RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
57 (4) 1535-1544, 2025
http://doi.org/10.54910/sabrao2025.57.4.19
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

MOLECULAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF WILD MUSHROOMS IN SULAIMANI PROVINCE, IRAQ

A.SH. ABDALRAHMAN[1*] and S.Q. SULIAMAN[2]

[1]Department of Maternity and Obstetric Nursing, Sulaimani Technical Institute, Sulaimani Polytechnic University, Sulaimani, Iraq
[2]Department of Biology, College of Science, Tikrit University, Tikrit, Iraq
*Corresponding author's email: adiba.sharif@spu.edu.iq
Email address of co-author: saraqahtan@tu.edu.iq

SUMMARY

Accurate species-level identification of mushrooms is essential due to the extensive diversity in their morphology and ecological characteristics. Consequently, this study utilized the amplification of the internal transcribed spacer (ITS) region via polymerase chain reaction (PCR) to facilitate the molecular identification of mushroom species. The rDNA-ITS (ribosomal DNA internal transcribed spacer) region of genomic DNA from 10 wild mushroom accessions collected in Sulaimani Province, Iraq, succeeded amplification using ITS1 and ITS4 primers. The PCR amplification products ranged in size from 680 to 800 bp and underwent comparison with sequences in the National Center for Biotechnology Information (NCBI) database. When comparing with GenBank data, BLAST (Basic Local Alignment Search Tool) analysis revealed the identified mushrooms had sequence identities ranging from 93.8% to 100%. All identified mushroom accessions were at the species level, with all being newly documented species in Iraq. These include *Volvariella bombycina* (PP921334.10), *Collybia nuda* (PP921333.1), *Amanita crocea* (PP921336.1), *Melanoleuca rasilis* (PP921332.1), *Stropharia coronilla* (PP921331.1), *Amanita lividopallescens* (PP410315.1), *Macrolepiota orientiexcoriata* (PP410313.1), and *Phallus hadriani* (PP410312.1). Two of them (PP921329.1 and PP921330.1) belonged to the same species, *Melanoleuca leucopoda*. Phylogenetic analysis revealed a close evolutionary relationship among the identified mushrooms. This work discovered the originality of the mushroom species, which expands the Iraqi mushroom diversity.

Keywords: Wild mushrooms, species, internal transcribed spacer (ITS), macrofungi, phylogenetic analysis, rDNA-ITS, evolutionary relationship

Communicating Editor: Dr. Irma Jamaluddin

Manuscript received: January 05, 2025; Accepted: March 04, 2025.

Citation: Abdalrahman ASH, Suliaman SQ (2025). Molecular identification and phylogenetic analysis of wild mushrooms in Sulaimani Province, Iraq. *SABRAO J. Breed. Genet.* 57(4): 1535-1544. http://doi.org/10.54910/sabrao2025.57.4.19.

Key findings: The molecular identification of wild mushrooms by internal transcribed spacer (ITS1 and ITS4 primers) facilitated precise taxonomic categorization and successfully distinguished the closely related species. Phylogenetic analysis elucidated genetic links, offering evolutionary insights and accurately confirming species identification.

INTRODUCTION

Macrofungi are fungi that produce visible fruiting bodies, with primary classifications of the phyla Basidiomycota and Ascomycota. These fungi's growth can be on several substrates, including soil surfaces, leaf litter, twigs, and both living and decaying wood (Ramadhani *et al.,* 2019). Mushrooms secrete various enzymes essential for their growth on diverse substrates, in addition to environmental factors. The enzymes include lignin peroxidases, quinone reductases, dehydrogenases, xylanases, cellulases, cellobiose dehydrogenases, and laccases (Carrasco *et al.,* 2018). Macrofungi exhibit a global distribution, with each species of mushroom occupying a distinct ecological niche conducive to its growth. Puffballs, bracket fungi, gilled fungi, and stinkhorns represent various types of macrofungi, with categories that may be edible, non-edible, or poisonous. (Appiah *et al.,* 2017).

Approximately 1.5 million species of fungi have current recognition globally, with total estimates varying between 3.5 and 5.1 million species. Fungi constitute the second biggest biotic group following insects, with an estimated 2.8 to 3.8 million species. At present, the discovery of 149,974 species of macrofungi has been successful, comprising 41,000 species categorized within basidiomycetes and ascomycetes. Numerous fungal species remain hidden within intricate taxonomies, with many others still undiscovered (Zeb *et al.,* 2023).

