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Identification and Genetic Diversity of Wild Mushroom Strains Using Nuclear Genes and Molecular Markers

Kazhan Mahmood Sleman [1], Nawroz Abdul-razzak Tahir [2,*]

[1] Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaimani, 46001, Iraq;[2] Food Science and Quality Control Department, Bakrajo Technical Institute, Sulaimani Polytechnic University, Sulaimani, 46001, Iraq

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Abstract

Wild mushroom species are integral to the steppe and mountain ecosystems in Iraq's Kurdistan area. Despite extensive investigation, the evolutionary relationships among the various strains remain mostly unknown. Three molecular techniques have been used to test sixty-three samples for identification and genetic diversity. Phylogenetic analysis using Internal Transcribed Spacer (ITS) sequences identified two primary clades. The BLASTn results identified seventeen *Agaricus litoralis*, two *Pleurotus columbinus*, three *Pleurotus eryngii*, three *Agrocybe dura*, eight *Amanita lividopallescens*, nine *Amanita crocea*, ten *Amanita vittadinii,* five *Agaricus pseudolutosus*, three *Agaricus campestris*, *two Agrocybe aegerita*, and one *Agaricus bisporus*. A substantial aggregation of taxa was associated with the genera *Agaricus, Agrocybe*, and *Amanita.* A total of 373 polymorphic fragments were identified using conserved DNA-derived polymorphism (CDDP) and start codon target (SCoT) markers. The CDDP and SCoT markers exhibited effects analogous to those of genetic diversity indices across several genera and species of mushrooms. CDDP (18 bands) and the SCoT (18 bands) markers exhibited high numbers of polymorphic bands per primer. CDDP exhibited a greater level of species-specific variance (23.74%) than SCoT markers (20.81%). The average polymorphism information content was 0.39 for both the CDDP and SCoT markers. In the clustering analysis, both methodologies categorized 63 samples into seven distinct groups. Three classes of mushroom species and genera were delineated using both markers. *A. lividopallescens* and *A. aegerita* showed the greatest genetic distance (0.48) for CDDP markers, while *A. aegerita* and *P. columbinus* showed the highest genetic dissimilarity (0.36) for SCoT markers. The combinatorial methods could effectively tell the difference between different strains of mushrooms. The significant genotypic diversity and sequenced genomes will give an ideal basis for functional genomics studies of the mushroom strains.

Keywords: Fungi, Species, Genus, DNA Identification, Molecular markers

1. Introduction

Eukaryotic heterotrophs called fungi have an estimated million species and are considered to be of tremendous economic and ecological importance (Blackwell, 2011; Hibbett *et al.*, 2016). The distribution of many fungal species is quite extensive, and it is possible that some of these species are truly cryptic or comprised of numerous genetic lineages. The fruiting bodies of macrofungi were vulnerable to environmental influences; therefore, morphological assessments typically cannot identify closely related genetic groupings (Zhao *et al.*, 2016). In the life cycle of *Basidiomycetes* and certain *Ascomycetes*, mushrooms are seen as visible fungi with unique carpophores, also called basidiocarps or fruiting bodies. They can be edible, inedible, or poisonous, and they are morphologically characterized as stinkhorns, puffballs, brackets, and gilled fungi (Adeniyi *et al.*, 2018). Mushrooms, which are fruiting bodies that develop from a subterranean mycelium and are widely consumed around the world, are produced by certain members of the macro-fungi group (Kalač, 2013). Rural areas and developing nations often gather wild mushrooms for culinary purposes or economic gain (Kotowski, 2016; Boa, 2004). Meanwhile, a select few mushroom species are farmed commercially and sold worldwide. The current culinary trends that prioritize health and sustainability are well aligned with mushrooms; thus, they are gaining in popularity. In reality, they are suitable for vegetarian diets, have ideal nutritional properties, and are compatible with a sustainable food supply (Giusti *et al.*, 2020).

Cultivated mushrooms are widely imported into Iraqi Kurdistan since mushroom growing cannot meet the high national demand. Wild mushrooms, on the other hand, have a long history of recreational collecting in Iraqi Kurdistan, and their users tend to value them more than the cultivated varieties. The months in concern are designated for this gathering. Mushrooms are not completely risk-free to eat. As a matter of fact, poisoning from mushrooms is a major kind of toxin-induced illness that can impact the neurological, respiratory, digestive, and liver systems (Giusti *et al.*, 2021; Govorushko *et al.*, 2019). When very toxic species are present, the liver sustains permanent damage that can be lethal. In addition, there are edible species that, if not handled correctly during collection, transportation, storage, and cooking, might turn hazardous (Gawlikowski *et al.*, 2015; Nieminen and Mustonen, 2020). They thrive on a variety of substrates and are often

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[*] Corresponding author. e-mail: nawroz.a.tahir@spu.edu.iq.

abundant during the rainy season in most regions. The many ecological, nutritional, physiological, and medicinal benefits of mushrooms have earned them the status of important bioresources (Ayimbila and Keawsompong, 2023). They help the ecosystem with nutrient recycling by breaking down dead organic materials, especially those that include lignin and cellulose (Kumla *et al.*, 2020). In addition to being rich in protein, crude fiber, vitamins, and minerals, they also have medicinal properties that make them useful in treating a variety of conditions, including high cholesterol, tumors, infections, cancer, ageing, anti-inflammatory, antidiabetic, antihyperglycemic, antiparasitic, anti-inflammatory, and antihypertensive conditions (Bhambri *et al.*, 2022; Titilawo *et al.*, 2022).

The majority of morphological and agronomic features, however, are quantitative variables that are highly responsive to environmental variation (Tahir *et al.*, 2021). Consequently, the genetic variety of germplasm resources cannot be directly reflected by phenotypic data (Sleman and Tahir, 2025; Lin *et al.*, 2022; Rokni *et al.*, 2019). Fungal morphological traits are highly variable and influenced by environmental factors, leading to overlapping or indistinct features among genetically distinct groups, as evidenced by the lack of consistent morphological traits among molecularly defined fungal species and intraspecific groups (Lin *et al.*, 2023). Conventional methods for identifying fungal species are typically characterized by indiscrimination, inconsistent morphology, and inconspicuousness; however, modern molecular techniques have mitigated these issues (Abdullah and Yasin, 2025). For precise species identification of mushrooms, however, DNA analysis in conjunction with phenotypic data is preferred because of its speed and low cost. Analyses using PCR-amplified nuclear rRNA genes, and more specifically the Internal Transcribed Spacer (ITS) region, which has been selected as a DNA barcode identifier for fungi because of its significant interspecies variation, constitute the majority of current approaches. The small subunit 5.8S rRNA gene separates the two non-coding parts (ITS-1 and ITS-2), which are in the ITS region and are about 600 to 800 kb in size. Genome sequencing at this marker and subsequent comparison to publicly available reference DNA libraries constitute the highest standard for taxonomic identification (Badotti *et al.*, 2017; Creedy *et al.*, 2020; Lücking *et al.*, 2020; Yuwa-Amornpitak *et al.*, 2023). Molecular markers, including inter simple sequence repeat (ISSR), sequence related amplified polymorphic (SRAP), random amplified polymorphic DNA (RAPD), and cleaved amplified polymorphic sequences (CAPs), are powerful methods for analyzing the genetic variation and strain relationships of organisms. It is also environmentally independent because of the high levels of detectable polymorphisms (Gu *et al.*, 2024; Hao *et al.*, 2022; Tahir, 2008; Tahir and Karim, 2011). In the past, different researchers employed molecular techniques for the identification and estimation of genetic variation within different mushroom strains (An *et al.*, 2021; Jiao *et al.*, 2024; Lin *et al.*, 2022; Liu *et al.*, 2018; Rokni *et al.*, 2019; Zhao *et al.*, 2016). The use of conserved DNA-derived polymorphism (CDDP) molecular markers is capable of capturing information uniquely offered by the targeted application of a single primer to a single locus within a gene of transcription factors (Ahmed *et al.*, 2023; Aziz and Tahir, 2023; Tahir *et al.*, 2023; Rasul *et al.*, 2022). Many studies have used SCoT markers, which are usually polymorphic and depend on shorter conserved regions close to the ATG start codon, to uncover genetic diversity and aid in conservation efforts. In this technique, similar to RAPD or ISSR markers, a single primer acts as both the forward and reverse primer (Majeed *et al.*, 2024; Tahir *et al.*, 2023; Rasul *et al.*, 2022).

Our literature review reveals an absence of published data about the in-silico analysis of the frequency of CDDP and SCoT markers across all fungal genomes. To our knowledge, this is a novel occurrence both regionally and globally for certain species. Iraqi Kurdistan possesses a wealth of mushroom genetic resources, despite having only one cultivated species. The presence of several names for identical or closely related strains results in difficulties in classification and ambiguity concerning genetic distinctiveness and relationships among strains. These challenges have complicated the categorization and preservation of genetic resources, the selection of appropriate breeding parents, and the advancement of improved cultivars. Therefore, the assessment of genetic diversity and the appraisal of germplasm resources are thus crucial. The aim of this study was to identify and assess the genetic diversity of 63 mushroom strains (62 wild and 1 cultivated) from the Iraqi Kurdistan region using the ITS gene and molecular markers (CDDP and SCoT). This comprehensive approach enabled the analysis of genetic variation data and improved understanding of the genetic diversity present in the germplasm resources from 63 mushroom strains. The identification of superior genetic resources established an outline for subsequent research aimed at enhancing and broadening current germplasm resources, breeding materials, and conservation strategy. To fully comprehend the potential of wild mushrooms and to strengthen their preservation, more research in this area is required.

2. Materials and Methods

2.1. Mushroom strains samples

In February 2024, at the end of winter, we collected sixty-two samples of wild mushrooms from eight different sites in the Kurdistan area of Iraq (Figure 1 and supplementary file, Figure S1). The distance between the sampling areas ranged from eighty to two hundred kilometers. To avoid sampling the same genotypes multiple times, we obtained each isolate at a minimum distance of 1 km from each other. For each geographical population, table S1 in the supplementary file displayed the sample size along with their names, strains, accession numbers, and altitudes. During sampling, the samples were maintained in the bag and placed in the chilled box. Afterwards, they were stored at 4 °C in the laboratory.

