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RESEARCH

Characterization of ectomycorrhizal fungal community of *Abies Pindrow* using sporocarp sampling, morphotyping, and metabarcoding through next-generation sequencing

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Abstract
Abies pindrow, commonly known as the West-Himalayan Fir, holds great ecological importance as a native tree species in the Himalayas. Beyond its value as a fuel and timber source, it serves as a keystone species within the ecosystem. However, over recent years, extensive degradation and deforestation have afflicted *A. pindrow* forests. Utilizing ectomycorrhizal fungal symbionts of *A. pindrow* could prove pivotal in restoring these deteriorated forests. This study aimed to evaluate the diversity and composition of the ectomycorrhizal fungal community associated with *A. pindrow*. We employed ectomycorrhizal root tip morphotyping, sporocarp sampling, and Illumina MiSeq metabarcoding of the ITS region of fungal nrDNA. The ectomycorrhizal root tips were categorized into 10 morphotypes based on their morphological characteristics, exhibiting an average colonization rate of 74%. Sporocarp sampling revealed 22 species across 10 genera, with *Russula* being the most prevalent. The metabarcoding yielded 285,148 raw sequences, identifying 326 operational taxonomic units (OTUs) belonging to 193 genera, 114 families, 45 orders, 22 classes, and 6 divisions. Of these, 36 OTUs across 20 genera were ectomycorrhizal, constituting 63.1% of the fungal community. Notably, *Tuber* was the most abundant, representing 37.42% of the fungal population, followed by *Russula* at 21.06%. This study provides a comprehensive understanding of mycorrhizal symbionts of *A. pindrow*. The findings hold significant implications for utilizing dominant ectomycorrhizal fungi in reforestation endeavors aimed at restoring this important Himalayan conifer.

Keywords West-Himalayan fir · Ectomycorrhiza · Molecular characterization · Morphotypes · Mycobiome · Himachal Himalaya

Introduction

Abies pindrow Royle, a vital conifer of economic significance, thrives in shaded and moist north-facing mountain slopes, typically ranging between 2000 and 3400 m above sea level (Kunwar et al. 2020). With towering heights of 40 to 60 m and diameters spanning 2.0 to 2.5 m (Ali et al. 2014), it forms dense stands either independently or in association with other trees such as *Pinus wallichiana*, *Picea smithiana*, *Aesculus indica*, *Quercus semecarpifolia*, *Taxus contorta*, *Prunus cornuta*, and *Acer ceasium* (Joshi and Samant 2004; Uddin et al. 2019). As a keystone species, *A. pindrow* significantly contributes to the structural integrity of temperate forest ecosystems, while also enhancing biological productivity through vegetative growth, biomass yield, and habitat creation (Singh et al. 2018). Its wood serves various purposes including fuel, timber, agricultural tools, furniture, and pulp production (Gairola et al. 2014). However, over the past few decades, fir forests have undergone extensive deforestation and degradation, primarily due to over-harvesting for building and carpentry, paper and pulp, matchwood, packing cases, and other uses, despite limited natural regeneration (Dar and Dar 2006). The removal of trees has not been synchronized with natural regeneration and seedling establishment processes. Challenges such as high litter fall, thick layers of humus accumulation, lean seed years, poor seed production, dense weed growth, grazing and trampling by livestock, and slow decomposition rates of their needles contribute to the obstacles faced in natural regeneration

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(Sufi 1970). Bakshi et al. (1972) have suggested that poor mycorrhizal association may be one of the reasons for the inadequate regeneration of *A. pindrow*, among other factors. The production of healthy and robust seedlings within a shorter nursery time can be achieved by utilizing appropriate mycorrhizal fungi. This approach holds promise for enhancing the restoration efforts of *A. pindrow* forests and ensuring the ecological balance of the Himalayan region.

Plants within the Pinaceae family, including *A. pindrow*, rely obligatorily on ectomycorrhizal fungi for their growth and survival, under both normal and stressful conditions (Policelli et al. 2020). The successful establishment of these plants in the field is heavily reliant on the presence of suitable ectomycorrhizal fungi (Argüelles-Moyao et al. 2017). In natural forest ecosystems, the root systems of trees almost invariably establish ectomycorrhizal relationships with various species of ectomycorrhizal mushrooms (Dahlberg 2001; Guidot et al. 2004; Tapwal et al. 2021). Mycorrhizae within plant roots play a crucial role in forest restoration through two primary mechanisms: mitigating root stresses and enhancing nutrient absorption by expanding root surface area (Itoo and Reshi 2013; Lakhanpal et al. 2021; Tapwal et al. 2023). Rinaldi et al. (2008) and Roy-Bolduc et al. (2016), indicate the presence of approximately 20,000 to 25,000 fungi establishing ectomycorrhizal associations with 6000 species of higher trees. The recent advancements in next-generation sequencing (NGS) have significantly contributed to our understanding of ectomycorrhizal fungal diversity, ecology, and biogeography (Nilsson et al. 2011; Smith and Peay 2014; Tyub et al. 2018; Tedersoo et al. 2014, 2022). Ectomycorrhizal symbiosis plays a pivotal role in forest ecosystems by facilitating essential functions such as carbon cycling, nutrient mobilization from soil organic matter and minerals, and establishing connections among trees through common mycorrhizal networks (Futai et al. 2008; Agarwal and Sah, 2009; Policelli et al., 2020; Usman et al., 2021). It also mediates plant responses to stressors like drought, soil acidification, toxic metals, and plant pathogens, while contributing to the rehabilitation and regeneration of degraded forest ecosystems and interacting with other soil microorganisms (Courty et al. 2010; Itoo and Reshi 2013). The ectomycorrhizal symbiosis offer a natural, cost-effective, and eco-friendly alternative to synthetic chemical inputs, enhancing productivity without causing ecological harm (Policelli et al. 2020; Assad et al. 2021). Similarly, *A. pindrow*, like most temperate and boreal tree species, forms mutualistic relationships with ectomycorrhizal fungi, which significantly contribute to the survival and growth of these trees. Despite being relatively understudied in terms of mycorrhizal association, the ectomycorrhizal fungi associated with *A. pindrow* show promise for effectively restoring and regenerating degraded fir forests. Presently, alongside sporocarp sampling and morphoanatomical methods, next-generation sequencing and bioinformatics tools are employed to investigate the mycorrhizal symbiosis in roots.