Mushrooms provide numerous benefits, including ease of cultivation, contributions to bioremediation and biodegradation, generation of extracellular enzymes, and the creation of nutraceutical characteristics. *Pleurotus* species, sometimes known as oyster mushrooms, are edible fungi widely grown worldwide, especially in Southeast Asia, India, Africa, and Europe. Oyster mushrooms are the world's third most commercially farmed fungus (Mala *et al.,* 2023). Given many types of mushrooms grow on fallen lumber, bark, and sapwood, very few assault living trees. Mycelium from mushrooms gradually breaks down wood, improving the soil in forests and supplying nutrients for healthy trees. As they break down decaying organic materials through a series of saprophytic fungi, mushrooms are essential to natural ecosystems (Kakraliya, 2020). Mushrooms have been beneficial as sustenance and medicinal agents since ancient times. Their importance in human health and nutrition is due to several pharmacological qualities, such as anticancer, antibacterial, antiviral, immune-stimulating, and blood lipid-lowering actions (Çayan *et al.,* 2021; Ambhore *et al.,* 2024). Moreover, mushrooms serve as a significant food source, supplying all nine essential amino acids necessary for human health: methionine, phenylalanine, leucine, lysine, isoleucine, tryptophan, valine, serine, and threonine (Nagy *et al.,* 2017).

Several studies have investigated macrofungi in Iraq and isolated mushrooms from mountainous regions in the Sulaimani and Erbil provinces, identifying 34 species belonging to 23 genera of woody and fleshy species of basidiomycetes (Aziz and Toma, 2012). Reports disclosed seven genera, six families, and two orders of basidiomycetous macrofungal species in Iraqi Kurdistan. These species include *Inocybe flocculosa, Pleurotus nebrodensis, Psathyrella spadiceogrisea, Schizophyllum commune*, *Volvopluteus gloiocephalus* (Agaricales), *Lentinus tigrinus,* and *Trametes trogii* (Polyporales) (Suliaman *et al.,* 2017). A study, conducted in the Amadiyah, Erbil, and Sulaimani districts of Northeast Iraq during 2015 and 2016, detected 14 taxa of basidiomycetous macrofungi (Agaricomycetes) (Al-Khesraji *et al.,* 2018). The ITS1 and ITS4 primers and morphological

data proved capable of identifying several mushroom types in the Basrah Province, Iraq (Alrubayae *et al.,* 2022).

Fungi exhibit considerable diversity in size, shape, and color, ranging from microscopic single-celled yeasts to complex multicellular mushrooms and molds. Identification based solely on morphological characteristics can be challenging due to phenotypic plasticity and genetic variability, which may lead to misidentification (Tekpinar and Kalmer, 2019). In overcoming this limitation, DNA barcoding has emerged as a highly effective tool for species-level identification in fungi. DNA sequences serve as a primary data source for species identification across all organisms, with the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (rDNA) being the most commonly used marker for accurate fungal classification (Dulla *et al.,* 2016). Consequently, macrofungi's reliable identification can use molecular techniques, such as sequence analysis and polymerase chain reaction (PCR).

The ITS region is a non-functional RNA sequence found within a common precursor transcript, situated among structural ribosomal RNAs. It remains highly conserved within the same species but varies between different species. Given its significant diversity among closely related species and its ease of amplification from small DNA samples, the ITS region is a widely used tool in taxonomic and phylogenetic analysis (Surawut *et al.,* 2021). Alongside the ITS region, many molecular markers employed for fungal identification include β-tubulin (TUB2), TEF1-α (translation elongation factor 1-alpha), RPB1 and RPB2 (RNA polymerase II subunits), LSU (28S rRNA), and SSU (18S rRNA). The ITS region, universally found in fungus, comprises both conserved and varied sequences, rendering it extensively utilized and prominently represented in databases, such as the NCBI GenBank. Nevertheless, ITS has constraints; for deep phylogenetic research, LSU, SSU, or RPB1/RPB2 may be more appropriate, while TEF1-α and β-Tubulin offer greater precision for the identification of *Fusarium* and *Aspergillus* (Lücking *et al.,* 2020). In this study, the use of ITS-PCR proceeded to investigate the molecular identification and phylogenetic analysis of wild mushrooms in Sulaimani Province, Iraq. Therefore, the presented study aimed to provide the accurate species identification and explore the knowledge about the mushroom taxonomy.

MATERIALS AND METHODS

Sample collection

Wild mushrooms randomly collected came from various regions in Sulaimani Province, Iraq. The said province covers over 1,200 km² in Northern Iraq (34° 42′ and 36° 35′ N, 44° 55′ and 46° 15′ E). The mushroom samples' harvest occurred during the rainy season, from November 2022 to May 2023. The growth habit, habitat, collection place, date, surrounding vegetation, and host plant species of ectomycorrhizal fungi preceded their recording in a field notebook. Gathering mature fruiting bodies of each species at different developmental phases helped show size and color variations. Photographs of mushrooms taken in their natural habitat ensued. Preserving specimen quality requires careful treatment after collection. Wax paper or aluminum foil can protect and preserve mushrooms. Cut paper or foil to the right size to roll the specimen inside and twist the ends to seal it. After wrapping, the item's delivery to the lab was in a robust box, bag, or basket. All the accession samples remained in labeled plastic bags before promptly transporting to the laboratory, then, kept in a refrigerator at 4 °C until further processing. The collected mushrooms obtained labels as E3, I1, B9, B16, I6, I3A, F1, B2, E4, and C2.