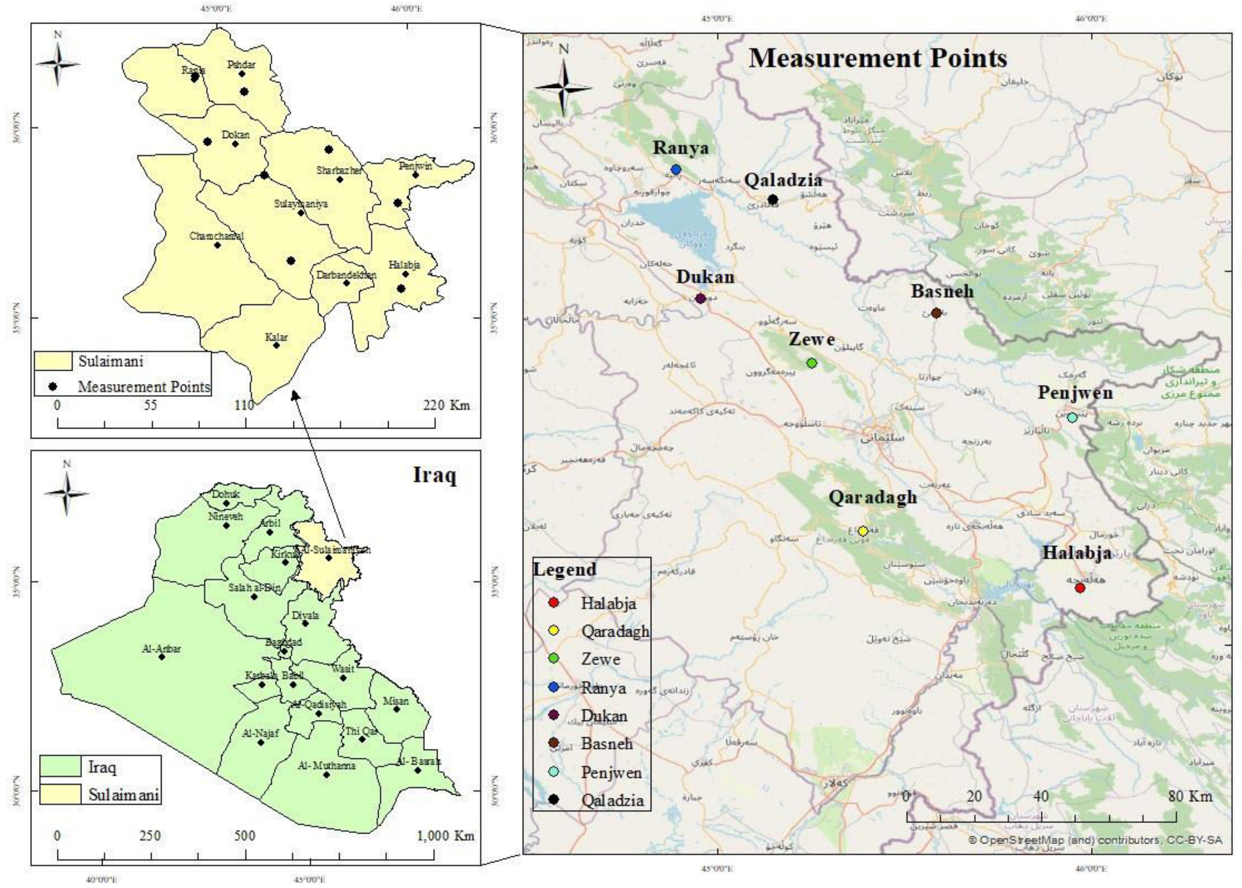

Figure 1. A map depicting the locations where distinct mushroom strains are harvested.

2.2. DNA extraction

The cap mushroom of samples (0.5 g) was ground using liquid nitrogen. DNA was extracted using the method described in Ahmed *et al.* (2023) and Aziz and Tahir (2023). The purity of genomic DNA was checked using gel electrophoresis on a 1.1% agarose gel (Addbio, Korea), and the quality was determined using Nanodrop spectrophotometry (NanoPLUS, MAALANLAB, Sweden). The DNA solution was maintained at a temperature of -17°C.

2.3. Polymerase chain reaction, purification, and DNA sequencing

The complete ITS1-5.8S-ITS2 region (supplementary file, Table S2) was amplified using PCR using a master mix kit (AddBio, Daejeon, Republic of Korea) in accordance with manufacturer protocols. The ITS primers, PCR circumstances, DNA separation, and DNA purification from agarose gel were detailed in our published article by Khal *et al.* (2023). The Macrogen Company in South Korea then sequenced the DNA and compared the results to those already recorded in the NCBI collection. GenBank assigned the sequences an accession number.

2.4. Genetic diversity analysis

Two molecular techniques, SCoT and CDDP markers, were employed to assess genetic diversity among various samples. A total of 24 PCR primers were used in the PCR method for SCoT and CDDP, comprising 12 primers for each marker type (Supplementary file, Table S2) (Aziz and Tahir, 2023; Collard and Mackill, 2009a; Collard and Mackill, 2009b; Majeed *et al.*, 2024; Rasul *et al.*, 2024). The conditions for PCR, DNA separation, and visualization are clarified in our published works by Tahir *et al.* (2023) and Ahmed *et al.* (2022).

2.5. Phylogenetic tree and statistical analysis

To ensure the highest quality of sequences, we implemented chromatograms of sequencing in the SnapGene version 3.1 software to verify the sequences and replicate them as necessary. The sequences were aligned using the Clustal W utility in Molecular Evolutionary Genetics Analysis 11 (MEGA 11). A phylogenetic tree was constructed with 1000 bootstrap replicates using the neighbor-joining tree technique to establish pretrial reliability. The detectable PCR products produced with SCoT and CDDP were subjected to an analysis. We only considered strong, distinct, and repetitive bands to decrease the chance of inaccuracy. We utilized numbers 1 and 0 to indicate the presence and absence of amplified bands, respectively, in order to generate a binary matrix for each sample. The radar chart was generated by XLSTAT version 2020.1.3. Principal component analysis and cluster analysis were conducted using the Ward method to generate the cluster diagram with JMP version 18. Using GenAIEx version 6.51b2, genetic distance, diversity indices, and analysis of molecular variance (AMOVA) were calculated. The polymorphism information content (PIC) was calculated using the formula outlined by Muhammad and Tahir (2026) and Aziz and Tahir (2023).

3. Results

3.1. Identification and phylogenetic analysis of mushroom specimens through nuclear DNA

The BLASTn results identified seventeen Agaricus litoralis, two Pleurotus columbinus, three Pleurotus eryngii, three Agrocybe dura, eight Amanita lividopallescens, nine Amanita crocea, seventeen Agaricus litoralis, ten Amanita vittadinii, five Agaricus pseudolutosus, three Agaricus campestris, two Agrocybe aegerita, and one Agaricus bisporus (Figure 2).

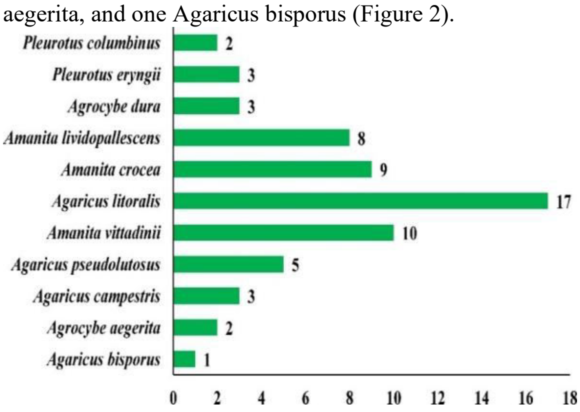

Figure 2. Number of identified strains in a collection of 63 samples through the ITS nuclear gene.

Phylogenetic analysis showed that there were two main groups of 63 isolates (Figure 3A). Five different strains of *P. columbinus* and *P. eryngii*, which belong to the genus *Pleurotus*, made up the first group (green line). In the second set of 58 isolates (red line), nine species were represented across three genera: *A. litoralis*, *A. dura*, *A. lividopallescens*, *A. crocea*, *A. vittadinii, A. pseudolutosus*, *A. campestris*, *A. aegerita*, and *A. bisporus.* After strain identification, the dendrogram (Figure 3B) divided the eleven species into three distinct categories based on the frequency of the nucleotides A, T, C, and G. *A. bisporus, A. litoralis, A. pseudolutosus*, and *A. aegerita* formed the initial group of taxa (red line). *A. vittadinii, A. crocea*, and *A. lividopallescens* were the members of the genus *Amanita*, forming the second clade (green line), while *P. columbinus, P. eryngii, A. dura*, and *A. campestris* were the members of the third clade (blue line). The four genera were categorized into two groups based on the frequency of four nucleotides. The first group consisted of three genera: *Agaricus, Agrocybe*, and *Amanita*; the second group was created by the genus *Pleurotus* (Figure 3C). As seen in figure 4, the range of C+G percentage was 0.43 to 0.50, whereas the A+T percentage was 0.50 to 0.57.

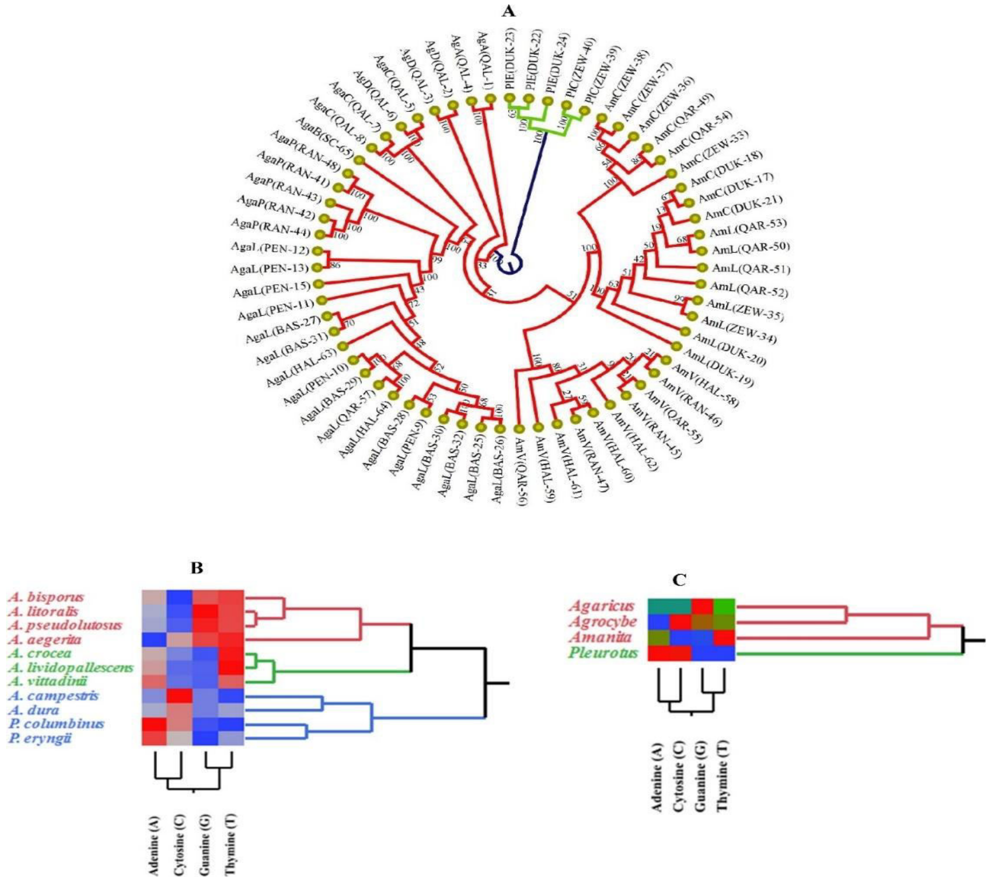

Figure 3. Clustering of mushroom specimens based on ITS gene sequencing data. A. Phylogenetic tree of all isolated samples. B. Classification of various mushroom species according to the prevalence of adenine, cytosine, guanine, and thymine. C. Clustering of diverse genera with respect to the frequency of adenine, cytosine, guanine, and thymine. The numbers on the node indicate the bootstrap percentage.

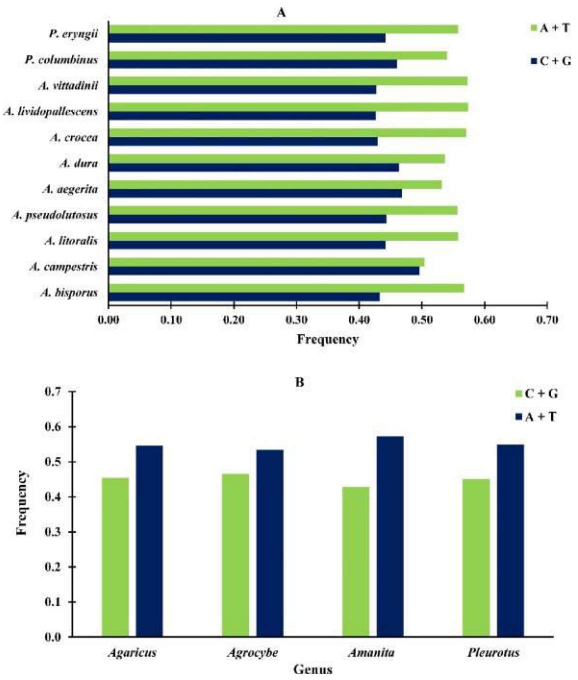

Figure 4. Illustrating the GC and AT contents in various species (A) and genera (B) of both wild and cultivated mushrooms.

