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Research Article

Taxonomic Description of *Conocybe telasar* Sp. Nov., a Newly Identified Species from Mansehra, Pakistan

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ABSTRACT

Conocybe telasar sp. nov. was identified as a distinct species of *Conocybe* from various locations in district Mansehra, including Telasar, Oghi, Sumb, Balakot, and Attar Sheesha. The novelty of this taxon was established based on morpho-anatomical characteristics and molecular phylogeny of the ITS region, in comparison with already described species retrieved from GenBank. Phylogenetic analysis revealed that our species formed a clade closely related to *Conocybe fuscimarginata* (JX968238) and *C. velutipes* (JX968233). It is distinguished from allied species by several morphological features, such as creamy white basidiomata with central discoloration, white stipe arising from non-fragile ground grassland habitat, smooth pileus lacking dust and hairs, and ovate-elliptical spores measuring 7.5-10 × 4-6 µm. Microscopic observations showed cheilocystidia measuring 20-33 × 6.2-8.9 µm, while pleurocystidia were absent. Both morpho-anatomical and molecular evidence support the novelty of this species. Detailed descriptions and comparisons with allied taxa are provided in the results section.

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Introduction

Conocybe is a genus comprising a wide range of mushroom species with distinctive characteristics that have long attracted the attention of mycologists (Smith et al., 2020). According to the Index Fungorum, 268 species within this genus had been described up to 2025. Belonging to the family Bolbitiaceae, most species of *Conocybe* have been reported from Europe, while about 30 species have been characterized in India (Kumar et al., 2014; Melo et al., 2016; Liu and Bau, 2018; Ullah, 2023).

Mushrooms of this genus are fragile yet striking in appearance and grow on diverse substrates, including soil, grasslands, wood, tree trunks of both deciduous and occasionally evergreen species, gardens, ponds, agricultural lands, and under dense crop canopies. Most species typically occur in clusters in moist habitats rich in vegetation. Mycologists remain particularly interested in their ecological role, diversification, and potential as a source of bioactive compounds (Kuo, 2005). Morphological investigations of the genus have primarily focused on hyphae, spores, basidia, and cystidia (Ullah, 2023).

The present study was conducted in district Mansehra (34°20′0″ N, 73°12′0″ E), located in the northern region of Khyber Pakhtunkhwa (KPK), Pakistan. The district is renowned for its diverse natural landscapes, including forests, plains, lakes, streams, rivers, and mountains. A novel species, *Conocybe telasar* N. Jabeen, was recorded from Telasar and Attar Sheesha, hilly areas of northern Mansehra, where it was found growing on soils densely vegetated with *Oxalis corniculata* (creeping woodsorrel) and *Cynodon dactylon* (Bermuda grass). Additional macrofungal species, including *Flammulina*, *Agrocybe*, and *Schizophyllum*, were also identified in the same region. The presence of sheep and goat herds in these areas enriches the soil with manure, providing favorable conditions for fungal growthThe region experiences summer temperatures ranging from 24-30°C, with relative humidity exceeding 70%, supporting the growth of various macrofungi from April to November. Mansehra city lies at an elevation of 1,069.9 m (3,510.17 ft) above sea level and has a humid subtropical climate without a distinct dry season. The district maintains an average annual temperature of 19.1 °C (66.38 °F), which is 1.79% lower than the national average of Pakistan, and receives approximately 128.22 mm (5.05 inches) of annual precipitation distributed over 141.7 rainy days, accounting for about 38.8% of the year.

This study aimed to investigate Basidiomycota species in the Mansehra region, documenting both previously reported and undocumented taxa. The characterized species appeared to be novel based on morphological and molecular analyses, with comparative assessments conducted against sequences available in NCBI and GenBank databases.

Materials and Methods

Specimen collection, preservation, and microscopic examination

This study was conducted from 2018 to 2024. More than 180 fungal specimens were collected and identified through morphological and molecular investigations. The collection site of *C. telasar* N. Jabeen is presented in Figure 1. Specimens of *C. telasar* sp. nov. were collected from Telasar, Oghi, Sumb, Balakot, and Attar Sheesha. The species were photographed *in situ* on their natural substrates, after which additional photographs were taken following collection. Basidiocarp features and stipe characteristics (color, shape, and size) were recorded. Photographs were captured with a Canon PowerShot A460 5.0 MP digital camera (Japan). GPS coordinates and altitude were recorded using a Garmin eTrex 10 (China).

