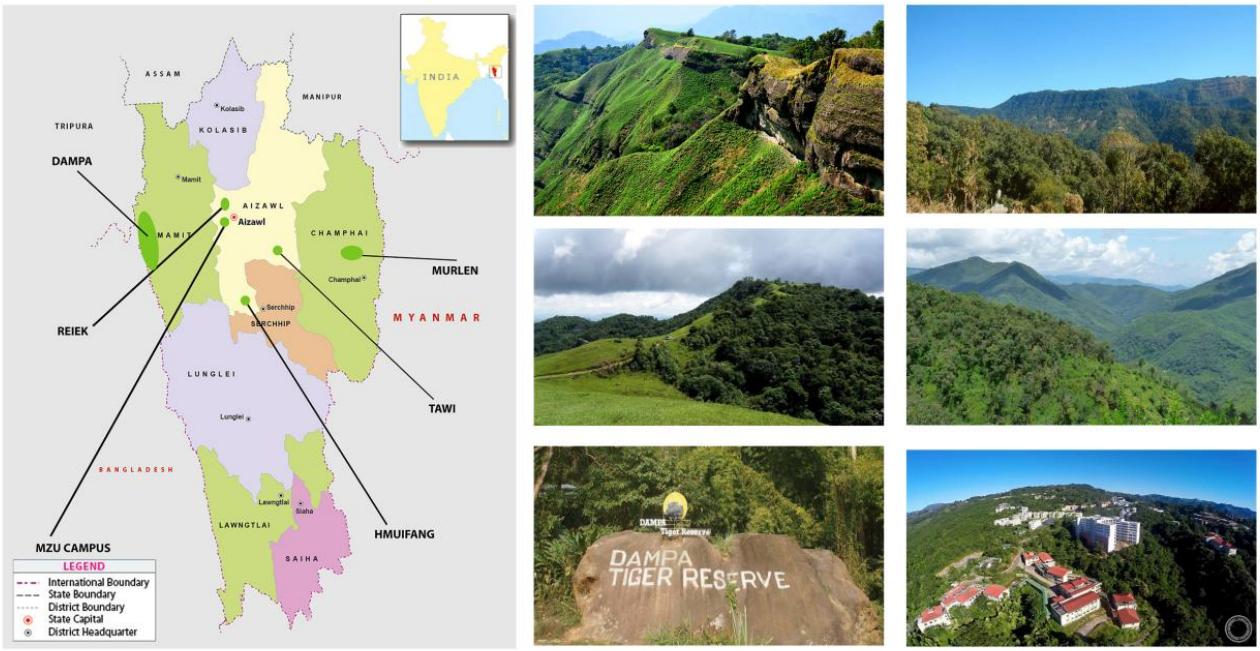

Fig. 1: Map indicating the collection sites

plucked with the bare hand. The collected samples were stored in airtight containers, and scale-assisted field and lab photographs of each sample were taken.[28] Deep freezing, air drying or liquid preservation is used to preserve the specimens, and the collected specimens are given voucher numbers and stored in the Department of Environmental Science, Mizoram University.

2.3. Morphological identification of collected specimens

In accordance with earlier morphological descriptions of standard macroscopic and microscopic criteria, all of the collected specimens were identified.[29,30] With the use of a razor blade, thin sections of dried specimens are cut for microscopic investigation. These sections are then mounted in 3% KOH solution and stained in 2% aqueous phloxine. Sections are mounted in 60% lactic acid + cotton blue or Lactophenol. By cutting the cap and placing it in a piece of white paper, spore prints of the collected specimens were obtained.[31]

2.4. DNA extraction

Using a CTAB Extraction specimen, DNA (deoxyribonucleic acid) was extracted from fruiting bodies of the samples.[32] 500 μL of CTAB lysis buffer with glass beads (212-150 μm size-fractionated acid-washed glass beads from Sigma) were added with a tiny amount of tissue from the inside of the fruiting body. To homogenize the fungal cells, the centrifuge tube was vortexed for one minute, and two minutes for drier and tougher fruiting bodies. The bigger tissue pieces were then removed from the tubes by immediately centrifuging them, and the supernatant was then transferred to a fresh, sterile micro centrifuge tube before being incubated for an additional 20 minutes at 65 °C. After adding 500 μL of chloroform, the mixture was centrifuged at 13,000 rpm for five minutes. Isopropanol was used to precipitate the DNA, which was then incubated for 5 minutes at room temperature and centrifuged for 7 minutes at 15,000 rpm. The DNA pellet was air-dried after being washed with 500 μL of 70% ethanol. Re-suspended DNA pellets were put in 100 μL of sterile milli-Q water. Using 0.8% agarose gel electrophoresis, the quality and quantity of the extracted DNA were visually observed.