3.2. Marker information and genetic diversity of CDDP and SCoT methods acquired from mushroom samples

Using 12 polymorphic CDDP primers, the present investigation produced 204 markers from 63 samples, covering 11 species across 4 genera of mushrooms. From 14 (MYB1) to 18 (WRKY-R3, ERF2, MYB1, ERF1, WRKY-R2B, and ABP1-1) polymorphic fragments were found. The PIC had a mean of 0.39 and a range of 0.35 to 0.43 (WRKY-R2B to ERF1), as shown in figure 5. Concerning SCoT markers (Figure 6), the number of polymorphic bands varied from 11 (SCoT23) to 18 (SCoT21), while the PIC ranged from 0.35 (SCoT35) to 0.44 (SCoT20).

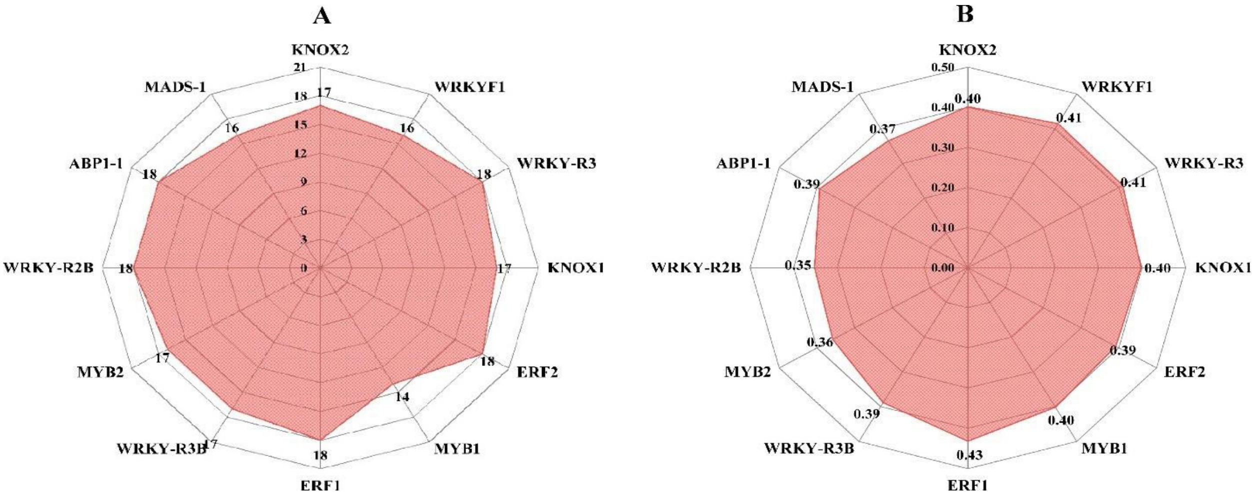

Figure 5. Number of polymorphic bands (A) and polymorphism information content (B) for various CDDP markers gathered from different species and genera of 63 mushroom samples.

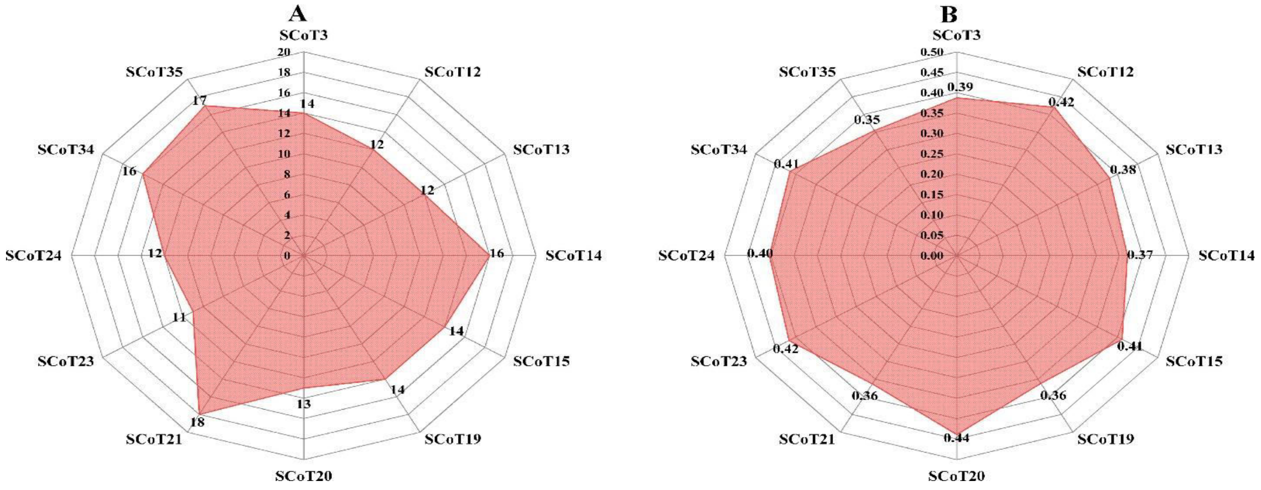

Figure 6. Polymorphic band number (A) and polymorphism information content (B) from various SCoT markers from 63 mushroom samples of different species and genera.

Species genetic diversity indicators, as determined by CDDP markers, are presented in table 1. The number of different alleles (Na), number of effective alleles (Ne), Shannon's information index (I), expected heterozygosity (He), and unbiased expected heterozygosity (uHe) varied from 1.01 (WRKY-R3) to 1.24 (ERF1), 1.23 (MADS-1) to 1.33 (MYB1), 0.21 (MADS-1) to 0.29 (MYB1), 0.14 (MADS-1) to 0.19 (MYB1 and KNOX1), and 0.15 (MADS-1 and KNOX1) to 0.22 (MYB1 and KNOX1), respectively. The Na, Ne, I, He, and uHe genetic diversity indices of the genera were as follows: 1.49 (WRKY-R3B) and 1.78 (KNOX1), 1.33 (WRKY-R2B) and 1.51 (ERF1), 0.33 (WRKY-R2B) and 0.44 (ERF1), 0.21 (WRKY-R2B) and 0.30 (ERF1), and 0.22 (WRKY-R2B) and 0.31 (ERF1), respectively. The genetic diversity indicators of species and genera, as determined by SCoT markers, are summarized in table 1. Na, Ne, I, He, and uHe genetic diversity indices of the species differed from 0.98 (SCoT35) to 1.35 (SCoT15), 1.24 (SCoT35) to 1.35 (SCoT20), 0.22 (SCoT35) to 0.32 (SCoT15), 0.15 (SCoT35) to 0.21 (SCoT15 and SCoT 20), and 0.17 (SCoT35) to 0.24 (SCoT15), respectively. The Na, Ne, I, He, and uHe genetic diversity indices of the genera were 1.38 (SCoT24) and 1.77 (SCoT13), 1.31 (SCoT35) and 1.52 (SCoT20), 0.30 (SCoT35) and 0.46 (SCoT20), 0.19 (SCoT35) and 0.31 (SCoT20), and 0.20 (SCoT35) and 0.33 (SCoT20), respectively.

Table 1. Genetic diversity indices of several markers of CDDP and SCoT derived from different genera and species of 63 mushroom samples.

	Species					Genus				
CDDP markers	Na	Ne	I	He	uHe	Na	Ne	I	He	uHe
KNOX2	1.08	1.29	0.26	0.17	0.20	1.59	1.39	0.38	0.24	0.26
WRKYF1	1.15	1.30	0.27	0.18	0.20	1.50	1.37	0.35	0.23	0.24
WRKY-R3	1.01	1.24	0.22	0.14	0.16	1.54	1.39	0.37	0.24	0.25
KNOX1	1.21	1.31	0.28	0.19	0.22	1.78	1.40	0.40	0.25	0.27
ERF2	1.17	1.31	0.27	0.18	0.21	1.61	1.42	0.38	0.25	0.27
MYB1	1.23	1.33	0.29	0.19	0.22	1.63	1.48	0.41	0.28	0.29
ERF1	1.24	1.31	0.28	0.18	0.21	1.71	1.51	0.44	0.30	0.31
WRKY-R3B	1.10	1.26	0.23	0.15	0.17	1.49	1.40	0.36	0.24	0.25
MYB2	1.08	1.27	0.25	0.16	0.18	1.50	1.37	0.36	0.23	0.24
WRKY-R2B	1.10	1.26	0.24	0.16	0.17	1.53	1.33	0.33	0.21	0.22
ABP1-1	1.16	1.32	0.27	0.18	0.21	1.56	1.47	0.40	0.27	0.29
MADS-1	1.04	1.23	0.21	0.14	0.15	1.56	1.40	0.37	0.24	0.26
Mean	1.13	1.28	0.25	0.17	0.19	1.58	1.41	0.38	0.25	0.26
SCoT markers	Species					Genus				
SCoT3	1.23	1.32	0.28	0.19	0.21	1.61	1.46	0.41	0.27	0.29
SCoT12	1.27	1.30	0.27	0.18	0.20	1.71	1.45	0.41	0.27	0.29
SCoT13	1.15	1.31	0.27	0.18	0.21	1.77	1.44	0.42	0.27	0.29
SCoT14	1.14	1.31	0.28	0.18	0.21	1.58	1.39	0.37	0.24	0.25
SCoT15	1.35	1.34	0.32	0.21	0.24	1.75	1.43	0.42	0.27	0.29
SCoT19	1.14	1.32	0.28	0.19	0.21	1.57	1.43	0.39	0.26	0.27
SCoT20	1.31	1.35	0.31	0.21	0.23	1.71	1.52	0.46	0.31	0.33
SCoT21	1.03	1.26	0.23	0.15	0.17	1.46	1.37	0.34	0.22	0.23
SCoT23	1.08	1.29	0.24	0.16	0.18	1.55	1.45	0.40	0.27	0.28
SCoT24	1.11	1.27	0.25	0.16	0.18	1.38	1.33	0.31	0.20	0.21
SCoT34	1.19	1.34	0.29	0.20	0.23	1.58	1.41	0.38	0.25	0.26
SCoT35	0.98	1.24	0.22	0.15	0.17	1.40	1.31	0.30	0.19	0.20
Mean	1.17	1.30	0.27	0.18	0.20	1.59	1.42	0.38	0.25	0.27

Na: number of observed alleles, Ne: effective number of alleles, He: expected heterozygosity or gene diversity, I: Shannon's information index, and uHe: unbiased expected heterozygosity

3.3. Genetic diversity parameters among different populations (species and genera) of mushroom strains

Gene diversity indicators at the population level (species and genera) exhibited variation among mushroom strains, as detailed in tables 2 and 3. The genetic diversity indices Na, Ne, I, He, and uHe for the species measured varied from 0.45 (*A. aegerita*) to 1.82 (*A. litoralis*), 1.04 (*A. aegerita*) to 1.47 (*A. vittadinii*), 0.00 (*A. bisporus*) to 0.43 (*A. litoralis*), 0.00 (*A. bisporus*) to 0.28 (*A. litoralis*), and 0.00 (*A. bisporus*) to 0.30 (*A. litoralis*), respectively. The measured genera' genetic diversity indices were 1.40 (*Pleurotus*) to 1.89 (*Agaricus*), 1.29 (*Agrocybe*) to 1.49 (*Amanita*), 0.27 (*Agrocybe*) to 0.46 (*Amanita*), 0.17 (*Agrocybe*) to 0.30 (*Amanita*), and 0.19 (*Agrocybe*) to 0.31 (*Amanita*), respectively. According to CDDP data, the average proportion of polymorphic loci (PPL) was 45.23% across 11 species and 75.98% across 4 genera, with species ranging from 0.00% (*A. bisporus*) to 91.18% (*A. litoralis*) and genera ranging from 52.45% (*Agrocybe*) to 94.61% (*Agaricus*). The average number of private bands across all species was 0.36, ranging from 1 (*A. dura*) to 2 (*A. litoralis*), but the average number of private bands across all genera was 2.75, with a range from 1 (*Agrocybe* and *Pleurotus*) to 5 (*Agaricus*).