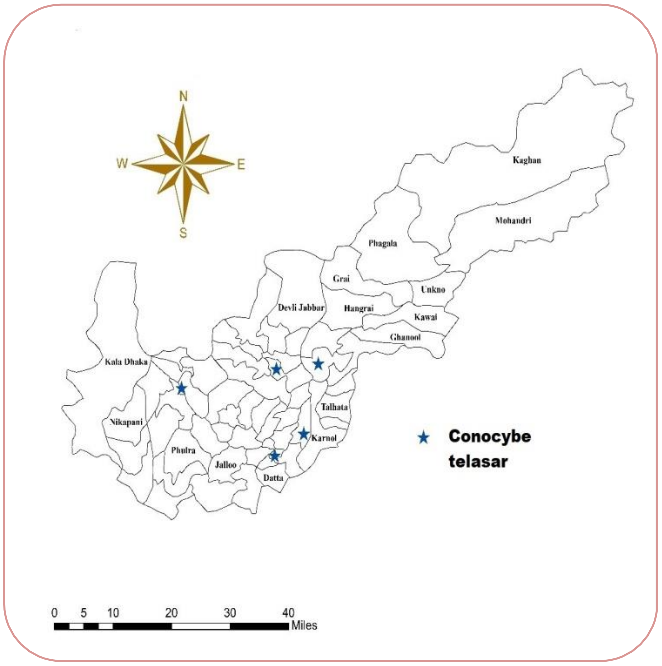

Figure 1. Prevalence of *C. telasar* sp. nov. in the Hazara Region.

Microscopic examinations were carried out with an MT4200EL Meiji Techno Binocular LED Microscope equipped with 40× and 100× oil immersion objectives and an optical camera (Japan). Spores and hyphal structures were examined using phenolphthalein blue dye.

Specimens were preserved both dry and wet. Dry preservation was achieved through oven drying and sun drying, after which samples were sealed in polythene bags. For wet preservation, specimens were washed with tap water, immersed in 3% formalin, and stored in glass jars. Permanent slides were prepared for the observation of spores, basidia, cystidia, and hyphal structures. Comparative analyses were conducted to evaluate unique morphological features following established protocols (Lodge et al., 2004; Kuo, 2005; Aman et al., 2022; Khalid, 2022). Specimens were deposited in the Botanical Herbarium of Hazara University (Dr. Alia) and the Plant Diagnostic Laboratory, Sindh Agriculture University Tandojam (Prof. Dr. Mubeen Lodhi).

Molecular analysis

Genomic DNA was extracted from all specimens using the EZ-10 Spin Column Genomic DNA Mini-Prep Kit (Bio Basic Inc., Canada), following the manufacturer's protocol (EZ-10 Genomic DNA Kit Handbook, 2013; Hameed et al., 2015). The internal transcribed spacer (ITS) region was amplified using primers ITS1 and ITS4. PCR amplification was performed, and products were purified using a single-step purification method (Ewerle et al., 1994).

Phylogenetic and evolutionary analysis

Amplified ITS sequences were compared with sequences in the NCBI database using BLAST. Closely related sequences were downloaded, trimmed in BioEdit, and aligned using MUSCLE in MEGA6. A total of 47 sequences were used to construct the phylogenetic tree. *Agaricus crocopeplus* (KF676446) and *A. biporiticus* (KJ575608) served as outgroups.

Evolutionary relationships were inferred using the Maximum Likelihood method with the Tamura 3-parameter model (Tamura, 1992). The phylogenetic tree with the highest log likelihood (-7069.74) was generated. Initial trees for heuristic searches were obtained automatically by applying Neighbor-Joining and BioNJ algorithms. Rate variation among sites was modeled using a discrete gamma distribution (+G, parameter = 0.3791). Codon positions included 1st, 2nd, 3rd, and noncoding. A total of 747 positions were included in the final dataset. All evolutionary analyses were performed in MEGA X (Kumar et al., 2018).

Results

The newly identified species was classified as follows:

Kingdom: Fungi
Phylum: Basidiomycota
Class: Agaricomycetes
Order: Agaricales
Family: Bolbitiaceae
Genus: *Conocybe*
Species: *Conocybe telasar* sp. nov. (2024)

Isolates

Isolate NJ201109, NJ201111, and NJ201120 were collected from Attar Sheesha (N=34°21′10.42425″, E=73°14′41.96846″, Altitude=1103 m). Isolates NJ201110 and NJ201121 were collected from Kotkay Telasar (N=34°28′0″, E=73°45′0″, Altitude=1070 m). Isolate NJ201123 was collected from Oghi (N=34°29′54″, E=73°0′51″, Altitude=1437 m). Isolate NJ201113D was collected from Sumb (N=34°28′0″, E=73°45′0″, Altitude=1060 m). Isolate NJ201125 was collected from Balakot (N=34°32′22″, E=73°21′0″, Altitude=974 m) (Figure 1).

