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Integrative Morphological and Molecular Taxonomy of 4 species of Macrofungi from High-Altitude Ecosystems of Hunza, Gilgit Baltistan

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Abstract

Fungus (plural fungi) is a Latin word meaning “mushroom” and is defined as a member of eukaryotes group that includes unicellular microorganisms, for example, yeast and molds, and multicellular fungi which produce fruiting forms commonly called mushrooms (Moore, 1980). The District Hunza represents a floristically rich area characterized mostly by moist and dry temperate forests with rich macrofungal diversity. Macrofungi are primary decomposers in alpine and subalpine ecosystems. They break down complex organic matter such as leaf litter, woody debris, and animal remains into simpler compounds. The study was carried out from March to August in the study area. Planned field visits were arranged to the selected localities during which macrofungal species were collected and identified using macro- and micro-morphological characters as well as at the molecular level. In this connection, DNA was extracted using standard molecular methodologies such as PCR amplification. The present study is the first documented attempt to estimate the genetic diversity of some important macrofungi from Gilgit-Baltistan using DNA-based markers. During the study, fourteen fungal species were analyzed using eleven Randomly Amplified Polymorphic (RAPD) primers. Bivariate data were used to estimate the genetic diversity in the four fungal accessions using unpaired group of arithmetic mean (UPGMA) procedure. A high level of genetic diversity ranging from 40% to 80 % was observed in the fungal species. The samples of accessions were also grouped into 7 clusters using dendrogram analysis. The present research highlights the critical importance of conserving fungal biodiversity while advancing taxonomic knowledge through genetic diversity analysis, providing a robust framework for accurate species identification, evolutionary understanding, and the sustainable management of ecologically vital fungal communities.

Keywords: Macrofungi, Genetic distance, Basidiomycetes, Ascomycetes, PCR, Phylogeny, RAPD.

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Introduction

The kingdom Fungi encompasses a wide range of organisms commonly referred to as mushrooms, boletes, bracket or shelf fungi, bread molds, yeasts, puffballs, morels, truffles, as well as smut and rust fungi. Macrofungi specifically include larger and visible forms such as mushrooms (toadstools), puffballs, bracket fungi, polypores, and coral fungi (Razaq et al., 2014). Approximately 100,000 species of fungi have been described in the literature, but the complete global biodiversity of fungi is not completely known (Mueller and Schmit, 2006). Mostly, classification of fungi has been done based on characteristics of their reproductive behavior. Presently, seven phyla have been proposed, viz.: Microsporidia, Chytridiomycota, Blastocladiomycota, Neocallimastigomycota, Glomeromycota, Ascomycota, and Basidiomycota (Hibbett et al., 2007).

In many Asian regions, these macrofungi have long been consumed as sources of essential minerals and vitamins. More recently, pharmacological research has increasingly focused on edible macrofungi due to their significant bioactive properties (Waseer et al., 2002). These organisms exhibit a range of beneficial effects, including chemopreventive, chemotherapeutic, immunomodulatory, and hypoglycemic activities (Khalaf and Remdan, 2025). Reports have also documented the diversity of macrofungi in Gilgit-Baltistan (Razaq et al., 2014).

Assessing genetic diversity is crucial for understanding variation and improving macrofungal resources in this region. Several studies have explored the morphological and molecular taxonomy of macrofungi from Gilgit-Baltistan, leading to the identification of new species such as Bjerkandera adusta (Yuan et al., 2025).

The international trade in wild edible fungi has taken place for many years. Major exporters of fungi in the world include China, France, Italy, Turkey, Mexico, Japan and Yugoslavia. The data on volumes of production, consumption and exports of macrofungi are patchy and uncertain, but it is estimated that world production of important macrofungi is more than 75000 tonnes per year with a commercial value of 22.5 billion US$ (FAO, 2015). The market price of morels has been studied in patchy detail in Pakistan. It varies from species to species, but the price of Morchella conica is always higher than other species, as a general observation in the market. Other parameters of the market price of macrofungi depend upon the stake in the market chain, e.g., one kg of dried fungi fetches up to Rs. 3000 to the collector, Rs. 9000 to the wholesaler, Rs. 12000 in the national market and Rs. 20000 in the international markets (Chaudhary et al., 2000; Hamayun et al., 2003).