Methodology

Study area

The current study was conducted in Hatu Forest, Narkanda, Shimla, Himachal Pradesh, India (2800–2900, Above Mean Sea Level (AMSL), 38° 03′ 23.8″ N, 128° 38′ 38.7″ E). The major canopy vegetation at the site comprised of *Abies pindrow* and *Picea smithiana* interspersed with *Quercus semecarpifolia*, *Taxus contorta*, *Pinus wallichiana*, and *Prunus cornuta*; the understory included the species of *Viburnum*, *Berberis*, and *Rosa*. The soil was acidic and moisture rich.

Sampling

Five individual trees, spaced at least 50 m apart, were randomly selected for root sample collection. Soil cores (10x10x10cm) were extracted using a spade, starting from the organic layer after clearing the litter. Mycorrhizal root samples were carefully collected from two points on opposite sides of each tree, sealed in sterilized polybags, and kept at 4°C until processing. Sporocarps of ectomycorrhizal fungi growing in the rhizosphere of *A. pindrow* were collected between July and October in during 2021 and 2022. Each sporocarp was carefully placed in a paper bag, transported to the laboratory, and identified.

Morphoanatomical characterization

Prior to analysis, the soil cores were immersed in water and soaked carefully. The roots were then placed in a 1-mm sieve and gently rinsed with tap water to remove any attached soil and debris. Fine root tips were meticulously sorted from the main roots, mycorrhizal roots identified by the absence of root hairs and the presence of somewhat enlarged tips (Menkis et al. 2005). Mycorrhizal root tips were further categorized into morphotypes based on morphological characteristics outlined by Agerer (1991, 2001, 2006) and DEEMY (http://www.deemy.de/). These morphotypes were differentiated by attributes such as color, shape, texture, ramification type, and the occurrence and abundance of emanating hyphae or rhizomorphs. Subsequently, different morphotypes were separated into two distinct vials: one for anatomical studies fixed in FAA, then stored in 50% ethanol for subsequent microscopic examination, and another for metagenomics analysis, preserved at −20°C.

To examine the surface features of ectomycorrhizal roots, tertiary roots with mantle sheaths were carefully excised and prepared for microscopic analysis. Macroscopic attributes

such as ramification, color, and the presence of rhizomorphs were observed under a stereo-zoom microscope (Nikon SMZ 1500). For detailed examination of internal structural features, roots were cut into 1 cm segments. These segments were then subjected to a series of treatments: they were first cleared using 10% KOH at 90°C for 3–4 h, followed by bleaching with a solution of 0.5% H_2O_2 and 0.5% NH_4OH for 30 min, and acidification with 5N HCl for 5 min. Subsequently, the roots were stained overnight with Trypan blue (0.1%), and any excess stain was removed with lactoglycerol. Thin sections of the root tips were cut, stained again with a 0.1% Trypan blue solution, and observed at different magnifications (×4, ×10, ×40, ×100) using a compound microscope (Nikon ECLIPSE E400) to examine the internal structures in detail.

Molecular characterization

Molecular characterization of *A. pindrow* root-associated ectomycorrhizal (EcM) symbiont was performed by extracting genomic DNA from the mycorrhizal root tips of the *A. pindrow*, followed by the amplification of the ITS region using universal primers (ITS1: 5′ TCCGTA GGTGAACCTGCGG3′ and ITS2: 5′ GCTGCGTTCTTC ATCGATGC3′).

Fungal DNA was isolated from the mycorrhizal root tips using the Xploregen gDNA extraction kit following the manufacturer's protocol. The integrity of the DNA was assessed using 0.8% agarose gel. Subsequently, the quality and quantity of the DNA were determined by measuring the optical density (OD) at 260/280 nm on a spectrophotometer. Samples with OD values ranging from 1.8 to 2.0 were selected for downstream PCR amplification. For the PCR amplification, 40 ng of extracted DNA was utilized along with 10 pM of each primer. The amplifications were conducted in a thermal cycler (MiniAmp™ Plus, Applied Biosystems, Thermo Fisher Scientific) with an initial denaturation step of 95°C for 10 min, followed by 25 cycles of 95°C for 15 s, 55°C for 15 s, and 72°C for 2 min, with a final extension step of 72°C for 10 min. The amplicons from each sample were purified using AMPure XP Beads to remove unused primers, and an additional 8 cycles of PCR were performed using Illumina barcoded adapters to prepare the sequencing libraries. Further purification was carried out using AMPure XP Beads, and the concentrations of the libraries were quantified using the Qubit dsDNA High Sensitivity assay. Subsequently, sequencing was conducted on the Illumina MiSeq platform using the 2x300PE ITS sequencing kit, which generated paired-end reads for each DNA fragment.