Based on the SCoT data, the maximum Na, Ne, I, He, and uHe of 1.75, 1.43, 0.41, 0.27, and 0.28 were recorded with *A. litoralis*, respectively. The highest PPL of 87.57% was seen with *A. litoralis*. The average number of private bands across all species was 0.36, ranging from 1 (*A. litoralis*) to 3 (*A. vittadinii*). At the genus level, the greatest values for Na, Ne, I, He, uHe, and PPL were 1.90, 1.48, 0.46, 0.30, 0.30, and 94.67% for the genus *Amanita*. A wide range of 2 to 5 specific bands appeared across all genera.

Table 2. Genetic diversity indices of various species and genera identified in a collection of 63 mushrooms based on CDDP data.

	Species						
Species	Na	Ne	I	He	uHe	PPL	PB
A. campestris	1.01	1.27	0.23	0.16	0.19	42.16	0.00
A. litoralis	1.82	1.45	0.43	0.28	0.30	91.18	2.00
A. pseudolutosus	1.28	1.35	0.32	0.21	0.23	59.31	0.00
A. aegerita	0.45	1.04	0.03	0.02	0.03	5.39	0.00
A. dura	0.50	1.06	0.06	0.04	0.05	11.76	1.00
A. crocea	1.50	1.36	0.33	0.21	0.23	71.57	0.00
A. lividopallescens	1.32	1.38	0.33	0.22	0.24	62.25	1.00
A. vittadinii	1.67	1.47	0.42	0.27	0.29	82.35	0.00
P. columbinus	0.73	1.16	0.14	0.10	0.13	23.04	0.00
P. eryngii	1.13	1.31	0.27	0.18	0.22	48.53	0.00
A. bisporus	1.00	1.26	0.00	0.00	0.00	0.00	0.00
Mean	1.13	1.28	0.23	0.15	0.17	45.23	0.36
Genus	Genus						
Agaricus	1.89	1.46	0.44	0.29	0.29	94.61	5.00
Agrocybe	1.18	1.29	0.27	0.17	0.19	52.45	1.00
Amanita	1.85	1.49	0.46	0.30	0.31	92.65	4.00
Pleurotus	1.40	1.39	0.34	0.23	0.26	64.22	1.00
Mean	1.58	1.41	0.38	0.25	0.26	75.98	2.75

Na: number of observed alleles, Ne: effective number of alleles, He: expected heterozygosity or gene diversity, I: Shannon's information index, uHe: unbiased expected heterozygosity, PPL: percentage of polymorphic loci, and PB: number of private bands.

Table 3. Genetic diversity indicators of different species and genera observed in a collection of 63 determined by data from SCoT markers.

	Species						
Species	Na	Ne	I	He	uHe	PPL	PB
A. campestris	1.10	1.31	0.27	0.18	0.22	46.75	0.00
A. litoralis	1.75	1.43	0.41	0.27	0.28	87.57	1.00
A. pseudolutosus	1.44	1.40	0.35	0.24	0.26	68.05	0.00
A. aegerita	0.69	1.15	0.13	0.09	0.12	21.30	0.00
A. dura	0.75	1.19	0.16	0.11	0.13	27.81	0.00
A. crocea	1.57	1.45	0.39	0.26	0.27	76.33	0.00
A. lividopallescens	1.43	1.37	0.34	0.22	0.24	68.05	0.00
A. vittadinii	1.64	1.45	0.40	0.27	0.28	81.07	3.00
P. columbinus	0.46	1.05	0.05	0.03	0.04	7.69	0.00
P. eryngii	0.98	1.27	0.24	0.16	0.19	42.01	0.00
A. bisporus	0.95	1.26	0.00	0.00	0.00	0.00	0.00
Mean	1.16	1.30	0.25	0.17	0.18	47.88	0.36
Genus	Genus						
Agaricus	1.87	1.46	0.44	0.29	0.29	93.49	2.00
Agrocybe	1.24	1.34	0.30	0.20	0.22	57.40	0.00
Amanita	1.90	1.48	0.46	0.30	0.30	94.67	5.00
Pleurotus	1.31	1.36	0.32	0.22	0.24	60.95	0.00
Mean	1.58	1.41	0.38	0.25	0.26	76.63	1.75

Na: number of observed alleles, Ne: effective number of alleles, He: expected heterozygosity or gene diversity, I: Shannon's information index, uHe: unbiased expected heterozygosity, PPL: percentage of polymorphic loci, and PB: number of private bands.

3.4. Molecular variation among different species and genera of mushroom strains

Analysis of molecular variance (AMOVA) was performed to evaluate the genetic variation among the species and genera identified from mushroom samples (Table 4). The variance analysis of CDDP and SCoT data indicated that 23.74% and 10.82% of the genetic variation stemmed from interspecific variation, respectively, while 76.26% and 89.18% derived from intraspecific variation, respectively, based on the CDDP and SCoT data. The PhiPT genus and species values of CDDP data were 0.11 and 0.24, respectively, whereas the PhiPT SCoT data were 0.10 and 0.21 for genus and species, respectively.

Table 4. Analysis of molecular variance among several species and taxa based on CDDP and SCoT marker data

			CDDP markers			
Source	Degree of freedom	Sum square	Mean square	Estimated variance	Percentage of variance	PhiPT
Among species	10.00	893.80	89.38	10.37	23.74	0.24
Within species	52.00	1732.93	33.33	33.33	76.26	
Total	62.00	2626.73		43.70	100.00	
	Genus					
Among genus	3.00	308.29	102.76	4.77	10.82	0.11
Within genus	59.00	2318.44	39.30	39.30	89.18	
Total	62.00	2626.73		44.07	100.00	
	SCoT markers					
	Species					
Among species	10.00	679.31	67.93	7.38	20.81	0.21
Within species	52.00	1459.86	28.07	28.07	79.19	
Total	62.00	2139.17		35.45	100.00	
	Genus					
Among genus	3.00	242.04	80.68	3.65	10.19	0.10
Within genus	59.00	1897.13	32.15	32.15	89.81	
Total	62.00	2139.17		35.80	100.00	

In relation to the CDDP data, Nei's genetic distances among species ranged from 0.07 (between *A. vittadinii* and *A. litoralis*) to 0.48 (between *A. lividopallescens* and *A. aegerita*) (Supplementary file, Table S3), whereas Nei's genetic dissimilarity among four genera varied from 0.03 (between *Amanita* and *Agaricus*) to 0.21 (between *Pleurotus* and *Agrocybe*). In terms of the species and genera, Nei's genetic distances derived from the SCoT data revealed the greatest distance between *P. columbinus* and *A. aegerita* (0.36), while the smallest distance was noted between *A. vittadinii* and *A. litoralis* (0.06). *Pleurotus* and *Agrocybe* exhibited the highest Nei's genetic dissimilarity at 0.16, while *Amanita* and *Agaricus* demonstrated the lowest at 0.04 (Supplementary file, Table S4).

3.5. Relationships and clustering of mushroom strains

The findings of the clustering and PCA plots generated from CDDP data indicated that the 63 strains were predominantly dispersed and categorized into seven principal groups (Figure 7A and B). The last group, designated as the seventh group and marked in a red circle, encompassed the majority of strains, totaling 24 strains. The clustering and PCA plots distinctly categorized 11 species into three clades at the species level. The PCA results for mushroom species samples revealed that the first two principal components represented 46.60% and 25.80% of the variation, respectively, for a cumulative contribution rate of 72.40%. Clade I, indicated by a red line, has six species: *A. campestris, A. litoralis, A. pseudolutosus, A. crocea, A. lividopallescens*, and *A. vittadinii*. Clade II possessed three species: *P. columbinus, P. eryngii*, and *A. bisporus*. *A. aegerita* and *A. dura* constituted the third clade (Figure 7C, D, and E). The WRKYF1 marker displayed the most variance across the three clades, followed by the WRKY-R3, ERF2, and MADS-1 markers, while the MYB1 marker demonstrated the least variation among the three clades (Figure 7F). The clustering results, as shown in figure 8, led to the classification of the 63 strains into four categories, generating the four genera. The PCA results for mushroom genera samples indicated that the first two principal components accounted for 59.30% and 40.5% of the variation, respectively, generating a cumulative contribution rate of 99.80%. The initial group comprised two genera: *Amanita* and *Agaricus*. The second and third categories consisted of the *Pleurotus* and *Agrocybe* genera, respectively (Figure 8).

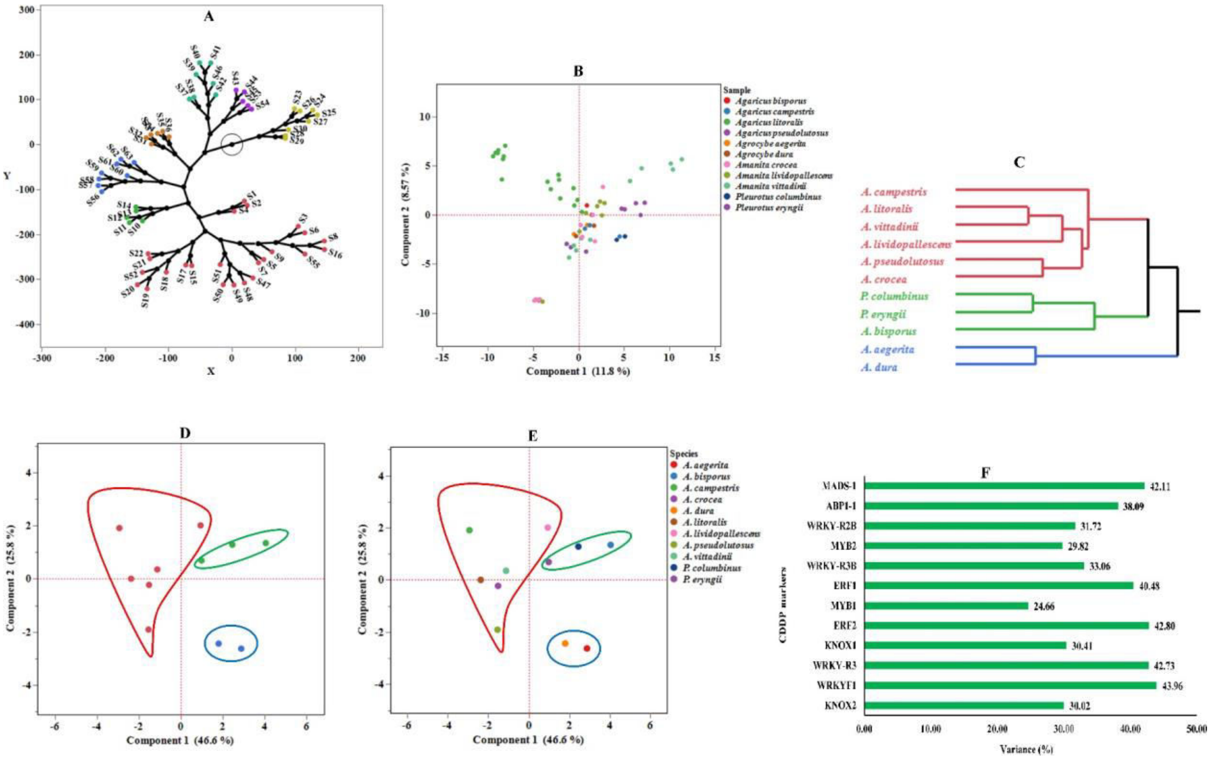

Figure 7. Clustering and interrelationships among 63 strains from various species and genera of mushrooms based on the CDDP data. A and B: Clustering and PCA plots illustrating the distribution and grouping of various strains; C, D, and E: grouping and PCA plots representing the classification of separate mushroom species; F: percentage of variation recorded by CDDP marker. S1-S63 represents the 63 mushroom strains.