In recent years, DNA-based markers have emerged as powerful tools for species identification because they are abundant and largely unaffected by environmental conditions. Molecular approaches have significantly advanced the systematics of fungi and other microorganisms (Jorgenson et al., 2001; Kirk et al., 208; Kaufman and Rousseeuw, 1990; Miller, 1997; Palsson et al., 1999). These techniques are highly sensitive, enabling detection of even single-base-pair variations, and are therefore effective in distinguishing closely related species (Schlick et al., 1994; Lanfranco et al., 1995). Additionally, several fungal species from the mountainous regions of Pakistan are exported to countries such as France, Belgium, Switzerland, Austria, and Germany, where they are valued for their medicinal properties, particularly as immune stimulants, antioxidants, and

anticancer agents (Pankaj et al., 2002; Khalid, 2022). Numerous protocols for DNA extraction and amplification from fungi and lichens have been developed (Bruns et al., 1990; Lee & Taylor, 1990; Armaleo & Clerc, 1991, 1995; Grube et al., 1995, 1997; Grube, 2005). The introduction of PCR with selective primers has largely resolved earlier challenges associated with isolating DNA from individual fungal species (Crespo et al., 1997).

Saprophytic fungi possess different life and dispersal strategies that combine various nutritional techniques and mechanisms meant to increase competitiveness towards other organisms. The versatility of the enzymatic system allows several species to survive under severe conditions such as low nutrient availability. As a result of the high competition for nutrients between the communities of microorganisms, saprotrophic fungi have developed various strategies to eliminate the antagonistic species. Depending on the substrate, environmental factors, incubation time, and the presence of other organisms, lignicolous basidiomycetes can synthesize secondary metabolites with different properties that have important biotechnological potential in numerous industrial branches: chemical, pharmacy, medicine, food industry, cosmetics and perfumery, and agriculture – as biocontrol agents of phytopathogens. Many lignicolous basidiomycetes that grow on dead wood or litter can synthesize toxic compounds that act against other species of fungi, including the plant pathogens. Moreover, these fungi use mechanisms other than the synthesis of toxic compounds to counteract the development of competitive organisms, such as: contact inhibition (mechanical inhibition involving the hyphae), extracellular enzyme 11 Lignicolous basidiomycetes as valuable biotechnological agents (hydrolases, peroxidases, oxidoreductases), modifying the properties of the substrate (changes in pH values, formation of hydrogen peroxide, discharging several ions and free radicals) and also a more efficient use of nutrients. The secondary metabolites synthesized by the lignicolous fungi are not vital for their survival but have important ecological functions, especially in inter- and intra-specific communication and defense against predators and parasites. Scientific studies revealed that the secondary metabolites produced by the lignicolous basidiomycetes are successfully used in pharmacy (their properties have been used for centuries in traditional medicine) for treating different conditions such as dysentery, headaches – Fomitopsis pinicola, tuberculosis - Trametes suaveolens, bleedings – Fomes fomentarius, Piptoporus betulinus, rheumatism – Phellinus igniarius, cancer – Bjerkandera fumosa, Ganoderma sp., Lentinula edodes, Lenzites betulina, Pleurotus ostreatus, Schizophyllum commune, Trametes versicolor, liver problems – Flammulina velutipes, Ganoderma lucidum.

Despite earlier morphological investigations by mycologists in Gilgit-Baltistan, comprehensive studies on genetic diversity—particularly for macrofungi in District Hunza—remain limited. Understanding this diversity is essential for revealing patterns of variation and supporting future conservation and utilization efforts.

The estimated number of world fungi is considered to be between 1–1.5 million species, many of them still unidentified. According to the 10th edition of Dictionary of Fungi, so far 97.330 species of fungi belonging to75.337 genera are described Crespo et al., 1997. Fungi belong to a very diverse group of eukaryotic organisms that