Bioinformatics analysis

The bcl data received from the sequencer was de-multiplexed into .fastq raw data. The de-multiplexed data quality was checked using Fastqc (Version 0.11.9) and Multiqc (Version 1.10.1) tools. Biokart Pipeline was used for metagenomics analysis. The workflow of the pipeline progresses through stages: ensuring data quality, detecting chimeric sequences, clustering operational taxonomic units (OTUs), selecting representative sequences, assigning taxonomic identities, and generating a concise OTU table. For visualization of data, Microsoft Excel (2010) was used. The dendrogram was constructed using MicrobiomeAnalyst (online tool: https://www.microbiomeanalyst.ca/) and MEGA 11. Taxonomic assignments to the representative sequence from each OTU were performed with the UNITE reference database. For functional detail analysis, the FunGUILD tool was used. Alpha diversity of the fungal community was determined by Shannon-Weiner and Simpson's index using PAST.

Results

Sporocarp Richness, diversity and community structure

The study identified a total of 22 species belonging to 10 genera in association with the roots of *Abies pindrow*. These genera include *Amanita*, *Boletus*, *Cortinarius*, *Helvella*, *Inocybe*, *Lactarius*, *Ramaria*, *Russula*, *Suillus*, and *Tuber*. The *Russula* genus was the most dominant, comprising 57 sporocarps and 6 species. To assess the diversity of the fungal community, various indices were calculated. The Shannon-Weiner index, which measures the richness and evenness of species in a community, was found to be 2.799. The Simpson's index, which quantifies the probability that two individuals randomly selected from a sample will belong to the same species, was calculated to be 0.927. These indices suggest a relatively diverse and evenly distributed fungal community associated with *Abies pindrow* roots. Additionally, the estimated richness of the fungal community, as predicted by the Chao1 index, was 22.25. Figure 1a illustrates the relative abundance of ectomycorrhizal (EcM) fungal sporocarps associated with *A. pindrow* roots. This figure provides insights into the distribution and abundance of different fungal species within the studied ecosystem, highlighting the dominance of certain genera and species in the fungal community. This information could be crucial for understanding the ecological dynamics and functioning of the fungal component within

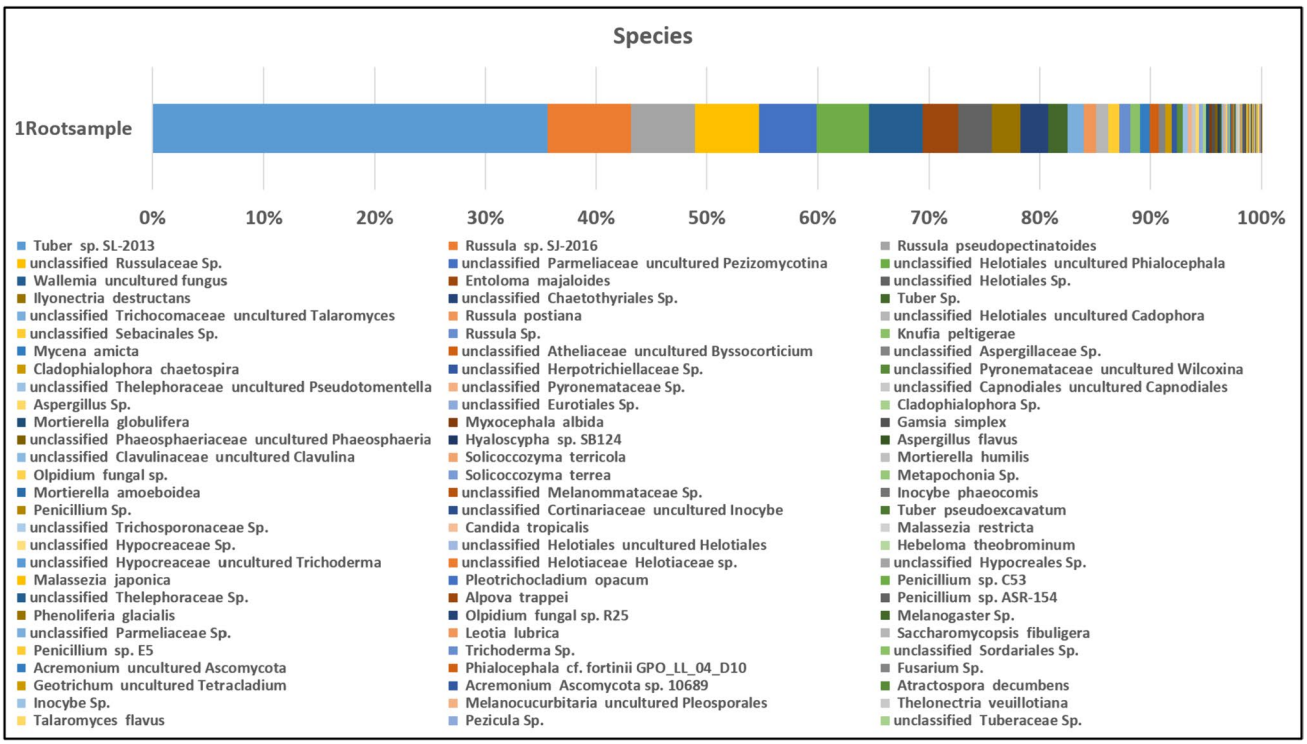


Fig. 1 EcM fungi associated with *Abies pindrow* as depicted by. **a** Sporocarp sampling. **b** Metagenomics analysis of EcM root tips (abundance represented as relative proportion of sequences for each OTU belonging to EcM guild)

the forest ecosystem and can inform conservation and management strategies aimed at preserving the diversity and health of these ecosystems.