2.5. PCR and sequencing

A 25 μl reaction mixture containing 1 μl of fungal DNA template, 5 μM of each primer, and 1X GoTaq Green Mastermix (Promega, Madison, WI) was used for the PCR setup. The PCR method involves a 5-minute initial denaturation stage at 94 °C, 35 cycles of 94 °C for 1 minute, 52 °C for 1 minute, and 72 °C for 1 minute, and a 72 °C extension step at the end. The amplicons' quality was examined using 1% agarose gel electrophoresis. PCR amplicons were sequenced using an ABI Prism Big Dye terminator cycle sequencing ready reaction kit and a 3730xl genetic analyzer at the DBT- State Biotech Hub, Department

of Biotechnology, Mizoram University.

2.6. Nucleotide analysis

Twenty-three local species of Polyporales were successfully sequenced to generate partial sequences of the 18S rRNA and 28S rRNA sandwiching full sequences of ITS1, 5.8 rRNA and ITS2. Generated ITS nucleotide sequences were edited using Bioedit 7.2,[33] aligned using BLAST[34] and were finally submitted to Genbank. Additional sequences were retrieved from GenBank to help taxonomic placement of locally available species. Sequence of *Dacryopinax spathularia* (GenBank Acc. No. AB712473) was used as outgroup to root tress. Sequences were aligned by MAFFT[35] in DAMBE.[36] The maximum parsimony (MP) analysis was performed in PAUP 4.0 beta.[37] Heuristic search using tree bisection-reconnection (TBR) swapping and 100 additional randomly selected replicates were employed to construct the tree. Gaps were considered as missing data, and all characters had the same weight.

MrModeltest 2.4[38] was used to find the best-fit evolution model using the Maximum Likelihood (ML) and Bayesian inference (BI) approaches. PAUP 4.0 beta was also used to build a maximum likelihood (ML) tree with the GTR+G+I site substitution model, which included estimating Gamma-distributed rate heterogeneity and a proportion of invariant sites. The bootstrapping approach with 1000 replicates was used to assess branch support. Bayesian analysis was carried out using Mrbayes 3.2.6,[39] for two independent runs using the command given by Mr.Modeltest for 100 million generations until the split deviation frequency value $\leq$ 0.001999. Trees were sampled every 1000 generations and the first 25% trees were discarded as burn-in. Convergence and mixing of the chains were evaluated using Tracer v1.7.1.[40] FigTree v. 1.4.3 was used to view the trees (http://tree.bio.ed.ac.uk/). The topologies of the consensus trees obtained from maximum parsimony and maximum likelihood are presented with bootstrap values (BS) and Bayesian inference (BI) with posterior probabilities (PP) and on or below branches as followed (MP/ML/BI). Finally, nucleotide sequence statistics of each clade were calculated with DAMBE.[36]

3. Results

3.1. Morphological identification and taxonomical placement

Twenty three species were collected from the study sites and morphological identification was first carried out according to characteristics of the spores, and reproductive structures if discernible using previously described methods.[41,42]

Most of the fungal isolates could be identified to the genus level using conventional morphological methods. The remaining were identified using molecular methods up to the species level. The identified species are *Fomitopsis dochmia*, *Fomitopsis feei*, *Favolus acervatus*, *Polyporus phillipinensis*, *Hexagonia tenuis*, *Lentinus squarrosulus*, *Lentinus badius*, *Abundisporus fuscopurpureus*, *Ganoderma subresinosum*, *Ganoderma mizoramense*, *Ganoderma australe*, *Microporus vernicipes, Pycnoporus coccineus, Trametes hirsuta*, *Lenzites warnieri*, *Trametes cubensis*, *Trametes maxima*, *Trametes elegans*, *Leotrametes flavida*, *Trichaptum biforme*, *Panus conchatus*, *Spongipellis delectans*, and *Ceriporiopsis semisupina*.