populate various habitats, and due to an extraordinary plasticity, they can colonize different substrates using resources that are inaccessible or hardly accessible to other species, like keratin, collagen, elastin, lignin, cellulose, hemicellulose, etc. Fungi are heterotrophic organisms with great adaptability and a high capacity to use every available resource. They can be saprotrophic species that decompose organic and inorganic substrates or can be part of symbiotic relations with photosynthetic organisms, and also parasitic species on plants and animals Dix & Webster, 1995. Because of their morphological and physiological versatility and their great biotechnological potential, these unique organisms captured the interest of the scientific community. The enzymes and secondary metabolites produced by fungi have been isolated and tested with significant results in various biotechnological processes from pharmacological industry and agriculture to habitat bioremediation. Used properly, fungi represent an important bioresource that can solve many of the environmental problems that society is facing nowadays, without involving products and techniques that would have additional negative effects. More than 1,000 different species of bacteria and fungi can be used to clean up various forms of pollution. These microorganisms contain enzymes involved in lignin metabolism. Laccase (Lac), lignin peroxidase (LiP), and manganese peroxidase (MnP) from white-rot fungi (WRF) are among the most studied enzymes in bioremediation, where Lac is involved in detoxification of phenols, triclorophenols, and PAHs (polycyclic aromatic hydrocarbons) distributed in terrestrial and aquatic environments. These enzymes, peroxidases and laccase, are the key lignin-degrading enzymes with great potential in industrial applications. The indigenously isolated white rot fungal strain T. versicolor *IBL-04* showed tremendous potential for LiP synthesis in SSF of corncobs at higher titers (592 U/mL) than other reported Trametes (Coriolus, Polyporus) species. The results obtained after dual-phase characterization suggested xerogel matrix entrapment as a promising tool for enzyme immobilization, hyper-activation, and stabilization against high temperature and inactivating agents. The pH and temperature optima, extra thermo-stability features, and kinetic characteristics of this novel LiP of *T.* versicolor IBL-04 make it a versatile enzyme for various industrial and biotechnological applications.

Material and Methods

Samples of four macrofungal species were collected from the regions of Nasirabad, Hunza $36^{0}16'00''''$ N latitude and $074^{0}40'60''''$ E longitude, and Nagar in Gilgit-Baltistan, located at an elevation of approximately 2327 meters. Each specimen was photographed in its natural habitat, and key morphological features such as color, shape, and texture of the fresh fruiting bodies were recorded. Field visits were conducted during the peak fruiting season of macrofungi. Collected fruiting bodies were stored individually in plastic bags to avoid cross-contamination and later preserved through air-drying at room temperature.

Genomic DNA was extracted from the fruiting bodies using a modified small-scale (miniprep) protocol based on the method described by Weining and Langridge (1991). Approximately 0.5 g of fresh fungal tissue was transferred into an Eppendorf tube and immediately frozen using liquid nitrogen. The frozen material was ground into a fine powder using a sterile needle. This powder was then mixed thoroughly with 500 µL of DNA extraction buffer. An equal volume (500 µL) of

phenol:chloroform:isoamyl alcohol (25:24:1) was added, followed by vortexing until a uniform mixture was achieved. The samples were then centrifuged at 5000 rpm for 5 minutes.

The upper aqueous phase was carefully transferred to a new tube, and 50 µL of 3M sodium acetate (pH 4.8) along with 500 µL of isopropanol was added to precipitate the DNA. After gentle mixing, the tubes were centrifuged again at 5000 rpm for 5 minutes to obtain a DNA pellet. The supernatant was discarded, and the pellet was resuspended in 50 µL of TE buffer.

PCR amplification was carried out under the following thermal cycling conditions: initial denaturation at 94°C for 4 minutes, followed by denaturation at 94°C for 1 minute, annealing at 20°C for 1 minute, and extension at 72°C for 2 minutes. This cycle was repeated for 40 cycles. The amplified PCR products were separated using 2% agarose gel electrophoresis in TBE buffer.

The resulting DNA bands were scored as binary data, where the presence of a band is recorded as "1" and its absence as "0," following the method outlined by Nei and Li (1997). One lane of PCR mixture without target DNA was included in the gels as a negative control

Results

1.Bjerkandraadusta (Willd. Ex Fr.)

Synonyms: PolyporusadustaWilled.ex. Fr.Bjerkanderaadusta (Wild)

Bjerkanderaadustaf.adusta (Wild)

Bjerkanderaadustaf.carpinea(Sowerby) Donk,

Bjerkanderaadustaf.resupinata(Bourdot and Galzin)

Bjerkanderaadustaf.solubilis (Velen)

Bjerkanderaadustaf.tegumentosa(Velen)

Description of species

Fruit body velvety, bracket-like. Flesh white, fibrous and leathery, partly bracket spores below and dark; margin at first paler, wavy. Flesh whitish, fibrous and leathery.