Morphoanatomical analysis

The root tips of *A. pindrow* underwent meticulous examination to investigate various morphological and anatomical features, revealing an average colonization rate of 74%. Through detailed observation of morphological characteristics, 10 distinct EcM morphotypes were discerned from *A. pindrow* roots. These morphotypes delineate a spectrum of structural features characterizing the mycorrhizal associations formed between *A. pindrow* and its fungal symbionts. The EcM morphotypes exhibited varying lengths, ranging from 0.3 to 1.9 cm, with the main axis diameter spanning from 0.4 to 1.6 mm. Extra-radicle hyphae and rhizomorphs were commonly observed in the majority of the morphotypes. Anatomical examination revealed a mantle thickness ranging from 14 to 63 µm, primarily organized in a plectenchymatous manner. Certain morphotypes also exhibited cystidia. Hartig net formation extended to a depth of up to 4 cortical layers. Further detailed insights from each morphotype is presented in Table 1.

Metagenomics analysis

A total of 285,148 raw sequences were obtained, representing 326 operational taxonomic units (OTUs) distributed across 193 genera, 114 families, 45 orders, 22 classes, and 6 divisions. The dataset encompassed OTUs from six taxonomic divisions: Ascomycota, Basidiomycota, Mucoromycota, Zoopagomycota, Chytridiomycota, and Alpidiomycota. Ascomycota emerged as the most prevalent division, with 211 identified OTUs (Fig. 2). Among the fungal classes, Pezizomycetes and Agaricomycetes were the most dominant. Dominant orders included Pezizales and Russulales, while Tuberaceae and Russulaceae stood out as the most dominant families. Notably, the genera *Tuber* and *Russula* exhibited high dominance, collectively constituting 53.5% of the fungal population.

Out of the 326 OTUs identified, 244 were successfully assigned a guild using FUNGuild. The prominent guilds observed included ectomycorrhizal, ectomycorrhizal-saprotrophic, endophytic, saprotrophic, pathogenic, and parasitic. Among these, 36 OTUs belonging to 20 genera and 15 families were categorized as ectomycorrhizal, representing 63.1% of the fungal community associated with *A. pindrow* roots. Of the ectomycorrhizal taxa, *Tuber* exhibited the highest absolute abundance, representing

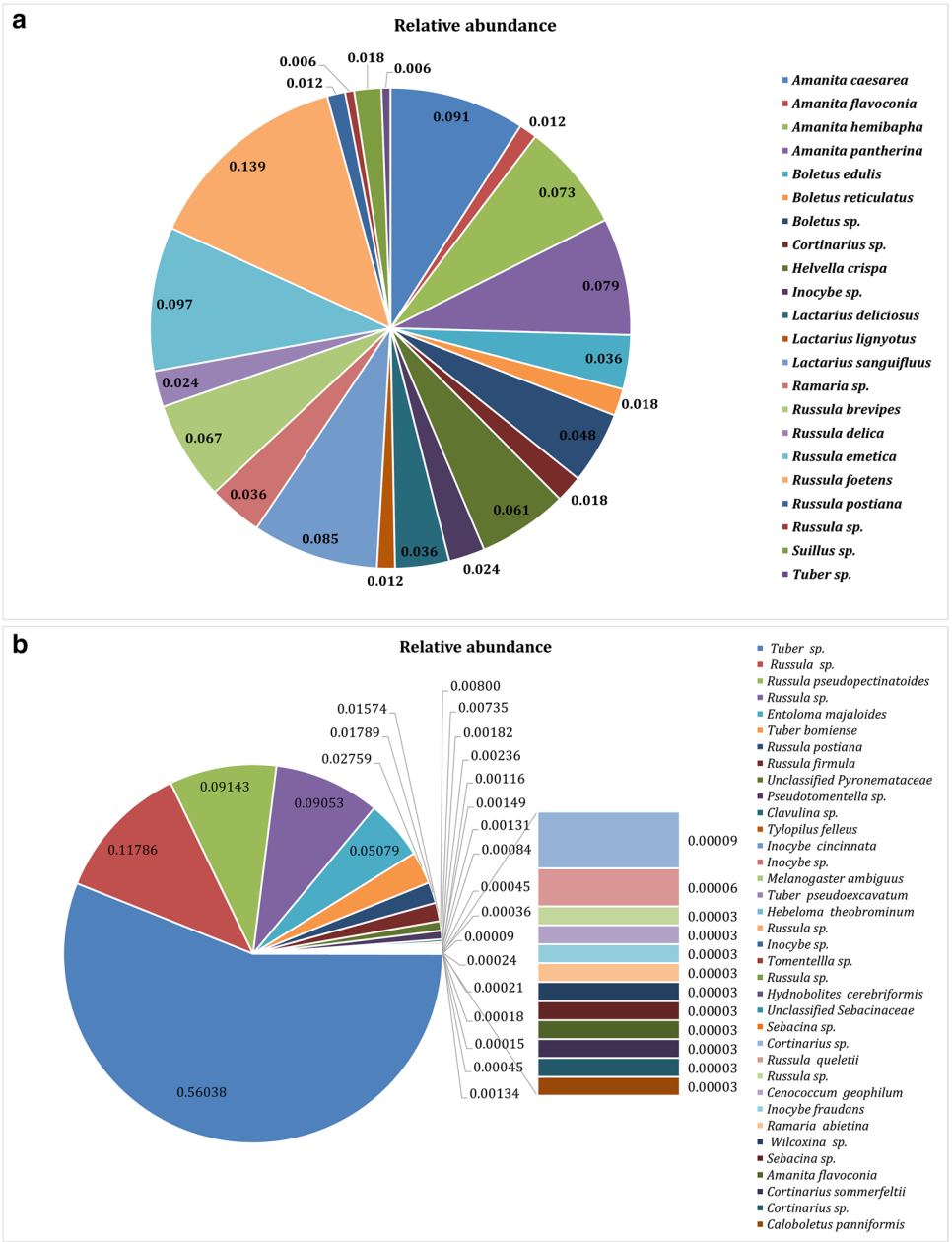
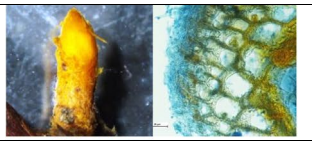
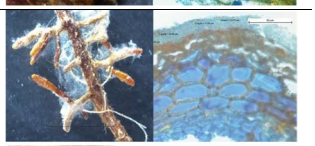
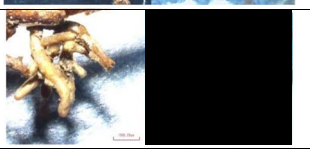
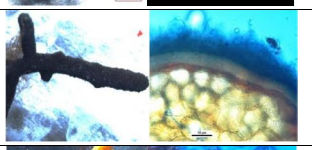
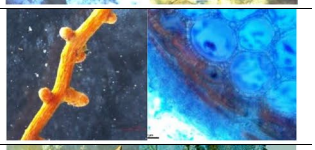
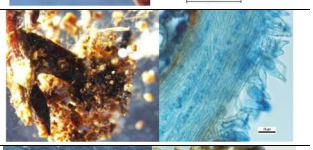
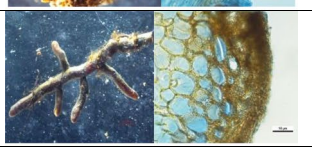
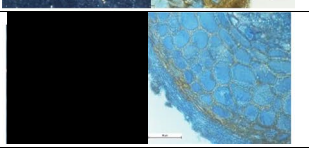