Species like *Lenzites warnieri* (*Cellularialla warnieri*), *Pycnoporus coccineus* (*Trametes coccinea*)and *Leiotrametes flavida* (*Trametes flavida*) were submitted to the GenBank as their previous name. Most of the new names given were proposed after our submission to GenBank. *Favolus acervatus, Ceriporiopsis semisupina* and *Lentinus squarrosulus* were only identified to the genus level using morphological techniques, which were later confirmed with molecular analysis to the species level. Morphological and microscopic characteristics of the 23 identified species are shown in Table 1 and their fruiting bodies in Figure 2.

Fig. 2: Basidiocarp of collected specimen

Table 1: Morphological and Microscopic characteristics of identified species

S. No.	Name of Species	Morphological and Microscopic characteristics
1	*Fomitopsis dochmia*	Basidiocarps annual, biennial or perennial, solitary or imbricate, sessile or effused-reflexed, Basidia subclavate, or narrowly clavate, 4-sterigmate, 12.0-19.0 x 4.0-5.0 with a basal clamp. Basidiospores oblong-ellipsoid, smooth-walled, hyaline, 4.0-6.0 (-7.0) x (1.5) 2.0-2.5
2	*Fomitopsis feei*	Basidiocarps annual, biennial or perennial, solitary or imbricate, sessile or effused-reflexed, corky to woody when dry; pore surface pale pinkish brown, whitish pink, or ochraceous pink, margin narrow, sterile; Basidia clavate, 4-sterigmate, 10.0-20.7 x 3.6-7.0 μm, with a basal clamp. Basidiospores cylindrical, hyaline, smooth-walled, somewhat pointed at the ends (4.0) 5.0-7.0 (-8) x (1.5) 2.0-3.5 (-4) μm.
3	*Favolus acervatus*	Basidiocarps annual, laterally stipitate; pileus flabelliform, up to 7 cm wide and 4 mm thick. Basidia clavate, 4-sterigmate, 18-25 x 5-8 μm. Basidiospores oblong ellipsoid to subellipsoid, (4.5)6-8(10) x 2.5-3 μm, varying in size within the same basidiocarp.
4	*Polyporus phillipinensis*	Basidiocarps annual, laterally stipitate; pileus flabelliform, up to 7 cm wide and 4 mm thick, Basidia clavate, 4-sterigmate, 18-25 x 5-8 mm. Basidiospores oblong ellipsoid to subellipsoid, (4.5)6-8(10) x 2.5-3 mm, varying in size in the same basidiocarp.
5	*Hexagonia tenuis*	Basidiocarps of Hexagonia tenuis is a thin, leathery bracket growing from the side, or adpressed to the underside, of dead branches. Spores are 9-13 × 2.5-4.5 μm, straight or slightly curved.
6	*Lentinus squarrosulus*	Basidia small, with 4 sterigmata. Cheilocystidia as sterile basidia along the sterile gill-edge. Pleurocystidia none. Hyphal pegs -60 (-120) x 15-25 (-38) μm abundant on the edges and sides of the gills, often with incipient sterile basidia.
7	*Lentinus badius*	Spores 3.7-5 x 2-2.7 μm white, smooth, ellipsoid, inamyloid (with 1-2 guttulae). Basidia 15-20 x 4-5 μm sterigmata.
8	*Abundisporus fuscopurpureus*	Basidiocarps perennial, effused-reflexed to pileate, soft, without odor or taste when fresh, becoming soft corky upon drying. Basidia barrel-shaped to pear-shaped, with four sterigmata and a basal clamp connection, 11–13 × 6–7 μm; basidioles dominant, similar to basidia in shape but slightly smaller. Basidiospores ellipsoid, yellowish, slightly thick-walled, smooth, non-dextrinoid, (3.5–)4–4.5(–5) ´(2.5–)3–3.5 μm, L = 4.3 μm, W = 3.3 μm, Q = 1.4–1.42 (n = 60/2).
9	*Ganoderma subresinosum*	Basidiome annual, sessile (with distinctly contracted base), weakly laccate, woody. Basidiospores ellipsoid to ellongate, pale orange to greyish orange, with a brown eusporium bearing fine, short, and distinct echinulae, overlaid by a hyaline myxosporium.
10	*Ganoderma mizoramense*	Basidiocarp annual, pileate, stipitate, soft when fresh and woody when dried, semi-circular, irregular surface, dark brownish to dark reddish brown. Stipe sometimes present and often prominent; twisted and irregular. Smooth pore surface creamy to snuff brown when dry, pores 4–5 per mm, round to somewhat slightly oval, 187–278 × 134–228 μm. Basidia tetrasterigmatic. Basidiospores ellipsoid, 10–12 × 6–9.5 μm in diameter.
11	*Ganoderma australe*	Pore surface white to cream in actively growing specimens, then dark when touched, in older and resting species, pale to umber-brown. Spores truncate, golden-brown, echinulate, 6-13 x 4.5-8 μm