Tubes and pores: The fertile surface is covered with tubes with pores measuring 4- 5 mm.

Spores: Spores ellipsoid, smooth, 3.5- 3 x 4-5.5 µm.

Spore print: White

Season of Fruiting: July –August

Edibility: Inedible, used for joint pain

Habitat: Usually in fused masses

Previous report from Pakistan: On decaying stumps of deciduous trees, Nathiagali(Muree), Kalam(Swat), Sharan Kagan, Changa Manga, Basidiomycota of Northern Areas, Gilgit-Baltista, Razaq et al. (2007).

Fig. 1. Bjerkandera adusta A- Fruiting body, B- Pores,

2.Fomesfomentarius (L.exFr) Kickx.

Synonyms: Polyporusfomentarrius L.,

Fomesabrabramisianus

Fomesalni (Sorokin)

Fomesamboinesis

Description of species

Fruiting body 13- 16 cm long, club-shaped, brown surface margin light brown.

Margin rounded and light colored. Tubes arranged in layers.

Pores and tubes: rounded and minute, with a thick wall which is first white, then brownish.

Spores: 13- 14 x 4- 5 µm in diameter.

Spore print: Pale

Occurrence: Aliabad

Edibility: Inedible

Habitat: Solitary on dead wood

Previous report from Pakistan: Shogran (Kagan valley); Nathiagali (Muree); Swat; Punjab;

Azad Kashmir, Quercusdilatata, Abbottabad (Razaq (2007) Basidiomycota of Gilgit-Baltistan).

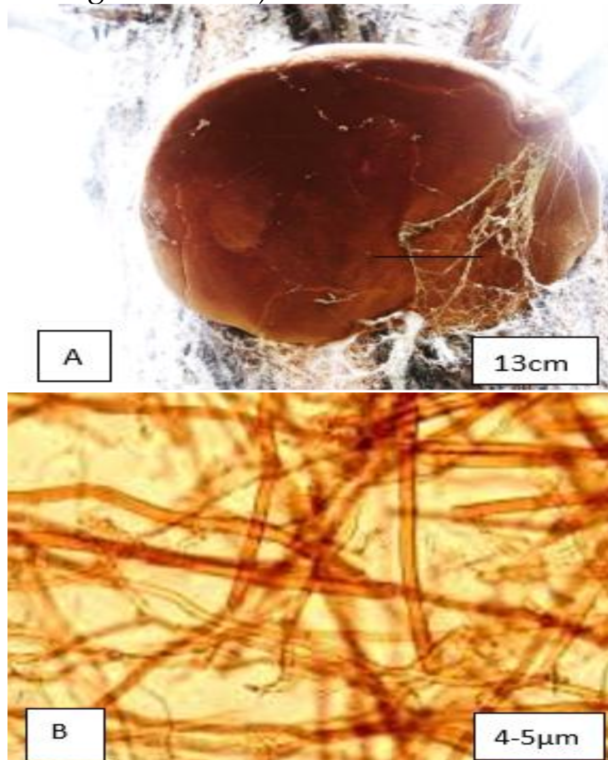

Fig. 2. Fomes fomentarius A- fruiting body B-Hyphae.

3.RidgidoporusulmariusFr. Imazeki, Bul

Synonymy: RigidoporusMurril,

Rigidoporusadnatus

Rigidoporusalbostygius var. parvulus(Corner)

Rigidoporusdimiticus(Corner)

Rigidoporusdurus(Jungh)

Rigidoporuspopulinus(Schumach)

Description of species

Fruiting body: flesh first cream–buff, tough fibrous, then very hard woody. Fruit body irregular, warty; concentric ridges; margin thick, rounded. Variable shape with 8-11cm, perennial.

Pores: pore surface at first pink and then brownish. Pores 5- 8 mm, rounded, angular.

Spore print: white.

Spores: spherical, smooth, 5-6x5µm in size, non-amyloid.

Season of fruiting: July – August

Occurrence: Altit, Hunza

Habitat: solitary or overlapping in small groups on wood

Edibility: Inedible

Previous report from Pakistan: Razaq et al. (2007).