Habitat

The fungus was found growing on rich organic soil and decaying tree trunks in forested habitats.

GenBank accession numbers

ITS sequences of *C. telasar* sp. nov. were submitted to GenBank under accession numbers: MZ044829 (NJ201109), MZ044830 (NJ201110), and MZ044831 (NJ201111).

Morphological characteristics

The pileus was conical to campanulate, smooth, creamy to whitish with pale margins, and exhibited discoloration toward the center. At maturity, it measured 1.5-4.5 cm in diameter and showed split margins. The stipe was cylindrical, slender, solid, and firm, measuring 3.5-5 × 1-3.5 cm.

Basidia measured 13.6-26.4 × 10.2-12.9 µm, were four-spored, thick-walled, and hyaline, with sterigmata 2-3 µm long. Spores were ovate-elliptical, measuring 7.5-10 × 4-6 µm. Cheilocystidia measured 15-25 × 2.8-5.6 µm, while pleurocystidia were absent (Figures 2 and 3).

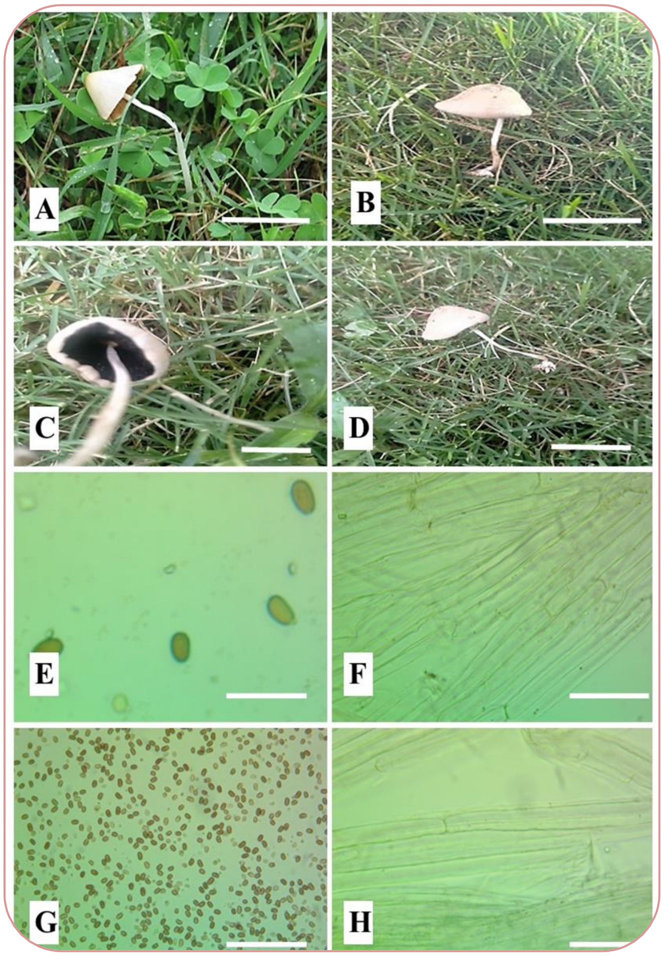

Figure 2. *C. telasar* sp. nov.: (A-B) basidiocarp; (C-D) hymenium; (E, G) basidiospores; (F, H) hyphae. Scale bars: A-B = cm; E, G = 8.5 × 5.3 µm; F, H = 7.8 µm.