Fig. 2 Taxonomic representation of fungal OTUs linked to the *Abies pindrow* (abundance represented as relative proportion of sequences for each OTU based on NGS)

37.42% of the fungal population, followed by *Russula* at 21.06% (Fig. 2b). Notably, the top four most abundant taxa belonged to the ectomycorrhizal guild, constituting 54.2% of the fungal community associated with *A. pindrow* roots. Among the 36 ectomycorrhizal taxa, 7 belonged to the Ascomycota group, representing approximately 59.7% of the EcM fungal population, while 29 belonged to the Basidiomycota group, representing about 40.3% of the population. Additionally, out of these 36 taxa, 19 were identified to the species level, 15 to the genus level, and 2 were classified only to the family level (Fig. 3 and 4). These taxa are included in the EcM guild, as all species within these genera and families are strictly mycorrhizal. Apart from the 36 ectomycorrhizal taxa, an additional 6 taxa could only be identified to the level of genus, family, or order (Entoloma, Pyrenomycetaceae, Pezizaceae, Thelephoraceae, Sebacinales, Agaricales, Heliotales), suggesting their likely association with the EcM guild. Detailed information regarding the taxonomy, abundance, and EcM status of all identified fungal OTUs can be seen in Supplementary Table 1 and Table 2.

In addition to EcM fungi, the fungal community included endophytes, saprophytic fungi, pathogenic fungi, and fungal parasites (*Hypomyces* spp.). The alpha diversity of the fungal community, as measured by the

Table 1 Morphoanatomical details of the ectomycorrhizal morphotypes of the *Abies pindrow*

Morpho-type	Ramification	Colour and surface	Length (cm)	Main axis diameter (mm)	Rhizomorphs	Exploration type	Extra radicle hyphae	Mantle	Hartig net	Morphology and Anatomy
I	Unbranched	Brown with orange tip, smooth	0.4-0.6	0.7-1.0	Present/yellow-orange, Rare	Short distance	Present/ white	14-17 µm thick, layers not differentiated, Plectenchymatous organization	3 cortical layers	
II	Monopodial pinnate	Yellowish brown to brown	0.8-1.5	0.4-0.7	Present/White Frequent, Highly branched	Medium to long distance	Present/ white	16-24 µm thick, layer undifferentiated	2 cortical layers	
III	Monopodial pinnate	Brown	0.5-1.7	0.5-0.8	Present/ dark brown	medium distance	Present/ white	39-69 µm thick 4 layered, plectenchymatous, Cystidia on outer mantle layer	2-3 cortical layers	
IV	Unbranched	Black	1.2-1.5	0.6-0.8	Present/ Dark brown	Medium distance	Absent	40-52 µm thick 2 layered, cystidia on outer layer	3-4 cortical layers	
V	Sessile	Orange	1.2-1.9	0.8-1.00	Present/White	contact	Absent	12-23 µm thick 2 layered	2-3 cortical layers	
VI	Irregular	Brownish	1.5-2.1	0.6-0.8	Present/Brown	Medium distance	Present/ Brown	56-63 µm thick 4 layered, cystidia on outer layer Plectenchymatous	1-2 cortical layers	
VII	Unbranched	Black	0.4-0.7	0.8-1.00	Present/Brown	Medium distance	Present/ Black	24-31 µm thick Layers not differentiated,	1-2 cortical layers	
VIII	Coralloid	Brownish	0.3-0.5	0.5-0.9	Present/ black	Long distance	Present/ creamish	50-70 µm thick, 3 layered, Plectenchymatous	3 cortical layers	
IX	Monopodial pinnate	Dark brown	0.7-0.8	1.3-1.6	Present/Brown	Long distance	Light brown	12-15 µm thick, Plectenchymatous, Layers not differentiated	2-4 cortical layers, patchy	
X	Monopodial pyramidal	Yellowish brown	0.5-0.6	0.6-0.9	Present/Cinnamon brown	Medium distance	White	26-30 µm thick, Plectenchymatous, layers not discernible, cystidia absent	1-3 cortical layer, patchy	