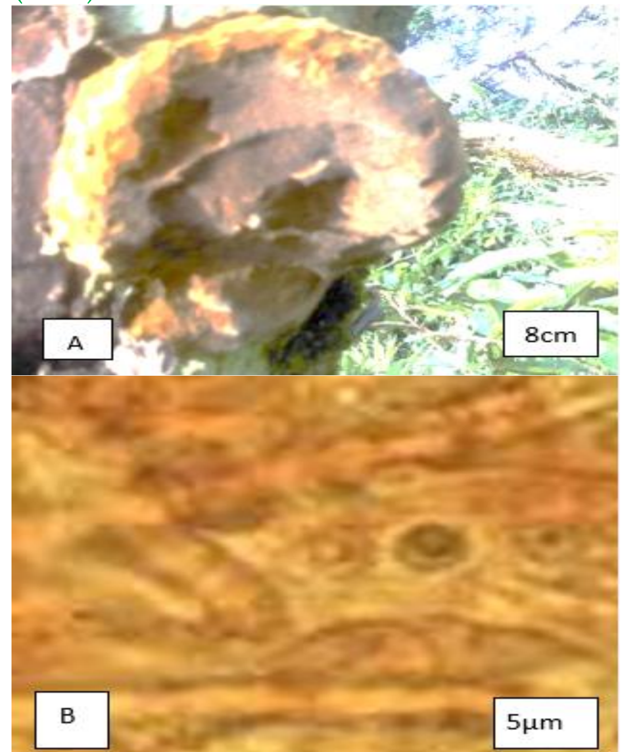

Fig. 3. Rigidoporus ulmarius A- Fruiting body, B-Basidiospore.

4. **Trametes versicolor**(L. ex Fr.) P.

Synonymy: Boletus versicolor, sp.

Poria Versicolor (L.) Scon.,Flcarniol.,

Trametes versicolor f. nigrozonata (Bondartey).

Trametesversicolorf.producta (Velen)

Trametesverscicolor f. tucumanesis(Rajchenb)

Trametesversicolorf.verscicolor(L.)

Description of species:

Fruit body 3- 7 cm wide, 1- 2 cm thick, and the overall bodies consisting of 12- 8 cm, leathery, bracket-like; surface velvet-like with concentric bands of brown-red-yellow-gray-blue colors.

Pores: surface first white and then cream. Pore 3-5mm angular.

Spores: Spores spherical, 1.5- 3 x 5- 6 µm in size, non-amyloid.

Spore print: white

Edibility: Inedible

Season of fruiting: June-July

Habitat: densely overlapping groups; fruit body attached to the tree trunk of broad-leaf trees.

Occurrence: Aliabad, Hunza

Previous report from Pakistan: Basidiomycota of Northern Areas Gilgit Baltistan (Razaq et al., 2007).

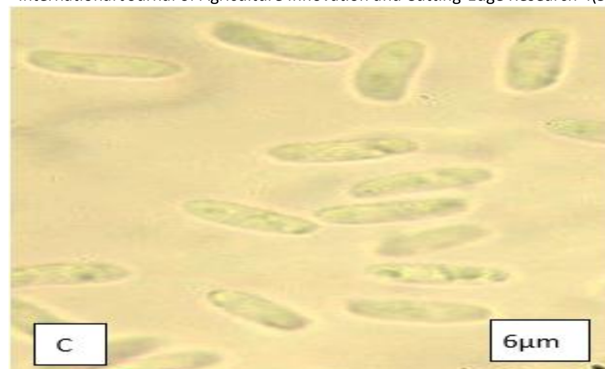

Fig.4 Trametes versicolor A- Fruiting body B-pore surface, C-Basidiospore

Sequence information of 11 RAPD primers used during the present study for estimation of genetic diversity in macrofungi is presented in Table 1. A representative gel of DNA amplification using RAPD primer GLI-09 is presented in Figure 5. Samples with faint or unclear bands were repeated. An approximately 42.05% polymorphism was estimated, as 119 out of 138 fragments were polymorphic with macrofungi samples used during the present study. The RAPD analysis shows that the accessions appeared to show difference/variability with the primer used. Although none of the primers was individually informative enough to differentiate all the accessions, highly polymorphic profiles were obtained with primers such as GL Decamer I-09, GL Decamer j-04, and GL Decamer j-05. The genetic distance for RAPD data for wheat accessions was constructed using the procedure outlined by Nei and Li (1979) (Table 2), and the relationships between accessions were presented graphically in the form of a dendrogram in Figure 6. The value of genetic distance ranging from 0.00 to 100% was observed among macrofungi accessions.