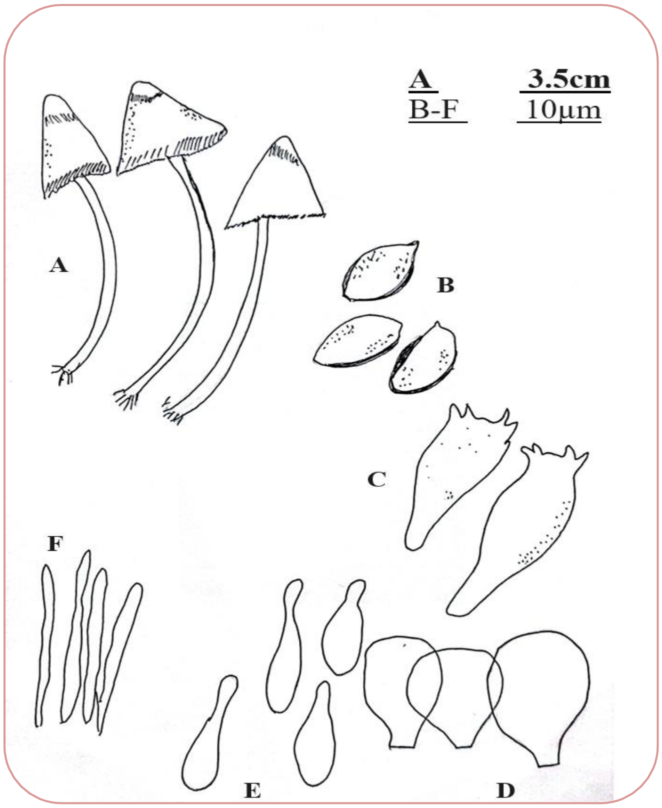

Figure 3. Sketches of *C. telasar* sp. nov.: (A) basidiocarp; (B) basidiospores; (C) basidia; (D-E) cheilocystidia; (F) pleurocystidia. Scale bars: A = 3.5 cm; B-F = 10 µm.

Properties

The genus *Conocybe* is generally non-edible, and *C. telasar* was also found to be non-edible, functioning as a saprophytic species with toxic properties (Wu et al., 2019).

Phylogenetic analysis

Phylogenetic analysis based on 47 ITS sequences revealed distinct clades. All three isolates of *C. telasar* sp. nov. (NJ201109, NJ201110, NJ201111) exhibited 100% sequence homology and formed a unique clade. The closest relatives were *C. pilosella* (JX968231, 97.25% similarity), *C. pseudocrispa* (JX968230, 97.2%), *C. rickenii* (AY194541, 96.9%), and *C. nitrophila* (KR998384, 95.7%). All other *Conocybe* species exhibited <95% similarity (Figure 4, Table 1).

Discussion

The phylogenetic analysis revealed that previously reported species from conserved regions, *C. fuscimarginata* and *C. velutipes*, clustered with our newly identified species (Figure 4). The genus *Conocybe* is a taxonomically complex and diverse group of mushrooms within the family Bolbitiaceae, order Agaricales. Members of this genus are widely distributed across ecosystems and are recognized for their delicate morphology and ecological versatility.

Historically, the taxonomic placement of Conocybe has undergone significant revisions due to advances in molecular phylogenetics. Earlier classifications placed the genus in Cortinariaceae because of morphological resemblance, particularly the presence of a cortina-like veil (Kornerup and Wanscher, 1978). However, subsequent phylogenetic analyses based on ribosomal DNA and multilocus sequencing provided strong evidence for its reclassification into Bolbitiaceae (Matheny et al., 2007; Vizzini, 2014), thereby improving the resolution of evolutionary relationships within the Agaricales.