Continued on next page

Table 1 continued

12	*Microporus vernicipes*	Basidiocarps annual, solitary or in smaller groups, often several pilei are grown together, laterally stipitate, pileus semicircular, often with the rear parts grown backwards so they almost meet over the stipe, or flabelliform to spathulate, up to 10 cm wide, thin, flexible and tough, rarely more than 1-2 mm thick. Basidiospores cylindrical, hyaline, smooth and non-amyloid and non-cyanophilous, 5-7 x 2-2.5 μm in diameter.
13	*Pycnoporus coccineus*	Basidia 10.5-13.2 x 4.0-5.2 μm, bearing four spores; basidiospores hyaline, even, non-amyloid, short cylindric, slightly flattened on one side, minutely apiculate, 4.0-4.6 x 1.6-2.0 μm.
14	*Trametes hirsuta*	Basidiocarps annual to perennial, pileate, sessile, dimidiate to fanshaped, single or imbricate, flexible to hard; upper surface hispid to glabrous, often zonate; pore surface white, cream to pale grayhe basidiocarps are often strongly susceptible to attack from insects.
15	*Lenzites warnieri*	Basidiocarps annual. Sessile to dimidiate, semicircular, applanate, 5-20 x 3-8 x 1-2 cm, coriaceous and tough Basidia clavate. 4-sterigmate, 15-25 x 5-6 μm. with a basal clamp. Basidiospores cylindric, straight or slightly curved, hyaline, thin-walled. 7-9 x 3-4 μm.
16	*Trametes cubensis*	Basidiocarps ranging from 6-7 inches down to 2 inches wide, showing concentric zones of cream and browns. Occurring singly or in groups; when in groups some brackets fused to others above/below. Some have very short stalk.
17	*Trametes maxima*	Basidiocarps annual, pileate, solitary or imbricate, without odour or taste when fresh, corky and light in weight when dry. Basidia clavate, with four sterigmata and a basal clamp connection, 10-15 x 3-5 μm; basidioles in shape similar to basidia, but slightly smaller. Basidiospores oblong ellipsoid, hyaline, thin-walled, smooth, IKI-, CB-, (4.1-) 4.2-5.1(-5.8) x 2-2.4(-2.5) μm, L = 4.78 μm, W = 2.18 μm, Q = 2.19 (n = 30/1).
18	*Trametes elegans*	Basidiocarps annual to perennial, sessile or with a short stipelike base, attached laterally or centrally, pileus 1-35 cm wide and long and 0.2-3 cm thick, corky and flexible when fresh, more rigid when dry. Basidiospores cylindric to oblong ellipsoid, hyaline, smooth and thin-walled, 5-7 x 2-3 μm, non-amyloid
19	*Leotrametes flavida*	Basidiomata probably perennial, sessile, pileate, applanate, semicircular. Basidia 12–15 × 3–5 μm, clavate, tetrasterigmatic, sterigmata 3 μm long. Basidiospores broadly ellipsoid, hyaline, thin-walled, smooth,
20	*Trichaptum biforme*	Basidiocarps pileate, annual or reviving next season, variable in form, partly dimidiate to broadly sessile, single or imbricate, or spathulate to flabelliform with a tapering base. Basidiospores cylindrical, hyaline, smooth, thinwalled and non-amyloid, 5-6.5 x 2-2.5 μm.
21	*Panus conchatus*	Gills deeply decurrent, rather crowded, 3-3.5 mm wide, 4-5 ranks, dingy cream to pale ochraceous bistre, pinkish near the entire edge, without hyphal pegs. Flesh thin, pliant, coriaceous, white. Basidia 20-28 x 5-6 μm sterigmata 4. Cheilocystidia 25-45 x 816 μm clavate or pyriform to ventricose and obtuse, smooth, thinwalled, as a sterile edge to the gill. Basidiospores 6-7 x 3-3.5 μm white, smooth, shortly subcylindric, inamyloid.
22	*Spongipellis delectans*	Basidiocarps annual, pileate, broadly attached, semicircular, reflexed to semiresupinate; pileus tomentose to smooth, white to ochraceous; hymenophore poroid to dentate. Basidiospores ellipsoid to globose, smooth, hyaline, thick-walled.
23	*Ceriporiopsis semisupina*	Basidiocarps annual, resupinate, mostly light-coloured; margin rhizomorphic or not. Basidiospores small to medium sized; context white to light-coloured, thin:

3.2. Molecular analysis: Nucleotide analysis and phylogeny

The ITS sequences acquired in this investigation contained partial 18S, complete ITSI, 5.8S, ITSII, and partial 28S sequences ranging in size from 441 to 722 bp from 23 fungal species. Along with 56 additional sequences retrieved from GenBank, The aligned length of the ITS dataset was 1386 characters, of which 829 are constant (proportion = 0.598124). 419 are parsimony-informative and 138 are variable and parsimony-uninformative. Maximum parsimony analysis under heuristic search retained 21118 trees of which 91% tress were equally parsimonious (tree length = 2266, consistency index = 0.421, retention indices = 0.777, rescaled consistency indices = 0.327 and homoplasy index = 0.579). GTR+ I +G and lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1, 1, 1, 1) were the best models for the ITS dataset in Maximum likelihood (ML) and Bayesian analysis (BI), with an average standard deviation of split frequencies of 0.002 (BI). Bayesian analysis and ML analysis produced a topology that was similar to MP analysis.

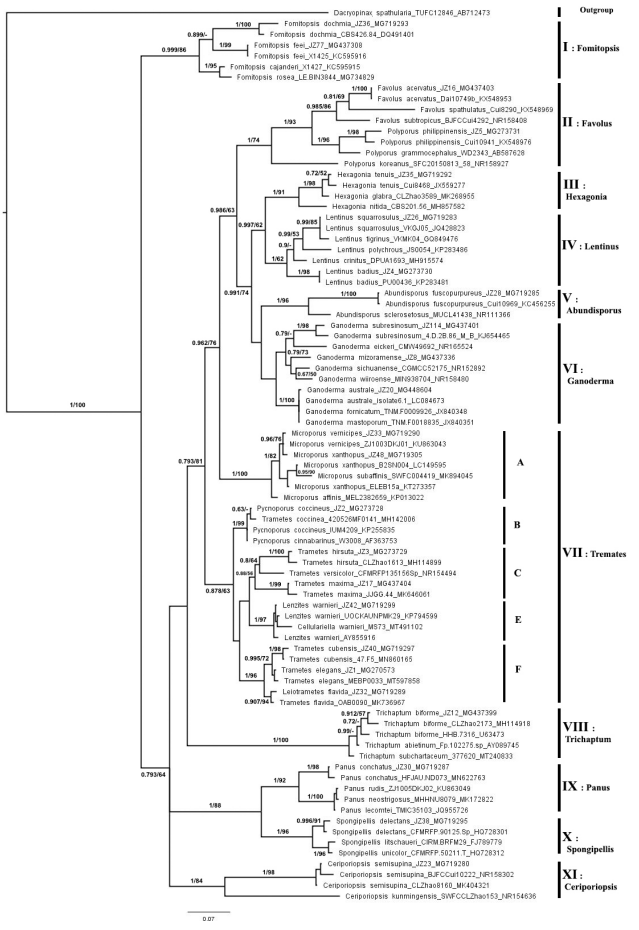

Fig. 3: Phylogenetic tree

The phylogeny inferred from the ITS dataset clustered to eleven distinct clades (Figure 3). Clade I consisted of species belonging to the genus *Fomitopsis* followed by *Favolus* in clade II, *Hexagonia* in clade III and *Lentinus* in clade IV. All the species of *Abundisporus, Ganoderma*, *Trametes* and *Trichaptum* grouped into clade V, clade VI, clade VII and clade VIII respectively meanwhile clade IX, clade X and clade XI was formed by species belonging to *Panus*, *Spongipellis* and *Ceriporiopsis*.

4. Discussion

The close relationships of the genera *Pycnoporus*, *Lenzites*, *Coriolopsis* and *Trametes* have been confirmed.[43–45] However, relationships within the core polyporoid group's subgroup "B" *Trametes-Lenzites-Pycnoporus-Coriolopsis*[43] remained unknown. Morphological traits characterizing these four genera, such as lamellate or pored hymenophores and hyphae color, have yet to be proven at the generic level. Based on these findings, two options can be considered: recognizing a unique genus Trametes, enlarged to include the three traditional genera mentioned above; or, as far as some monophyletic clades can be supported by morphological features, splitting this clade into different genera, each defined by a thorough combination of characters.

Wood decaying fungi hold a vital ecological role in nutrients recycling through the action of enzymatic and mechanical processes to break down the complex structure of wood[46] and significantly contributing to the soil ecosystem. Wood rotting fungi degrade wood's lignocellulosic components into simpler compounds, while their growth patterns and physical structures aid in the mechanical disruption of wood tissues. This process contributes to the release of essential nutrients and carbon back into the ecosystem, facilitating nutrient cycling and maintaining the ecological balance of forest ecosystems. Due to the phenotypic plasticity, the phenotypes of the same species of mushroom may exhibit variable phenotypes in different locations.[47] This may be cause by the change in environment which alters the morphology, physiological state or behavior of the mushroom which results in different names of the same species.