Table 1: Sequence of RAP primers used during the present study

Primer name	%GC	Sequences	Molecula r weight
GL Decamer I-07	60%	CAGCGAC AAG	3117.04

GL Decamer I-09	60%	TGGAGAG CAG	3053.01
GL Decamer I-10	60%	ACAACGC GAG	3068.02
GL Decamer I-11	60%	ACATGCC GTG	2987.98
GL Decamer I-15	60%	TCATCCGA GG	2947.96
GL Decamer I-17	60%	GGTGGTG ATG	3019
GL Decamer I-20	60%	AAAGTGC GGG	3019
GL Decamer J-04	70%	CCGAACA CGG	3108.04
GL Decamer J-05	70%	CTCCATGG GG	2954.97
GL Decamer J-06	60%	TCGTTCCG CA	2954.97
GL Decamer J-14	70%	CACCCGG ATG	2994.99

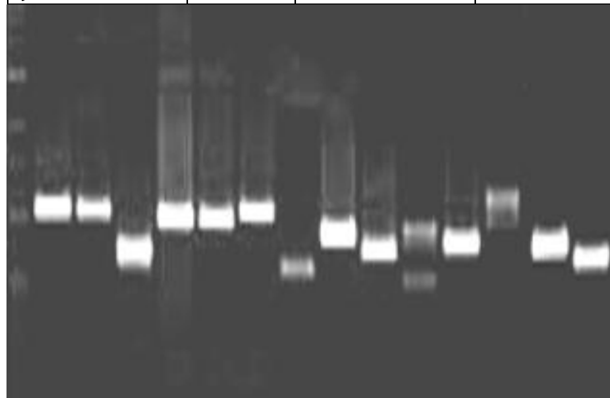

Figure 5: PCR amplification profile of samples of macro fungi using RAPD Primer GL I-09.

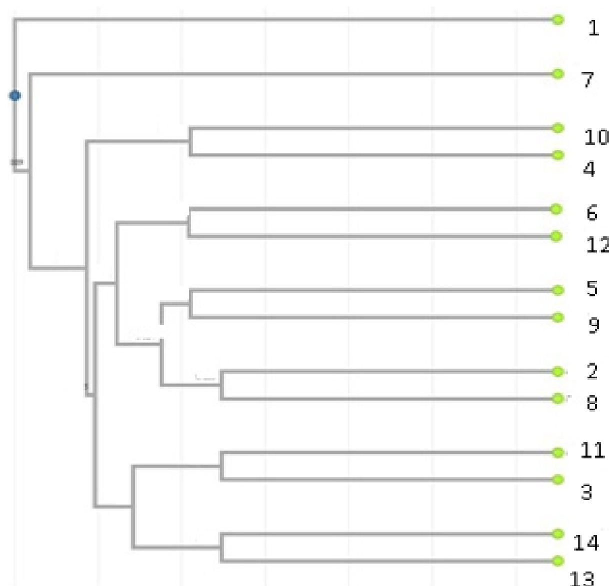

Fig 6. Dendrogram constructed for macrofungi accessions using 11 RAPD primers

Discussion

Genetic studies based on Polymerase Chain Reaction (PCR) have greatly advanced the understanding of macrofungi by providing rapid, reliable, and sensitive tools for the analysis of fungal diversity, taxonomy, phylogeny, and population genetics. Traditional identification of macrofungi mainly depends upon morphological characters such as shape, size, color, spore structure, and fruiting body characteristics. However, these features are often influenced by environmental conditions and developmental stages, leading to difficulties in accurate species identification. PCR-based molecular techniques have therefore become essential in modern mycological research because they enable direct analysis of fungal DNA independent of environmental variation.

PCR techniques amplify specific regions of genomic DNA, allowing researchers to detect genetic polymorphism among fungal species and populations. Among the various molecular markers, Random Amplified Polymorphic DNA (RAPD), Internal Transcribed Spacer (ITS) analysis, Inter Simple Sequence Repeat (ISSR), and Simple Sequence Repeat (SSR) markers are widely used in macrofungal studies. RAPD-PCR is particularly useful because it does not require prior knowledge of DNA sequence information and can generate multiple polymorphic bands quickly and economically. These amplified fragments help in evaluating genetic diversity, evolutionary relationships, and species differentiation among macrofungi.