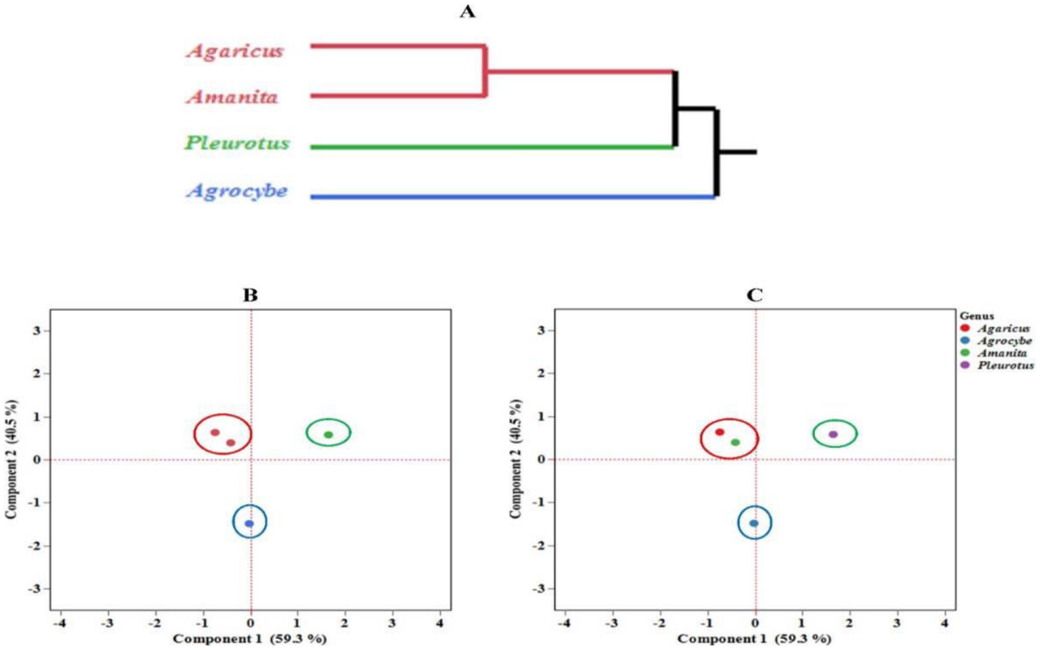

Figure 8. Clustering and interrelationships across four genera of mushrooms observed across 63 strains based on the CDDP data.

The results from the dendrogram and PCA plots derived from SCoT data revealed that the 63 strains were primarily distributed and classified into seven main categories (Figure 9A and B). The last group, identified as the seventh group and highlighted in red, included the majority of strains, reaching 19 strains. The clustering and PCA plots clearly divided 11 species into three clades at the species level. The PCA results for mushroom species samples revealed that the first two principal components represented 45.50% and 22.30% of the variation, respectively, for a cumulative contribution rate of 67.80%. Clade I, denoted by a red line, comprises five species: *A. campestris, A. litoralis, A. crocea, A. lividopallescens,* and *A. vittadinii. A. pseudolutosus, A. aegerita*, and *A. dura* formed clade II, indicated by the green line. Clade III, indicated by a blue line, comprised three species: *P. columbinus, P. eryngii*, and *A. bisporus* (Figure 9C, D, and E). The SCoT13 marker exhibited the highest variance (38.26%) across the three clades, followed by the SCoT21 marker (37.20%), whilst the SCoT3 marker revealed the lowest variation (26.68%) among the three clades (Figure 9F). The four genera were established from the 63 strains categorized into four classifications based on the results. The PCA results for mushroom genera samples indicated that the first two principal components accounted for 70.00% and 29.99% of the variation, respectively, generating a cumulative contribution rate of 99.99%. The original group consisted of two genera: *Amanita* and *Agaricus*. The second and third categories comprised the *Pleurotus* and *Agrocybe* genera, respectively (Figure 10).

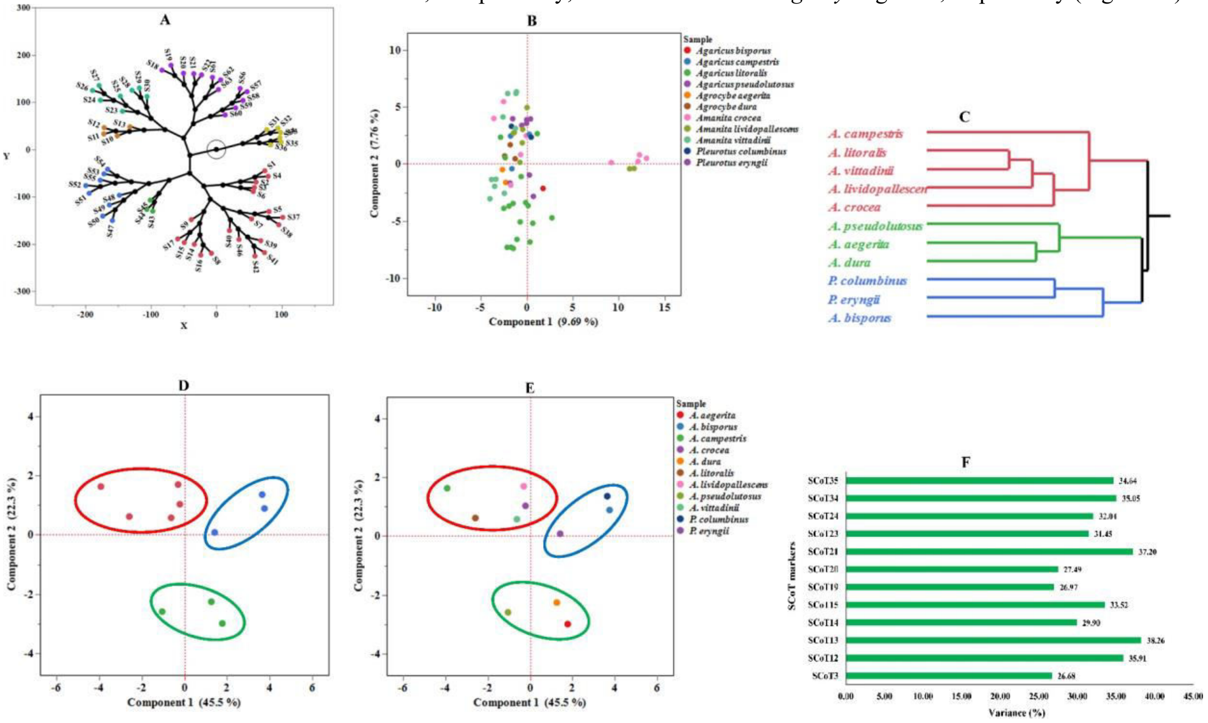

Figure 9. Clustering and relationships among 63 strains from several species and genera of mushrooms based on the SCoT data. A and B: Clustering and PCA plots depicting the dispersion and clustering of diverse strains; C, D, and E: clustering and PCA plots displaying the classification of distinct mushroom species; F: proportion of variation documented by SCoT markers. S1-S63 denote the 63 mushroom strains.

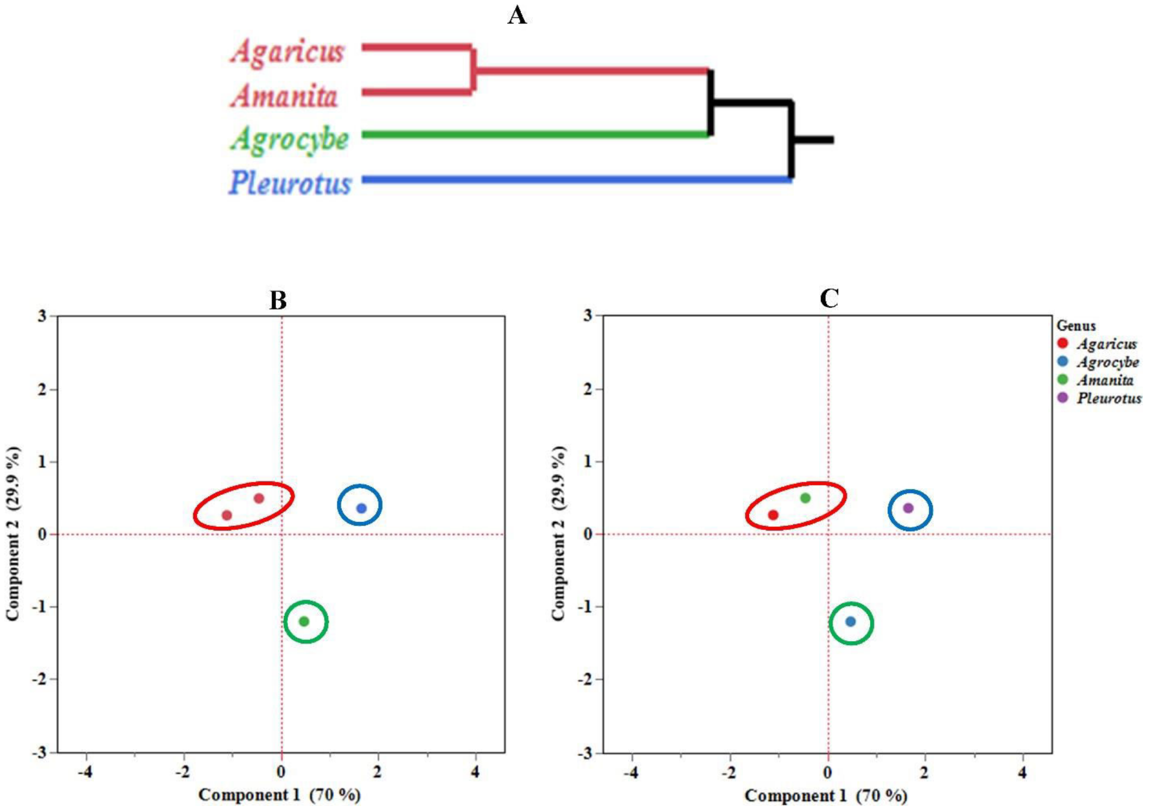

Figure 10. Grouping and relationships over four genera of mushrooms assessed 63 strains based on the SCoT data.

4. Discussion

Research on any kind of organism cannot proceed without first establishing an accurate taxonomy. Conventional mushroom identification relies on morphological characteristics such as fruit body size, shape, length, diameter, and any other distinguishing characteristics. The contradiction in macrofungi taxonomy at the species and genus levels has been corroborated by the internal transcribed spacer (ITS) region of nuclear ribosomal DNA, encompassing ITS1 and ITS4. The current study involves the PCR product of mushrooms, measuring roughly 550-750 base pairs. Fujita *et al.* (2001) documented fragment sizes ranging from 350 to 880 bp for fungi using ITS1 and ITS4. Following PCR amplification using ITS1 and ITS4 primers, Adeniyi *et al.* (2018) reported a product of approximately 850 bp. Ab Rhaman *et al.* (2021) reported an amplicons size between 500 and 800 bp among mushroom species. The DNA gel electrophoresis of eight species with a molecular weight of 700–750 bp was presented in a study by Jalil *et al.* (2025).

Our study attempted to resolve the phylogenetic relationship of the 63 strains invested in the region of Iraqi Kurdistan using the ITS rDNA. Eleven species distributed in four genera were revealed by this study from the collected samples of wild mushrooms. The second group of this study included strains from the genera *Agaricus, Amanita*, and *Agrocybe*, whereas the *Pleurotus* strains were placed in the first group. The strains of species in the *Amanita* genus were stratified in the beginning subgroup of the second group. Subsequently, the species that were placed under the genera *Agaricus* and *Agrocybe* were clustered together with the same secondary subgroup. The second group also consists of the cultivated mushroom *A. bisporus*. The ITS region of the strains in the same clade group was, however, highly homogenous within the group, which is the reason for putting them grouped together (Stackebrandt *et al.*, 2002). Moreover, the bootstrap in this investigation was 9 and 100%. The BLASTn analysis of the ITS data showed that the dominant strains in all the identified mushroom samples corresponded to *A. litoralis,* followed by *A. vittadinii* and *A. crocea,* which indicates that the species adapt to different environments.