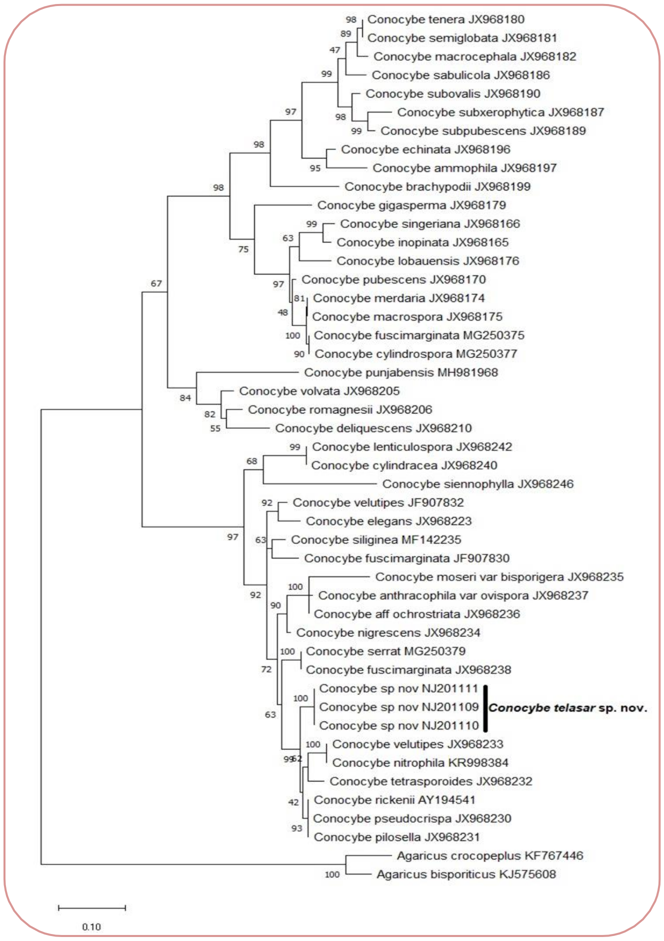

Figure 4. ITS phylogenetic tree of *Conocybe* spp. constructed using Maximum Likelihood (Tamura 3-parameter model) with 1000 bootstrap replicates in MEGA X. Branches with <50% bootstrap support were collapsed. The analysis included 47 nucleotide sequences with 747 positions in the final dataset. *Agaricus crocopeplus* (KF676446) and *A. bisporiticus* (KJ575608) were used as the outgroup.

Table 1. Pairwise patristic distances between Conocybe spp. sequences and related GenBank entries based on the Kimura 2-parameter model (MEGA11).

GenBank Accessions	Isolates of *Conocybe* spp. of present study		
	MZ044829 (NJ201109)	MZ044830 (NJ201110)	MZ044831 (NJ201111)
	Sequence homology (%)		
*Conocybe_volvata*_JX968205	76.726	76.939	76.968
*Conocybe_velutipes*_JX968233	95.347	95.379	95.250
*Conocybe_velutipes*_JF907832	92.865	92.916	92.802
*Conocybe_tetrasporoides*_JX968232	94.617	94.654	94.529
*Conocybe_teneroides*_JX968264	55.962	56.450	56.762
*Conocybe_tenera*_JX968180	71.274	71.547	71.636
*Conocybe_subxerophytica*_JX968187	67.472	67.788	67.918
*Conocybe_subpubescens*_JX968189	70.813	71.090	71.186
*Conocybe_subovalis*_JX968190	70.456	70.738	70.839
*Conocybe_singeriana*_JX968166	72.090	72.350	72.427
*Conocybe_siliginea*_MF142235	90.923	90.923	90.923
*Conocybe_siennophylla*_JX968246	80.671	80.829	80.820
*Conocybe_serrat*_MG250379	93.262	93.309	93.194
*Conocybe_semiglobata*_JX968181	70.992	71.268	71.360
*Conocybe_sabulicola*_JX968186	73.566	73.809	73.867
*Conocybe_romagnesii*_JX968206	75.984	76.205	76.241
*Conocybe_rickenii*_AY194541	96.881	96.903	96.761
*Conocybe_punjabensis*_MH981968	70.622	70.908	71.012
*Conocybe_pubescens*_JX968170	72.560	72.817	72.890
*Conocybe_pseudocrispa*_JX968230	97.129	97.148	97.008
*Conocybe_pilosella*_JX968231	97.307	97.325	97.183
*Conocybe_nitrophila*_KR998384	95.509	95.540	95.555
*Conocybe_nigrescens*_JX968234	93.647	93.692	93.574
*Conocybe_moseri*_var_*bisporigera*_JX968235	83.786	83.918	83.877
*Conocybe_merdaria*_JX968174	72.377	72.636	72.709
*Conocybe_macrospora*_JX968175	72.715	72.969	73.039
*Conocybe_macrocephala*_JX968182	72.689	72.944	73.015
*Conocybe_lobauensis*_JX968176	70.278	70.561	70.663
*Conocybe_lenticulospora*_JX968242	84.933	85.050	85.000
*Conocybe_inopinata*_JX968165	73.523	73.770	73.832
*Conocybe_gigasperma*_JX968179	69.447	69.741	69.852
*Conocybe_fuscimarginata*_MG250375	72.523	72.786	72.858
*Conocybe_fuscimarginata*_JX968238	93.262	93.309	93.194
*Conocybe_fuscimarginata*_JF907830	90.621	90.689	90.594
*Conocybe_elegans*_JX968223	90.625	90.696	90.594
*Conocybe_echinata*_JX968196	71.019	71.293	71.384
*Conocybe_deliquescens*_JX968210	75.297	75.526	75.572
*Conocybe_cylindrospora*_MG250377	72.377	72.636	72.709
*Conocybe_cylindracea*_JX968240	84.912	85.030	84.981
*Conocybe_brachypodii*_JX968199	71.299	71.569	71.656
*Conocybe_anthracophila*_var_*ovispora*_JX968237	91.667	91.727	91.624
*Conocybe_ammophila*_JX968197	72.639	72.893	72.960
*Conocybe*_aff_*ochrostriata*_JX968236	91.860	91.919	91.813
*Agaricus_crocopeplus*_KF767446	41.540	42.253	42.819
*Agaricus_bisporiticus*_KJ575608	46.468	47.085	47.540