PCR-based studies have revealed significant genetic variability within and among macrofungal species. Such

variability reflects adaptation to diverse ecological conditions and contributes to the evolutionary success of fungi. Molecular characterization has also helped resolve taxonomic ambiguities in morphologically similar species that are difficult to distinguish through conventional methods alone. In addition, PCR analysis has proven valuable in identifying medicinally and economically important mushrooms, including edible and poisonous species, thereby contributing to biodiversity conservation and public health awareness.

Another important application of PCR in macrofungi is phylogenetic analysis. Sequencing of amplified DNA regions, especially the ITS region of ribosomal DNA, has become a standard method for determining evolutionary relationships among fungal taxa. Phylogenetic trees generated from PCR-derived data provide insights into the origin, divergence, and classification of fungal groups. These studies have improved the systematic organization of macrofungi and strengthened fungal databases worldwide.

Furthermore, PCR-based genetic studies play a significant role in ecological and conservation research. Assessment of genetic diversity helps identify rare and endangered fungal species and supports conservation strategies for preserving fungal biodiversity. Molecular markers are also useful in studying fungal distribution, adaptation, and interactions with environmental factors.

Despite these advantages, PCR techniques may have certain limitations such as sensitivity to contamination, reproducibility issues in some marker systems like RAPD, and dependence on DNA quality. Nevertheless, continuous improvements in molecular methodologies and sequencing technologies have enhanced the accuracy and reliability of PCR-based analyses.

Genomic DNA was successfully extracted from four macrofungal samples using the small-scale isolation method described by Weining and Langridge (1991). DNA quality was confirmed through electrophoresis on a 0.8% agarose gel, which showed clear and intact bands. Comparable amounts of DNA were obtained from each sample. No RNA contamination was detected; therefore, RNase treatment was not required. The DNA samples were diluted (1:4) with double-distilled, deionized, autoclaved water and used directly for Polymerase Chain Reaction (PCR).

Genetic distances for all possible pairwise combinations were calculated following the method of Nei and Li (1979), and the relationships among accessions were illustrated as a dendrogram (Figure 3). The genetic distance among macrofungal accessions ranged from 40.2% to 71.8%. Overall, the applied protocol proved effective for isolating genomic DNA from different genera, consistently yielding DNA of sufficient quality for PCR amplification.

Conclusion:

This study identifies the Hunza District of Gilgit-Baltistan, Pakistan, as a biologically important area rich in macrofungal diversity. By combining morphological characterization with molecular approaches, especially RAPD-based DNA analysis, it offers the first genetic characterization of macrofungi from this region. The considerable genetic variation detected among the examined taxa highlights their evolutionary diversity and ecological adaptability. In addition, clustering patterns generated through UPGMA analysis reveal significant genetic relationships, aiding in more accurate taxonomic classification. Overall, the findings deepen scientific knowledge of fungal diversity in high-altitude

environments and stress the need for effective conservation measures to safeguard these ecologically vital organisms.

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References

Armaleo, D., & Clerc, P. (1991). Lichen chimeras: DNA analyses suggest that one fungus forms two morphotypes. Experimental Mycology, 15(1), 1–10.

Armaleo, D., & Clerc, P. (1995). A rapid and inexpensive method for the purification of DNA from lichens and their symbionts. The Lichenologist, 27(3), 207–213.

Bruns, T. D., White, T. J., & Taylor, J. W. (1991). Fungal molecular systematics. Annual Review of Ecology and Systematics, 22, 525–564.

Crespo, A., Bridge, P. D., & Hawksworth, D. L. (1997). Amplification of fungal rDNA ITS regions from non-fertile specimens of the lichen-forming genus Parmelia. The Lichenologist, 29, 275–282.

Chaudhary, M. S., Ahmad, A. Ali, H., & Malik, S. (2000). Technical Report on Market study of medicinal herbs in Malakand, Peshawar, Lahore and Karachi. SDC-Intercooperation, Peshawar.

Grube, M., DePriest, P. T., Gargas, A., & Hafellner, J. (1995). DNA isolation from lichen ascomata. Mycological Research, 99, 1321–1324.

FAO. 2015. Wild edible fungi: a global overview of their use and importance to people. http://www.fao.org/3/ay5489e/y5489e08.htm. Division of Plant Production and Protection. ISSN: 1020-337. Retrieved on September, 26, 2015

Grube, M. (2005). Nucleic acid isolation from ecological samples: Fungal associations and lichens. Methods in Enzymology, 395, 48–57.

Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F. & Eriksson, O.E. (2007). A higher level phylogenetic classification of the Fungi. Mycological Research 111 (5): 509–547

Hamayun, M., Khan, M. A., & Begum, S. 2003. Marketing of medicinal plants of Utror-Gabral Valleys, Swat, Pakistan. Journal of Ethnobotanical Leaflets, SIUC, USA.

Kaufman, L., & Rousseeuw, P. J. (1990). Finding groups in data: An introduction to cluster analysis. Wiley.

Khalaf, A. S., & Remdan, Y. M. (2025). New records with an updated checklist of aphyllophoroid fungi (Basidiomycota) in Iraq. Journal of Macrofungi, 1(1), 1–7.

Khalid, A. N. (2022). A checklist of macrofungi of Pakistan published from 1998–2020. Pakistan Journal of Botany, 54(5), 1947–1962.

Kirk, P. M., Cannon, P. F., Minter, D. W., & Stalpers, J. A. (2008). Dictionary of the fungi (10th ed.). CAB International.

Lanfranco, L., Wyss, P., Marzachi, C., & Bonfante, P. (1995). Generation of RAPD-PCR primers for the identification of isolates of Glomus mosseae, an arbuscular mycorrhizal fungus. Molecular Ecology, 4, 61–68.

Lee, S. B., & Taylor, J. W. (1990). Isolation of DNA from fungal mycelia and single spores. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), PCR protocols: A guide to methods and applications (pp. 282–287). Academic Press.

Mueller, G. M., Schmit, J. P., Leacock, P. R., Buyck, B., et al. (2007). Global diversity and distribution of macrofungi. Biodiversity and Conservation, 16(1), 37–48. https://doi.org/10.1007/s10531-006-9108-8

Miller, M. P. (1997). Tools for population genetic analysis (TFPGA): A Windows program for the analysis of allozymes and molecular population genetic data (Version 1.3).

Moore, R.T. (1980). Taxonomic proposals for the classification of marine yeasts and other yeast-like fungi including the smuts. Botanica Marina 23: 361–373.

Pankaj, P., Kusum, C., Kandari, L. S., Maikhu, R. K., Aditya, R. P., Bhatt, P. R., & Rao, K. S. (2002). Morchella esculenta (Guchhi): Need for scientific intervention for its cultivation in Central Himalaya. Current Science, 82(9), 1098–1108.

Nei, M., & Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the United States of America, 76(10), 5269–5273.

Palsson, B., Palsson, F., Perlin, M., Gudbjartsson, H., Steffansson, K., & Gulcher, J. (1999). Using quality measures to facilitate high-throughput genotyping. Genome Research, 9, 1151–1159.

Razaq, A., & Shahzad, S. (2012). New records of Agaricaceae from Pakistan. Pakistan Journal of Botany, 44, 1475–1477.

Razaq, A. (2007). Taxonomic studies on Basidiomycota from northern areas of Pakistan (Doctoral dissertation, University of Karachi, Karachi, Pakistan).

Schlick, A., Kuhls, K., Meyer, W., Lieckfeldt, E., Borner, T., & Messner, K. (1994). Fingerprinting reveals gamma-ray-induced mutations in fungal DNA: Implications for identification of patent strains of Trichoderma harzianum. Current Genetics, 26, 74–78.

Weining, S., & Langridge, P. (1991). Identification and mapping of polymorphisms in cereals based on polymerase chain reaction. Theoretical and Applied Genetics, 82, 209–216.

Wasser, S. P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Applied Microbiology and Biotechnology, 60, 258–274.

Vellinga, E. C. (2001). Lepiota. In M. E. Noordeloos, T. W. Kuyper, & E. C. Vellinga (Eds.), Flora Agaricina Neerlandica (Vol. 5, pp. 109–151). A. A. Balkema Publishers.

Vellinga, E. C., de Kok, R. P. J., & Bruns, T. D. (2003). Phylogeny and taxonomy of Macrolepiota (Agaricaceae). Mycologia, 95, 442–456.

Yuan, T. J., Luo, H., Su, K., Li, S. H., & Li, E. (2025). Two novel Tuber species (Tuberaceae, Pezizales) from southwestern China based on morphological and molecular evidence. MycoKeys, 119, 295–314.