Understanding genetic diversity within and between species or genera is essential for creating efficient conservation management strategies for endangered and threatened species. There is a broad and varied genetic availability of wild mushroom strains in Iraqi Kurdistan. In order to find resources, find new genes, and breed new types, it is essential to study the genetic diversity of germplasms. Since critical mushroom species are still rarely bred, most cultivated strains are derived from tamed wild varieties. Problems like recurrent strains and unclear genetic links across varieties emerged as a consequence of the limitations in genetic diversity between parent strains brought about by isolating tissue culture and rebranding. Genomic research into the relationships between wild mushroom strains collected from various parts of Iraqi Kurdistan is crucial. Future breeding and use of germplasm also necessitate genetic diversity studies among strains (Gu *et al.*, 2024; Tahir *et al.*, 2015). Conventional molecular markers may not consistently elucidate phenotypic variations as effectively as genetic diversity assessment using CDDP and SCoT. There has been no prior mention of applying CDDP and SCoT to create fingerprints of wild mushroom germplasm for the analysis of genetic associations (Gao *et al.*, 2018). Fungi typically exhibit significant levels of genetic variety in their wild populations when they are sexually reproducing, inhabiting diverse ecological niches, and/or found all over the world. This investigation used two different types of molecular markers to generate 373 bands. Comparatively, SCoT primers detected 14.08 polymorphic bands from various markers, but CDDP primers averaged 17 bands. Polymorphism in various parts of the mushroom's genome likely causes this variation, which is contributed by SCoT markers that specifically target the highly conserved region surrounding the start codon (ATG) in mushroom genes, while CDDP markers amplify portions of conserved functional genes (transcription factors). The high levels of polymorphism exhibited by various markers clearly indicated their applicability in genetic variability investigations in wild mushroom strains, and they could be used to identify plant materials that are related in different species and genera (Golian *et al.*, 2022; Khandayataray *et al.*, 2022).

Gene diversity determines the degree of genetic variety within breeding population accessions. The primary focus here is the evolutionary restrictions on alleles and the probable mutation rate for a certain locus. Genomic diversity reflects genetic diversity for haploid markers and provides an estimate of anticipated heterozygosity and genetic distance among population members (Salem and Sallam, 2016). Several researchers used the molecular markers and demonstrated their usefulness in evaluating the genetic diversity of the species (Khal *et al.*, 2023; Akhtar *et al.*, 2021; Shekhar *et al.*, 2014). Gene-targeted markers are especially useful for studies that seek to measure functional genetic diversity because they sample genomic regions that are important for traits or subject to selection more directly than anonymous markers, which mainly represent neutral variation. The purpose of gene-targeted markers like SCoT and CDDP is to amplify regions of DNA linked to coding or regulatory sequences. These areas are more likely to be linked to adaptive responses and phenotypic traits, which offer information on genetic diversity that is essential for selection and functionality (Platten *et al.*, 2019).

Our research indicates that CDDP and SCoT markers are effective for assessing genetic diversity in mushroom samples and validating the presence of significant genetic variation. In this investigation, the use of SCoT and CDDP markers with polymorphic variation possessed higher PIC parameters. This allowed for efficient selection of the most appropriate markers for the research on genetic divergence. A diversity index allows assigning strain members to different groups, enabling a quantitative measure of the diversity of the species or genus range. Variegated indices are used to indicate the differing degrees of diversity, where lower values indicate low diversity while higher values suggest high diversity (Oluyinka Christopher, 2020). There is a notable amount of variability in SCoT genome analysis between species. For instance, Shannon’s information index mean value ‘I’, which is calculated from SCoT markers, is more than that which is computed from CDDP markers, suggesting that there exist much variation and ecological complexity impacting the SCoT loci target area. Expected heterozygosity or gene diversity values were mostly low for both species and genus for most markers, supporting the evidence from AMOVA analysis of high diversity among various species. Average gene diversity values for 4 genera of mushrooms were higher than those of 11 species of mushrooms: 0.30 for both markers versus 0.17 and 0.18 for CDDP and SCoT, respectively. Results of AMOVA analysis demonstrated that the PhiPT value for CDDP data was higher than that of the SCoT marker, which means that CDDP markers are more informative than the SCoT approach for revealing the variation between different taxa. High variability within the species and genus of mushrooms, based on CDDP and SCoT data, in this study suggests that strains of the same species present significant variation, indicating the effects of environmental factors on the strains' genetic structure. Mushroom species require molecular markers for genetic characterization in order to better understand the variety of mushrooms available and develop strategies for their breeding (Lee *et al.*, 2019; Khatun *et al.*, 2017; Singh and Gupta, 2017). With CDDP data, our study found that the genus *Agaricus* had the largest number of unique bands (5), while the genus *Amanita* had only four. On the other hand, SCoT data identified four maximum specific bands linked to the *Amanita* genus. This study postulates that these unique bands associated with the *Agaricus* genus could potentially serve as biomarkers for differentiating strains of mushrooms across different taxa. In addition, these unique bands can be linked to phenotypic and other enhancements and to the construction of a new marker that evaluates genetic diversity (Jiao *et al.*, 2024; Liu *et al.*, 2022). Cluster analyses are important in aiding interspecies mapping investigations because they assist in understanding genetic diversity (Eltaher *et al.*, 2018). The dendrogram produced confirms a large diversity of mushroom strains and taxa. The diversity among strains, species, and genera points to an abundance of genetic material and an elevated amount of genetic variation. The CDDP markers had a wider spectrum of dissimilarities than the SCoT markers, indicating a high degree of structural variability in transcription factor genes. It was considered more effective to discriminate between the strains of different taxa and genera using markers CDDP and SCoT. The *A. litoralis* isolates located within the Basneh region were determined from the CDDP plot (group 1; olive circle), while those found in the Penjwen region belonged to group 5 (mint circle). All *A. pseudolutosus* strains ranged within the same group (group 3; teal circle). The isolates of *A. vittadinii* were positioned in two groups: group 2 (purple circle) and group 5 (blue circle). The different SCoT markers used in the clustering plot depicted the allocation of strains of different species and genera into different clades; for example, the *A. litoralis* strains collected from different areas of the regions were arranged in clades 3 and 4. The differences existing among strains of a particular mushroom species collected from different regions can be largely accounted for by the different environmental conditions of those regions. These environmental factors have an influence on not only the growth of mushrooms but also their productivity as well as the genetic diversity and adaptability of the fungi (Vieira Junior *et al.*, 2022; Bellettini *et al.*, 2019). This diversity in strains distribution denotes a considerable level of genetic variation existing among members of the same taxa.

One of the reasons for the discrepancy between the CDDP and SCoT clustering, as well as with the ITS method, is that different techniques increase the genomic variation of the different strains of the same taxon and genus. Gap spacer DNA that is amplified by the universal barcode sequence (ITS) separates the large subunit rRNA from the small subunit rRNA genes on the chromosome. SCoT markers focus on the highly conserved area not far from the ATG in the fungal genes, while CDDP markers focus on the regions of conserved functional genes, which are transcription factors (Aziz and Tahir, 2023; Khal *et al.*, 2023; Tahir *et al.*, 2023).

5. Conclusions

In this study, the molecular phylogenetic analysis was found to be useful for tracing the relationships of the 63 strains of mushrooms from the Iraqi Kurdistan region. According to the clustering analysis, there is a high level of divergence in both the species and genus levels. The species of mushrooms displayed the presence of AT content in greater quantities compared to the GC content. The genetic relationships of mushroom samples at the strain, species, and genus levels are highly facilitated by the CDDP and SCoT markers, which are very efficient in polymorphism detection. The results also verified the possibility of markers CDDP and SCoT being able to differentiate strains of the same species from different regions. In Iraq, there are still many wild types of mushrooms that are yet to be studied, and there is a need to carry out research that aims to discover new species in this area. The study of mushroom strains should involve studying not just the rDNA ITS region and some molecular markers but also the morphological and chemical profile of the mushroom samples to elucidate evolutionary relations among the strains, species, and genera.

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Mushroom accession numbers

The profiles of strain DNA sequences scrutinized in this study can be found in the databases of the National Center for Biotechnology Information.

Contribution statement

Nawroz A. Tahir: Supervision, Resources, Project administration, Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Conceptualization, and Validation. Kazhan M. Sleman: Data curation, Methodology, and Investigation. Writing – review & editing, Visualization.

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Declaration of competing interest

The authors affirm that they have no known opposing financial interests or personal relations that could have seemed to affect the data described in this article.

Footnote

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References

Ab Rhaman SMS, Naher L and Siddiquee S. 2021. Mushroom quality related with various substrates' bioaccumulation and translocation of heavy metals. *J Fungi*, **8(1)**: 42.

Abdullah SK and Yasin MR. 2025. New Records with an Updated Checklist of Aphyllophoroid Fungi (Basidiomycota) in Iraq. *J Macrofun.*, **1(1)**: 1–7.

Adeniyi M, Titilawo Y, Oluduro A, Odeyemi O, Nakin M and Okoh AI. 2018. Molecular identification of some wild Nigerian mushrooms using internal transcribed spacer: polymerase chain reaction. *Amb Express*, **8**: 1–9.

Ahmed AA, Qadir SA and Tahir NA-R. 2022. CDDP and ISSR markers-assisted diversity and structure analysis in Iraqi Mazu (*Quercus infectoria* Oliv.) accessions. *All Life*, **15(1)**: 247–61.

Ahmed AA, Qadir SA and Tahir NA-R. 2023. Genetic variation and structure analysis of Iraqi Valonia oak (*Quercus aegilops* L.) populations using conserved DNA-derived polymorphism and inter-simple sequence repeats markers. *Plant Mol Biol Rep.*, **41(1)**: 1–14.

Akhtar N, Hafiz IA, Hayat MQ, Potter D, Abbasi NA, Habib U, Hussain A, Hafeez H, Bashir MA and Malik SI. 2021. ISSR-based genetic diversity assessment of genus *Jasminum* L. (Oleaceae) from Pakistan. *Plants*, **10(7)**: 1270.

An H, Lee H-Y, Shim D, Choi SH, Cho H, Hyun TK, Jo I-H and Chung J-W. 2021. Development of CAPS markers for evaluation of genetic diversity and population structure in the germplasm of button mushroom (*Agaricus bisporus*). *J Fungi*, **7(5)**: 375.

Ayimbila F and Keawsompong S. 2023. Nutritional quality and biological application of mushroom protein as a novel protein alternative. *Curr Nutr Rep.*, **12(2)**: 290–307.

Aziz RR and Tahir NA-R. 2023. Genetic diversity and structure analysis of melon (*Cucumis melo* L.) genotypes using URP, SRAP, and CDDP markers. *Genet Resour Crop Evol.*, **70(3)**: 799–813.

Badotti F, Oliveira FS de, Garcia CF, Vaz ABM, Fonseca PLC, Nahum LA, Oliveira G and Góes-Neto A. 2017. Effectiveness of ITS and sub-regions as DNA barcode markers for the identification of *Basidiomycota* (Fungi). *BMC Microbiol.*, **17**: 1–12.

Bellettini MB, Fiorda FA, Maieves HA, Teixeira GL, Ávila S, Hornung PS, Júnior AM and Ribani RH. 2019. Factors affecting mushroom *Pleurotus* spp. *Saudi J Biol Sci.*, **26(4)**: 633–46.