Shannon-Weiner (H) and Simpson's (D) indices, was 2.81 and 0.85, respectively. The estimated richness of the fungal community was 507.6 species according to the CHAO1 index. For the ectomycorrhizal community specifically, the Shannon-Weiner (H) and Simpson's (D) indices were 2.659 and 0.897, respectively.

Discussion

The current study offers a thorough examination of both the morpho-anatomical and molecular aspects, shedding light on the functional roles and diversity of the fungal community associated with *A. pindrow* roots. Our findings reveal a rich

Table 2 Taxonomic position and relative abundance of EcM fungi associated with *Abies pindrow*

S. No.	EcM fungi	RA	Matched BLAST accession number	Family
1.	*Tuber* sp.	35.338	KC51748.1	Tuberaceae
2.	*Russula* sp.	7.428	MK346330.1	Russulaceae
3.	*Russula pseudopectinatoides*	5.763	MK860689.1	Russulaceae
4.	*Russula* sp.	5.706	GQ219863.1	Russulaceae
5.	*Entoloma majaloides*	3.201	OR419861.1	Entolomataceae
6.	*Tuber bomiense*	1.739	OM265247.1	Tuberaceae
7.	*Russula postiana*	1.127	MH930211.1	Russulaceae
8.	*Russula firmula*	0.992	JF834342.1	Russulaceae
9.	Unclassified Pyronemataceae	0.504	AY634151.1	Pyronemataceae
10.	*Pseudotomentella* sp.	0.463	EU563488.1	Thelephoraceae
11.	*Clavulina* sp.	0.149	KX444218.1	Clavulinaceae
12.	*Tylopilus felleus*	0.115	MW899062.1	Boletaceae
13.	*Inocybe cincinnata*	0.094	OM964555.1	Inocybaceae
14.	*Inocybe* sp.	0.083	KX444235.1	Inocybaceae
15.	*Melanogaster ambiguus*	0.077	MN994353.1	Melanogastraceae
16.	*Tuber pseudoexcavatum*	0.073	GU979042.1	Tuberaceae
17.	*Hebeloma theobrominum*	0.053	NR_120177.1	Cortinariaceae
18.	*Russula* sp.	0.028	KX095026.1	Russulaceae
19.	*Inocybe* sp.	0.023	AM882848.1	Inocybaceae
20.	*Tomentellla* sp.	0.019	KC152248.1	Thelephoraceae
21.	*Russula* sp.	0.013	OR168893.1	Russulaceae
22.	*Hydnobolites cerebriformis*	0.011	KR019789.1	Pezizaceae
23.	Unclassified Sebacinaceae	0.009	EU526853.1	Sebacinaceae
24.	*Sebacina* sp.	0.008	KU141302.1	Sebacinaceae
25.	*Cortinarius* sp.	0.006	MW472144.1	Cortinariaceae
26.	*Russula queletii*	0.004	MG255230.1	Russulaceae
27.	*Russula* sp.	0.004	KX441145.1	Russulaceae
28.	*Cenococcum geophilum*	0.002	KX449189.1	Gloniaceae
29.	*Inocybe fraudans*	0.002	KM873362.1	Inocybaceae
30.	*Ramaria abietina*	0.002	JX310378.1	Gomphaceae
31.	*Wilcoxina sp.*	0.002	DQ069051.1	Pyronemataceae
32.	*Sebacina* sp.	0.002	OR435789.1	Sebacinaceae
33.	*Amanita flavoconia*	0.002	MH910544.1	Amanitaceae
34.	*Cortinarius sommerfeltii*	0.002	MN047061.1	Cortinariaceae
35.	*Cortinarius badiovinaceus*	0.002	NR_131815.1	Cortinariaceae
36.	*Caloboletus panniformis*	0.002	KU317758.1	Boletaceae

diversity of fungi inhabiting the roots, with a significant portion belonging to the EcM guild. Given the limited correlation between the diversity of EcM fungi, as determined by visible above-ground sporocarps (Richard et al. 2005), and their actual presence in host roots, we conducted a detailed investigation involving the collection and analysis of *A. pindrow* roots to identify associated EcM fungi. Morphotyping of EcM alone proves insufficient, as characteristics such as color, ramification, and mycorrhizal system size can vary depending on growth conditions and host plant species (Agerer 1991; Horton and Bruns 2001; Burke et al. 2005). Therefore, our study combines comprehensive morphoanatomical and molecular analyses of *A. pindrow* roots to gain deeper insights into the genetic basis of *A. pindrow* mycorrhizae. Moreover, this approach enables the identification of EcM fungi that produce inconspicuous or hypogeous sporocarps.