Polypores species hold cultural significance, medicinal potential and contribute to bioremediation efforts. Moreover, their complex enzymatic systems break down lignocellulosic materials and provide energy and nutrients to the ecosystems. With their remarkable medicinal properties, polypore species are extensively used in folk medicines for the treatment of several diseases[48] and some edible polypores have high nutritional properties[49] and exhibit a symphony of healing potential showcasing their ability ranging from combating microbes and viruses to thwarting cancer, atherosclerosis and allergies. Furthermore, their multifaceted benefits extend to regulating blood sugar,

quelling inflammation, safeguarding the liver and potent anticancer activity.[50,51]

5. Conclusion

Combining both morphological and molecular methods enable mycologists to delve into the intricate understanding of fungal distribution. Since, traditional sampling technique can be considerably time consumption and the scarcity of proficient taxonomists can gently stretch the time to identify all the collected samples, many researchers ignore the classical method of identifying mushroom. Despite recent advances in the use of molecular methods, there are still many advantages to classical methods for studying fungal diversity. Even with the modern molecular techniques, traditional methods for studying fungal diversity remain valuable due to their advantages of longstanding reference and cost-effectiveness.

This work materially adds to our knowledge of polyporales from all over Mizoram in one sight. This could be the first contribution from Mizoram in which total overview of the polyporales flora has been taken extensively. It can also be concluded that molecular methods described serve as an important new tool for studying the taxonomy and diversity of the fungi in Mizoram so that a more comprehensive inventory for the fungi of northeastern India can be realized.

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7. Conflict of Interest

None.

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Author biography

Zohmangaiha Chawngthu, Research Scholar https://orcid.org/0000-0001-5762-2490

Lalnun Tluanga, Professor

John Zothanzama, Professor https://orcid.org/0000-0003-1418-9185

V L Thachunglura, Research Scholar https://orcid.org/0000-0003-1182-776X

Benjamin Lalbiakmawia, Research Scholar https://orcid.org/0000-0002-4436-0206

Laltanpuia Renthlei, Research Scholar https://orcid.org/0000-0002-6653-6268

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