Bhambri A, Srivastava M, Mahale VG, Mahale S and Karn SK. 2022. Mushrooms as potential sources of active metabolites and medicines. *Front Microbiol.*, **13**: 837266.

Blackwell M. 2011. The Fungi: 1, 2, 3… 5.1 million species? *Am J Bot.*, **98(3)**: 426–38.

Boa ER. 2004. *Wild edible fungi: a global overview of their use and importance to people.* Food & Agriculture Org

Collard BCY and Mackill DJ. 2009a. Conserved DNA-derived polymorphism (CDDP): a simple and novel method for generating DNA markers in plants. *Plant Mol Biol Rep.*, **27**: 558–62.

Collard BCY and Mackill DJ. 2009b. Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Mol Biol Rep.*, **27**: 86–93.

Creedy TJ, Norman H, Tang CQ, Qing Chin K, Andujar C, Arribas P, O'Connor RS, Carvell C, Notton DG and Vogler AP. 2020. A validated workflow for rapid taxonomic assignment and monitoring of a national fauna of bees (Apiformes) using high throughput DNA barcoding. *Mol Ecol Resour.*, **20(1)**: 40–53.

Eltaher S, Sallam A, Belamkar V, Emara HA, Nower AA, Salem KFM, Poland J and Baenziger PS. 2018. Genetic diversity and population structure of F3: 6 Nebraska winter wheat genotypes using genotyping-by-sequencing. *Front Genet.*, **9**: 76.

Fujita S-i, Senda Y, Nakaguchi S and Hashimoto T. 2001. Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. *J Clin Microbiol.*, **39(10)**: 3617–22.

Gao W, Qu J, Zhang J, Sonnenberg A, Chen Q, Zhang Y and Huang C. 2018. A genetic linkage map of Pleurotus tuoliensis integrated with physical mapping of the de novo sequenced genome and the mating type loci. *BMC Genomics*, **19**: 1–14.

Gawlikowski T, Romek M and Satora L. 2015. Edible mushroom-related poisoning: A study on circumstances of mushroom collection, transport, and storage. *Hum Exp Toxicol.*, **34(7)**: 718–24.

Giusti A, Ricci E, Gasperetti L, Galgani M, Polidori L, Verdigi F, Narducci R and Armani A. 2020. Molecular identification of mushroom species in Italy: An ongoing project aimed at reinforcing the control measures of an increasingly appreciated sustainable food. *Sustainability*, **13(1)**: 238.

Giusti A, Ricci E, Gasperetti L, Galgani M, Polidori L, Verdigi F, Narducci R and Armani A. 2021. Building of an Internal Transcribed Spacer (ITS) gene dataset to support the Italian Health Service in mushroom identification. *Foods*, **10(6)**: 1193.

Golian M, Chlebová Z, Žiarovská J, Benzová L, Urbanová L, Hovaňáková L, Chlebo P and Urminská D. 2022. Analysis of Biochemical and Genetic Variability of Pleurotus ostreatus Based on the β-Glucans and CDDP Markers. *J Fungi*, **8(6)**: 563.

Govorushko S, Rezaee R, Dumanov J and Tsatsakis A. 2019. Poisoning associated with the use of mushrooms: A review of the global pattern and main characteristics. *Food Chem Toxicol.*, **128**: 267–79.

Gu M, Chen Q, Zhang Y, Zhao Y, Wang L, Wu X, Zhao M and Gao W. 2024. Evaluation of genetic diversity and agronomic traits of germplasm resources of *Stropharia rugosoannulata*. *Horticulturae*, **10(3)**: 213.

Hao H-b, Zhao J, Yang H, Zhang J-x, Wei Y-h, Kuai B-k, Zhang J-j and Chen H. 2022. Comprehensive evaluation of main agronomic traits and nutritional components of different *Stropharia rugosoannulata* strains. *Acta Edulis Fungi*, **38(3)**: 41–49.

Hibbett D, Abarenkov K, Kõljalg U, Öpik M, Chai B, Cole J, Wang Q, Crous P, Robert V and Helgason T. 2016. Sequence-based classification and identification of Fungi. *Mycol.*, **108(6)**: 1049–68.

Jalil SJ, Kateeb AIA and Al-Juboory HH. 2025. Investigation of macrofungi in the middle of Iraq. *Bull Iraq Nat Hist Mus.*, **18(3)**: 665–87.

Jiao L, Han C, Zhu J, Zhang P, Ma Y, Dai X and Zhang Y. 2024. Transcriptome analysis and development of EST-SSR markers in the mushroom Auricularia heimuer. *Sci Rep.*, **14(1)**: 12340.

Kalač P. 2013. A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. *J Sci Food Agric.*, **93(2)**: 209–18.

Khal LH, Tahir NA-R and Abdul-Razaq RT. 2023. Molecular variation in some taxa of genus *Astragalus* L. (Fabaceae) in the Iraqi Kurdistan region. *Horticulturae*, **9(10)**: 1110.

Khandayataray P, Murthy MK, Giri A, Samal D and Laskar SA. 2022. Genetic diversity analysis of the wild edible mushroom *Termitomyces heimii* by using RAPD markers. *Res J Biotech.*, **17(5)**: 115–29.

Khatun S, Islam A, Gupta K and Gupta B. 2017. Detection of Edible Mushroom Species by Using Molecular Markers. In *Molecular Markers in Mycology: Diagnostics and Marker Developments*, ed. BP Singh, VK Gupta, pp. 201–24. Cham: Springer International Publishing

Kotowski M. 2016. Differences between European regulations on wild mushroom commerce and actual trends in wild mushroom picking. *Slov národop.*, **64(2)**: 169–78.

Kumla J, Suwannarach N, Sujarit K, Penkhrue W, Kakumyan P, Jatuwong K, Vadthanarat S and Lumyong S. 2020. Cultivation of mushrooms and their lignocellulolytic enzyme production through the utilization of agro-industrial waste. *Molecules (Basel, Switzerland)*, **25(12)**: 2811.

Lee SH, Ali A, Ha B, Kim M-K, Kong W-S and Ryu J-S. 2019. Development of a molecular marker linked to the A4 locus and the structure of HD genes in *Pleurotus eryngii*. *Mycobiol.*, **47(2)**: 200–06.

Lin P, Kook M, Yi T-H and Yan Z-F. 2023. Current fungal taxonomy and developments in the identification system. *Curr Microbiol.*, **80(12)**: 375.

Lin P, Yan Z-F, Kook M, Li C-T and Yi T-H. 2022. Genetic and chemical diversity of edible mushroom *Pleurotus* species. *Biomed Res Int.*, **2022(1)**: 6068185.

Liu J-H, Ding F-H, Song H-Y, Chen M-H and Hu D-M. 2022. Analysis of genetic diversity among Chinese *Cyclocybe chaxingu* strains using ISSR and SRAP markers. *PeerJ*, **10**: e14037.

Liu XB, Li J and Yang ZL. 2018. Genetic diversity and structure of core collection of winter mushroom (*Flammulina velutipes*) developed by genomic SSR markers. *Hereditas*, **155**: 1–8.

Lücking R, Aime MC, Robbertse B, Miller AN and Ariyawansa HA, et al. 2020. Unambiguous identification of fungi: where do we stand and how accurate and precise is fungal DNA barcoding? *IMA Fungus*, **11**: 14.

Majeed HO, Faraj JM, Rasul KS, Lateef DD and Tahir NA-R. 2024. Evaluation of the genetic diversity and population structure of *reticulated iris* accessions in the Iraqi Kurdistan region using SCoT and SRAP markers. *Genet Resour Crop Evol.*, **71**: 3705–20.

Muhamad AO and Tahir, NA. 2026. Unraveling the genetic variation and population structure in okra genotypes using ISSR, SCoT, and CDDP markers. *Plant Mol. Biol. Rep.,* **44 (91)**: 1-15.

Nieminen P and Mustonen A-M. 2020. Toxic potential of traditionally consumed mushroom species—a controversial continuum with many unanswered questions. *Toxins (Basel)*, **12(10)**: 639.

Oluyinka Christopher A. 2020. Comparative analyses of diversity and similarity indices of west bank forest and block a forest of the International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State, Nigeria. *J For Res.*, **2020(1)**: 4865845.

Platten JD, Cobb JN and Zantua RE. 2019. Criteria for evaluating molecular markers: Comprehensive quality metrics to improve marker-assisted selection. *PLoS One*, **14(1)**: e0210529.

Rasul KS, Grundler FMW and Tahir NA-R. 2022. Genetic diversity and population structure assessment of Iraqi tomato accessions using fruit characteristics and molecular markers. *Hortic Environ Biote.*, **63(4)**: 523–38.

Rasul KS, Majeed HO, Faraj JM, Lateef DD and Tahir NA-R. 2024. Genetic diversity and relationships among *Iris aucheri* genotypes determined via ISSR and CDDP markers. *Genet Resour Crop Evol.*, **72**: 3235–3248.

Rokni N, Sadati SAA, Safaie N, Ebrahimi MA and Samimifar M. 2019. Assessment of genetic relatedness among commercial and wild strains of *Agaricus bisporus* using repetitive extragenic palindromic sequences and polymerase chain reaction. *Mycoscience*, **60(5)**: 293–97.

Salem KFM and Sallam A. 2016. Analysis of population structure and genetic diversity of Egyptian and exotic rice (*Oryza sativa* L.) genotypes. *C R Biol.*, **339(1)**: 1–9.

Shekhar S, Prasad MP and Sriram S. 2014. Detection of genetic diversity in Jasmine species by DNA fingerprinting using RAPD molecular markers. *Int. J. Pure Appl. Biosci.*, **2**: 312–17.

Singh BP, Gupta VK, eds. 2017. *Molecular Markers in Mycology: Diagnostics and Marker Developments.* Cham: Springer International Publishing

Sleman KM and Tahir NA. 2025. A comprehensive evaluation of the morpho-biochemical and chemical constituent profiles in various wild mushroom species: Investigating their potential benefits and limitations. *J. King Saud Univ. Sci.,* **37 (4)**: 4232025.

Stackebrandt E, Frederiksen W, Garrity GM, Grimont PAD, Kämpfer P, Maiden MCJ, Nesme X, Rosselló-Mora R, Swings J and Trüper HG. 2002. Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol.*, **52(3)**: 1043–47.

Tahir N, Hamakareem H and Amin BH. 2015. Differentiate of ten pea cultivars (*Pisum sativum* L.) by RAPD markers and seed storage proteins. *JJAS*, **11(1)**: 95–107.

Tahir N, Lateef DD, Rasul KS, Rahim D, Mustafa KM, Sleman S, Mirza A and Aziz R. 2023. Assessment of genetic variation and population structure in Iraqi barley accessions using ISSR, CDDP, and SCoT markers. *Czech J Genet Plant Breed.*, **59(3)**: 148–59.

Tahir NA-R. 2008. Assessment of genetic diversity among wheat varieties in Sulaimanyah using random amplified polymorphic DNA (RAPD) analysis. *Jordan J Biol Sci.*, **1(4)**: 159–64.

Tahir NA-R, Ahmad NS, Mustafa KM and Kareem DDL. 2021. Diversity maintenance of some barley (*Hordeum* spp) genetic resources using SSR-based marker. *J Anim Plant Sci.*, **1(1)**: 221–34.

Tahir NA-R and Karim HFH. 2011. Determination of genetic relationship among some varieties of chickpea (*Cicer arietinum* L.) in Sulaimani by RAPD and ISSR markers. *Jordan J Biol Sci.*, **4(2)**: 77–86.

Titilawo MA, Faseun AO, Akinde SB, Olaitan JO and Odeyemi O. 2022. Bioactivity of four Nigerian wild mushrooms against some typed clinical isolates. *Jordan J Biol Sci.*, **15(1)**: 67–73.