Morphologically, *Conocybe* species are generally small to medium-sized mushrooms characterized by a conical to campanulate pileus, often with a pointed umbo, and brown to cinnamon-brown gills that are adnate to adnexed. The stipe is slender, fragile, and occasionally bears a membranous or fugacious annulus. Microscopically, the genus is distinguished by ovoid to ellipsoid spores with a distinct apical pore and non-diverticulate cheilocystidia (Pegler, 1986; Hausknecht, 2009). Some species also possess lecythiform cystidia, an important diagnostic feature for species delimitation.

Ecologically, *Conocybe* species exhibit wide adaptability. Many are saprotrophic, growing on soil, decaying organic matter, or dung, and contribute to nutrient cycling (Gulden et al., 2005). Others are found in association with grass roots in lawns, pastures, and agricultural fields. Though less well documented, some taxa are suspected of forming ectomycorrhizal associations with trees (Tedersoo et al., 2010). Toxicological interest in the genus stems from species such as *C. filaris*, which contains deadly amatoxins similar to those in *Amanita phalloides* (Beug et al., 2006; Michelot, 2008), emphasizing the importance of accurate species identification.

From a biogeographical perspective, *Conocybe* is cosmopolitan but remains underexplored in Asia. In Pakistan, reports of this genus have been scarce. Ahmad (1980a, b) and Iqbal and Khalid (1996) documented several species, including *C. khasiensis*, *C. macrocephala*, *C. mesospora*, *C. pubescens*, *C. rickenii*, *C. semiglobata*, and *C. tenera* from Punjab province, mostly from soils and dung substrates. More recently, two novel taxa, *C. punjabensis* (Izhar et al., 2019) and *C. karakensis* (Song et al., 2023), were described from Pakistan, highlighting the country as a potential hotspot of fungal diversity.

Our combined morphological and molecular analyses, based on ITS and LSU rDNA sequences, revealed that our isolates are distinct from closely related species such as *C. fuscimarginata*, *C. velutipes*, *C. serrata*, *C. nitrophila*, *C. tetrasporoides*, *C. rickenii*, *C. pseudocrispa*, and *C. pilosella*. Differences were especially evident in spore dimensions, cystidial morphology, and pileus coloration, all of which are crucial diagnostic traits in *Conocybe* taxonomy (Singer, 1986; Hausknecht and Krisai-Greilhuber, 2000). These results justify the recognition of *C. telasar* N. Jabeen as a new species from Pakistan.

The discovery of *C. telasar* highlights the value of integrative taxonomy, combining morphological and molecular data, in resolving species boundaries within *Conocybe*, where convergent morphologies often obscure identification. It also highlights the need for further fungal biodiversity surveys in underexplored regions such as Pakistan, where macrofungal diversity is likely underestimated. With advances in molecular sequencing and phylogenomics, future studies are expected to uncover even greater diversity in *Conocybe* and clarify its ecological significance in grassland, forest, and agricultural ecosystems (Tedersoo et al., 2014; He et al., 2019).

Conclusions

This study explored mushroom diversity in multiple locations, including Kotkay Telasar, Hazara University, Attar Sheesha, and Jaba. *C. telasar* N. Jabeen was identified from Kotkay Telasar and Attar Sheesha through a combination of morphological and molecular analyses. Phylogenetic reconstruction demonstrated that three collected specimens represented the same taxon, confirming the novelty of this species within *Conocybe*. This research expands current knowledge of fungal diversity in Pakistan and provides a foundation for future ecological and taxonomic studies of macrofungi in the region.

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Authors' Contributions

NJ and NS designed the study; NJ, RM, MI and KB collected samples, prepared the materials, identified the specimens, collected and analyzed the data; BZ, MS, AG, A and HM helped in sampling, identification; NS supervised the studies; NJ, NS and HM wrote the manuscript; All the authors proofread and approved the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 12: Responsible Consumption and Production
SDG 13: Climate Action
SDG 15: Life on Land

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