The morphoanatomical characterization followed the DEEMY guidelines. However, within this database, comprising 554 entries, only 13 descriptions were associated with the genus *Abies*, and none specifically pertained to *A. pindrow* ectomycorrhizae. Due to the limited literature on *A. pindrow* mycorrhizae, precise classification of morphotypes into distinct ectomycorrhizal types was challenging. Consequently, mycorrhizae of *A. pindrow* were categorized into

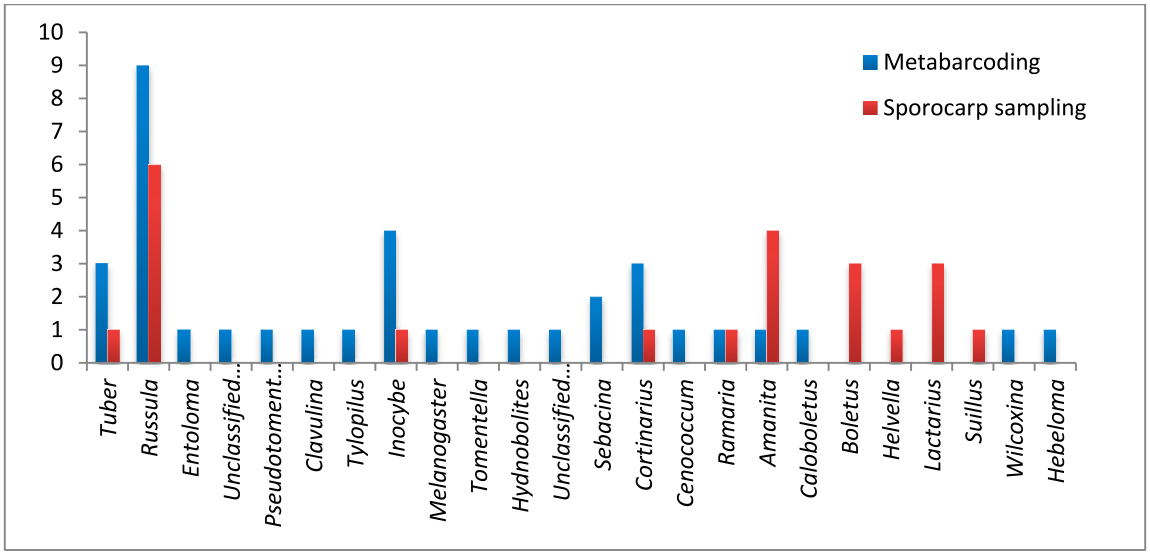


Fig. 3 EcM species richness as depicted by sporocarp sampling and metabarcoding of mycorrhizal roots

various groups based on their morphological traits. In total, ten different morphotypes (labeled as A to J) were identified within *A. pindrow* roots; however, metagenomics analysis revealed the presence of 36 EcM fungi associated with *A. pindrow*. This underscore previous observations suggesting that similar morphotypes could potentially arise from different fungal species (Menkis et al. 2005; Pestana-Nieto and Santolamazza-Carbone 2009).

Metabarcoding analysis revealed a dominance of fungal operational taxonomic units (OTUs) belonging to Ascomycota (65.7%) and Basidiomycota (33.5%). Notably, a significant portion of the Ascomycota was attributed to *Tuber* sp., constituting 37.4% of this group. The higher relative abundance of ascomycetes in roots might indicate their better adaptation compared to basidiomycetes (Durand et al. 2017; Dao et al. 2023). Additionally, various fungal types such as endophytes, saprophytes, and pathogens were documented, suggesting the coexistence of functionally diverse fungal taxa within mycorrhizal root tips (Menkis et al. 2005). The fungal community was predominantly composed of ectomycorrhizal fungi, consistent with previous research findings (Argüelles-Moyao and Garibay-Orijel 2018). Several OTUs were detected that could not be classified at the species level, hinting at the possibility of representing previously undescribed taxa.

Sporocarp sampling revealed 22 EcM species, a count comparable to 21 species reported by Sharma and Lakhanpal (1988), 21 species by Thakur (1990), and 18 species by Beig et al. (2011). However, this count is lower than the 36 species identified through metagenomics analysis. In this study, only 33.3% of the genera described by metagenomics analysis of mycorrhizal root tips were found as sporocarps. The variation in species count between sporocarp sampling and metagenomics analysis stems from the fact that some fungi, like *Tuber*, *Melanogaster*, and *Hydnobolites*, are hypogeous, while others form inconspicuous fruiting bodies, such as *Cenococcum*, *Tomentella*, and *Pseudotomentella*. These inconspicuous species constitute a sizable portion of the EcM community associated with *A. pindrow*, as revealed by metagenomics analysis. This supports previous findings indicating that species not producing obvious fruiting bodies might be highly prevalent in mycorrhizal root tips (Tedersoo et al. 2003; Pestana-Nieto and Santolamazza-Carbone 2009). However, despite being the most abundant sporocarps, *Amanita* and *Lactarius* are nearly non-existent in mycorrhizal roots. This may be due to succession of EcM fungi in the mycorrhizal roots.

The presence of ectomycorrhizal truffles in the genus *Tuber* is notable, constituting a significant portion of the ectomycorrhizal community associated with *A. pindrow*. Within the genus *Tuber*, three taxa were identified, with one only classified up to the genus level, while the other two were identified as *Tuber pseudoexcavatum* and *Tuber bomiense*. Reports of *Tuber* species from India are limited, highlighting the need for further studies focusing on these important mycorrhizal and culinary mushrooms.

In a parallel investigation conducted by Assad et al. (2021) in the Kashmir Himalaya, 14 morphotypes were identified, with the monopodial pyramidal type being the most common. Molecular analysis revealed 251,158 reads, 136 OTUs, and 62 confirmed EcM fungi. Abundant EcM genera included *Inocybe*, *Russula*, *Otidea*, *Chalara*, *Sebacina*, *Tomentella*, *Cenococcum*, and *Wilcoxina*. In our study, *Russula* was the second dominant and most diverse genus. In comparison, our study yielded a higher number of OTUs, but a smaller proportion of them were classified within the ectomycorrhizal guild. This variation might stem from differences in the sampling approach.