Vieira Junior WG, Centeio Cardoso RV, Fernandes Â, Ferreira ICFR, Barros L, Pardo-Giménez A, Mendel Soares DM, Stevani CV and Zied DC. 2022. Influence of strains and environmental cultivation conditions on the bioconversion of ergosterol and vitamin D2 in the sun mushroom. *J Sci Food Agric.*, **102(4)**: 1699–706.

Yuwa-Amornpitak T, Butkhup L and Chookietwattana P-NY. 2023. Cultivation of edible ectomycorrhizal mushrooms (*Phelobopus portentosus*) associated with *Sesbania javanica* Miq. *Jordan J Biol Sci.*, **16(1)**: 125–30.

Zhao M-R, Huang C-Y, Wu X-L, Qiang C, Qu J-B, Li Y-C, Wei GAO and Zhang J-X. 2016. Genetic variation and population structure of the mushroom *Pleurotus ferulae* in China inferred from nuclear DNA analysis. *J Integr Agric.*, **15(10)**: 2237–46.

Supplementary data

Figure S1. Wild and cultivated mushrooms species.

Table S1. Name, strains, accessions number, and altitudes of different mushroom samples collected from different locations.

Location of sampling	Number of samples	Code	Identified sample	Strain	Average of altitude (m)	Accession number
Qaladiza	8	S1	*Agrocybe aegerita*	AgA-QAL-1	586	PQ679358
Qaladiza		S2	*Agrocybe dura*	AgD-QAL-2		PQ680084
Qaladiza		S3	*Agrocybe dura*	AgD-QAL-3		PQ680083
Qaladiza		S4	*Agrocybe aegerita*	AgA-QAL-4		PQ680085
Qaladiza		S5	*Agaricus campestris*	AgC-QAL-5		PQ679044
Qaladiza		S6	*Agrocybe dura*	AgD-QAL-6		PQ679043
Qaladiza		S7	*Agaricus campestris*	AgaC-QAL-7		PQ679045
Qaladiza		S8	*Agaricus campestris*	AgaC-QAL-8		PQ679046
Penjwen	8	S9	*Agaricus litoralis*	AgaL-PEN-9	1272	PQ680647
Penjwen		S10	*Agaricus litoralis*	AgaL-PEN-10		PQ680646
Penjwen		S11	*Agaricus litoralis*	AgaL-PEN-11		PQ680649
Penjwen		S12	*Agaricus litoralis*	AgaL-PEN-12		PQ680655
Penjwen		S13	*Agaricus litoralis*	AgaL-PEN-13		PQ680656
Penjwen		S14	*Agaricus litoralis*	AgaL-PEN-15		PQ680668
Dukan	8	S15	*Amanita crocea*	AmC-DUK-17	798	PQ686237
Dukan		S16	*Amanita crocea*	AmC-DUK-18		PQ686016
Dukan		S17	*Amanita lividopallescens*	AmL-DUK-19		PQ685979
Dukan		S18	*Amanita lividopallescens*	AmL-DUK-20		PQ681289
Dukan		S19	*Amanita crocea*	AmC-DUK-21		PQ681290
Dukan		S20	*Pleurotus eryngii*	PlE-DUK-22		PQ681292
Dukan		S21	*Pleurotus eryngii*	PlE-DUK-23		PQ685981

Dukan		S22	*Pleurotus eryngii*	PlE-DUK-24		PQ681296
Basneh	8	S23	*Agaricus litoralis*	AgaL-BAS-25	1573	PQ682616
Basneh		S24	*Agaricus litoralis*	AgaL-BAS-26		PQ682630
Basneh		S25	*Agaricus litoralis*	AgaL-BAS-27		PQ682658
Basneh		S26	*Agaricus litoralis*	AgaL-BAS-28		PQ682661
Basneh		S27	*Agaricus litoralis*	AgaL-BAS-29		PQ682665
Basneh		S28	*Agaricus litoralis*	AgaL-BAS-30		PQ682666
Basneh		S29	*Agaricus litoralis*	AgaL-BAS-31		PQ683183
Basneh		S30	*Agaricus litoralis*	AgaL-BAS-32		PQ685808
Zewe	8	S31	*Amanita crocea*	AmC-ZEW-33	1583	PQ686266
Zewe		S32	*Amanita lividopallescens*	AmL-ZEW-34		PQ686317
Zewe		S33	*Amanita lividopallescens*	AmL-ZEW-35		PQ686318
Zewe		S34	*Amanita crocea*	AmC-ZEW-36		PQ686633
Zewe		S35	*Amanita crocea*	AmC-ZEW-37		PQ686958
Zewe		S36	*Amanita crocea*	AmC-ZEW-38		PQ686964
Zewe		S37	*Pleurotus columbinus*	PlC-ZEW-39		PQ686965
Zewe		S38	*Pleurotus columbinus*	PlC-ZEW-40		PQ687023
Ranya	8	S39	*Agaricus pseudolutosus*	AgaP-RAN-41	882	PQ720781
Ranya		S40	*Agaricus pseudolutosus*	AgaP-RAN-42		PQ720782
Ranya		S41	*Agaricus pseudolutosus*	AgaP-RAN-43		PQ722530
Ranya		S42	*Agaricus pseudolutosus*	AgaP-RAN-44		PQ722533
Ranya		S43	*Amanita vittadinii*	AmV-RAN-45		PQ720786
Ranya		S44	*Amanita vittadinii*	AmV-RAN-46		PQ720789
Ranya		S45	*Amanita vittadinii*	AmV-RAN-47		PQ720788
Ranya		S46	*Agaricus pseudolutosus*	AgaP-RAN-48		PQ722534
Qaradagh	8	S47	*Amanita crocea*	AmC-QAR-49	1708	PQ720780
Qaradagh		S48	*Amanita lividopallescens*	AmL-QAR-50		PQ720981
Qaradagh		S49	*Amanita lividopallescens*	AmL-QAR-51		PQ721015
Qaradagh		S50	*Amanita lividopallescens*	AmL-QAR-52		PQ721021
Qaradagh		S51	*Amanita lividopallescens*	AmL-QAR-53		PQ721023
Qaradagh		S52	*Amanita crocea*	AmC-QAR-54		PQ721027
Qaradagh		S53	*Amanita vittadinii*	AmV-QAR-55		PQ722535
Qaradagh		S54	*Amanita vittadinii*	AmV-QAR-56		PQ721036
Qaradagh		S55	*Agaricus litoralis*	AgaL-QAR-57		PQ721038
Halabja	8	S56	*Amanita vittadinii*	AmV-HAL-58	900	PQ721310
Halabja		S57	*Amanita vittadinii*	AmV-HAL-59		PQ721312
Halabja		S58	*Amanita vittadinii*	AmV-HAL-60		PQ721315
Halabja		S59	*Amanita vittadinii*	AmV-HAL-61		PQ721317
Halabja		S60	*Amanita vittadinii*	AmV-HAL-62		PQ721318
Halabja		S61	*Agaricus litoralis*	AgaL-HAL-63		PQ721319
Halabja		S62	*Agaricus litoralis*	AgaL-HAL-64		PQ721320
Sulaimani Center	1	S63	*Agaricus bisporus*	AgaB-SC-65	882	PQ895553

Table S2 Sequences and annealing temperatures of ITS gene, CDDP and SCoT markers.

Primers	Sequence (5' to 3')	Annealing temperature (°C)
ITS gene		
ITS 1	TCCGTAGGTGAACCTGCGG	55
ITS 4	TCCTCCGCTTA TTGATATGC	55
CDDP markers		
KNOX2	CACTGGTGGGAGCTSCAC	50
WRKYF1	TGGCGSAAGTACGGCCAG	50
WRKY-R3	GCASGTGTGCTCGCC	50
KNOX1	AAGGGSAAGCTSCCSAAG	50
ERF2	GCSGAGATCCGSGACCC	50
MYB1	GGCAAGGGCTGCCGC	50
ERF1	CACTACCCCGGSCTSCG	50
WRKY-R3B	CCGCTCGTGTGSACG	50
MYB2	GGCAAGGGCTGCCGG	50
WRKY-R2B	TGSTGSATGCTCCCG	50
ABP1-1	ACSCCSATCCACCGC	50
MADS-1	ATGGGCCGSGGCAAGGTGC	50
SCoT markers		
SCoT3	CAACAATGGCTACCACCG	51.27
SCoT12	ACGACATGGCGACCAACG	55.93
SCoT13	ACGACATGGCGACCATCG	55.39
SCoT14	ACGACATGGCGACCACGC	58.6
SCoT15	ACGACATGGCGACCGCGA	59.9
SCoT19	ACCATGGCTACCACCGGC	57.1
SCoT20	ACCATGGCTACCACCGCG	57.5
SCoT21	ACGACATGGCGACCCACA	56.7
SCoT23	CACCATGGCTACCACCAG	52.43
SCoT24	CACCATGGCTACCACCAT	51.6
SCoT34	ACCATGGCTACCACCGCA	56.3
SCoT35	CATGGCTACCACCGGCCC	57.9

Table S3. Nei's genetic distance among different species (A) and genera (B) of mushroom samples based on CDDP data.

A

Species	*A. campestris*	*A. litoralis*	*A. pseudolutosus*	*A. aegerita*	*A. dura*	*A. crocea*	*A. lividopallescens*	*A. vittadinii*	*P. columbinus*	*P. eryngii*	*A. bisporus*
A. campestris	0.00										
A. litoralis	0.15	0.00									
A. pseudolutosus	0.20	0.10	0.00								
A. aegerita	0.43	0.30	0.16	0.00							
A. dura	0.35	0.24	0.16	0.09	0.00						
A. crocea	0.23	0.12	0.11	0.30	0.28	0.00					
A. lividopallescens	0.27	0.14	0.23	0.48	0.40	0.21	0.00				
A. vittadinii	0.16	0.07	0.13	0.31	0.27	0.16	0.14	0.00			
P. columbinus	0.28	0.25	0.26	0.42	0.38	0.25	0.34	0.24	0.00		
P. eryngii	0.23	0.16	0.20	0.35	0.32	0.20	0.26	0.16	0.08	0.00	
A. bisporus	0.32	0.26	0.30	0.40	0.35	0.30	0.32	0.27	0.28	0.17	0.00

B

Genus	*Agaricus*	*Agrocybe*	*Amanita*	*Pleurotus*
Agaricus	0.00			
Agrocybe	0.11	0.00		
Amanita	0.03	0.15	0.00	
Pleurotus	0.12	0.21	0.12	0.00

Table S4. Nei's genetic distance among various species (A) and genera (B) of mushroom samples derived from the SCoT data.

A

Species	*A. campestris*	*A. litoralis*	*A. pseudolutosus*	*A. aegerita*	*A. dura*	*A. crocea*	*A. lividopallescens*	*A. vittadinii*	*P. columbinus*	*P. eryngii*	*A. bisporus*
A. campestris	0.00										
A. litoralis	0.14	0.00									
A. pseudolutosus	0.20	0.11	0.00								
A. aegerita	0.31	0.22	0.15	0.00							
A. dura	0.29	0.18	0.16	0.11	0.00						
A. crocea	0.24	0.11	0.17	0.29	0.23	0.00					
A. lividopallescens	0.21	0.09	0.21	0.33	0.28	0.14	0.00				
A. vittadinii	0.20	0.06	0.14	0.26	0.22	0.14	0.10	0.00			
P. columbinus	0.29	0.27	0.29	0.36	0.30	0.32	0.27	0.23	0.00		
P. eryngii	0.27	0.15	0.16	0.25	0.22	0.24	0.21	0.15	0.16	0.00	
A. bisporus	0.30	0.23	0.25	0.33	0.27	0.28	0.32	0.25	0.35	0.17	0.00

B

Genus	*Agaricus*	*Agrocybe*	*Amanita*	*Pleurotus*
Agaricus	0.00			
Agrocybe	0.10	0.00		
Amanita	0.04	0.11	0.00	
Pleurotus	0.11	0.16	0.11	0.00