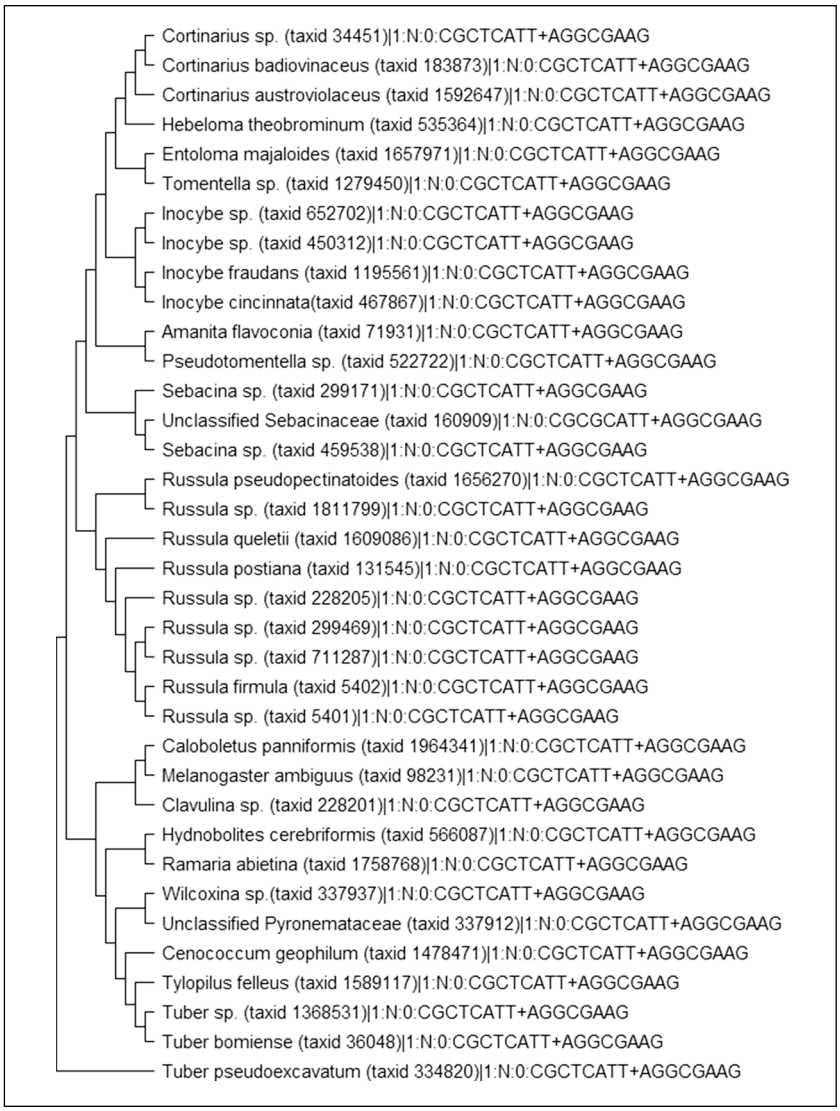


Fig. 4 Phylogenetic tree with the maximum likelihood analysis of the ITS region of EcM fungi associated with *Abies pindrow*

In nursery and field experiments, prioritizing the use of native microbiomes is essential for improving plant establishment and consistently achieving superior outcomes (Koziol et al. 2018; Policelli et al. 2020; Singh et al. 2020; Tapwal et al. 2022). Ectomycorrhization of seedlings plays a pivotal role in the restoration of conifer forests (Assad et al. 2022). The current research has unveiled that *Tuber*, *Russula*, and *Entoloma* are the most prevalent genera of ectomycorrhizal symbionts of *A. pindrow* roots. Consequently, it is worthwhile to consider these Ectomycorrhizal fungi for their potential utility in promoting ectomycorrhization, and restoring fir forests.

Conclusion

In forest ecosystems, EcM fungi play an essential role in supporting plant and soil health. Next-generation sequencing tools offer a comprehensive examination of diverse belowground microbiota, making them invaluable for assessing the structure of EcM communities within forest ecosystems. Scanty information is available on the mycorrhizal morphotypes and metagenomics of EcM communities associated with *A. pindrow*. The current study comprising combined morphoanatomical and molecular characterization of *A. pindrow* roots deepens our understanding of the diversity and community structure of its ectomycorrhizal symbionts, as well as provides insights into the structural aspects of the ectomycorrhizae. Our findings revealed a total of 326 OTUs belonging to 193 genera, with 36 OTUs across 20 genera being ectomycorrhizal fungi, representing a substantial portion (63.1%) of the fungal community. The findings from this research hold significant relevance for assessments of biodiversity and ecosystem conservation, the potential use of mycorrhizal fungi for the inoculation of tree seedlings in nursery settings and field out planting, and therefore play an important role in the success of reforestation programs. By

understanding the EcM associates of *A. pindrow*, we can better design and implement strategies to enhance the success of reforestation initiatives, ultimately contributing to the conservation and preservation of this valuable Himalayan conifer species and its associated ecosystem.

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Author contributions Ashwani Tapwal conceptualized the research and reviewed the manuscript, Neha Sharma performed the experiment and wrote the manuscript.

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Data availability Data is provided within the supplementary files.

Declarations

Conflict of interest The authors declare no competing interests.

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