DNA extraction

Following the manufacturer's instructions, the fruiting bodies of the collected mushroom accessions received drying in an oven at 40 °C to extract the DNA using the AddPrep Genomic DNA extraction kit (www.addbio.net). The 10–20 mg of dried fruit bodies sustained grinding by using a mortar, followed by transferring into

a microcentrifuge tube (1.5 ml in size). Adding the 20 µl of proteinase K solution (20 mg/ml) and 200 µl of lysis solution to the tube continued. The mixture then underwent pulse-vortexation and incubation at 65 °C for 30 min, with inverting the tube 2–3 times during the incubation period to facilitate the lysis process. After centrifuging and mixing 100 µl of the precipitation solution into the sample tube, the transfer of 200–300 µl of the supernatant transpired to a 1.5 ml micro-centrifuge tube. Subsequently, adding 200 microliters of binding solution and 200 microliters of absolute ethanol incurred thorough mixing and centrifugation for one minute at 13,000 rpm. Following two rounds of washing with washing solutions 1 and 2, the samples' centrifugation took place for one minute at 13,000 rpm after each wash. After drying, the introduction of a microcentrifuge tube containing 100 µl of elution solution followed to the spin column. Centrifugation helped to elute the genomic DNA for one minute at 13,000 rpm. Then eluted DNA remained at -20 °C until further use.

Amplification of the ITS region

The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) was the most common region for amplification in the sample DNA. A primer set consisting of ITS1 (5´TCCGTAGGTGAACCTGCGG-3´) as a forward primer, and ITS4 (5´TCCTCCGCTTATTGATATGC-3´) as the reverse primer, were the universal primers for fungi (Sulaiman *et al.,* 2024).

The PCR reactions, carried out in a total volume of 50 µL, had each reaction consisting of 4.0 µL of genomic DNA, 2.0 µL of each primer (ITS1 and ITS4), 0.5 µL of Taq DNA polymerase reaction, 25 of master mix (HSTM Kit) containing requirements for PCR reaction, and 16.5 µL of nuclease-free water. DNA amplification proceeded in a thermocycler. The initial denaturation step commenced at 95 °C for 2 min. Thereafter, there were 35 cycles of denaturation lasting 1 min at 95 °C, annealing for 45 s at 55 °C, and extension lasting 1 min and 30 s at 72 °C. The last extension took place for 5 min at 72 °C (Alrubayae *et al.,* 2022).

The PCR products' analysis employed electrophoresis, with subsequent running in agarose gel (1%). Five microliters (5 µl) of the PCR products, along with 5 µl of 100 bp DNA ladder, succeeded in loading into the agarose gel wells. The gel, as immersed in TBE buffer (0.09 M Tris, 0.09 M boric acid, 2 mM EDTA, and pH 8.3), sustained a run at 90 volts for 45 min. The gel's subsequent visualization proceeded in photography and submission in the gel documentation system.

DNA sequencing and phylogenetic analysis

The primers used for PCR amplification also served to sequence the PCR products before submitting to Macrogen, Inc., Korea. Sanger DNA sequencing took place with an Applied Biosystems 3500xl Genetic Analyzer. Ensuring maximum sequence similarity, the study used the BioEdit program for the assembly and analysis of the sequence data. The National Center for Biotechnology Information (NCBI) database, consulted for nucleotide sequence comparisons, used the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST).

In addition to aligning the nucleotide sequences, the same sequences were applicable for phylogenetic analysis. A resulting optimal tree, with a branch length sum (31.85435830), manifested. Phylogenetic trees are assumptions based on evolutionary distances among the branches. The distances, as expressed in base substitutions per site, reached calculations using the maximum composite likelihood technique, with expressions as the quantity of base substitutions made at each site. Nineteen nucleotide sequences attained analysis, including noncoding sections and the first, second, and third codon positions. The final dataset comprised 509 locations overall, after gaps and missing data succeeded removal. The use of MEGA6 helped carry out the evolutionary study (Tamura *et al.,* 2013).

RESULTS AND DISCUSSION

Molecular identification of the mushrooms has become increasingly important because it can overcome the limitation of morphological detection and can even identify the incomplete mushroom bodies and characterize the mushrooms (Kaewgrajang *et al.,* 2020). The recognition of mushrooms based exclusively on morphological traits entails numerous uncertainties, especially due to the presence of various cultivated species exhibiting similar morphology within related taxa. Moreover, discrepancies in nomenclature, drying and preservation techniques, geographic provenance, climatic variances, ecological factors, and harvesting periods can all result in morphological variations within the same mushroom species, causing confusion in species identification (Yan *et al.,* 2021). The morphological characteristics of the collected mushroom accessions were beneficial to differentiate them in the context of the current study (Figure 1). Morphological identification is crucial for understanding the evolution of these characters, with frequent employment to categorize fungi at the ordinal and family levels in taxonomic investigations. However, the examination through morphological parameters could be less effective for a more precise classification at the species level (Raja *et al.,* 2017).

DNA barcoding is an effective technique for precise species identification, often resolving closely related and cryptic species that are otherwise difficult to distinguish. Crucially, the inconsistent taxonomy of macrofungi at the species and subspecies levels has attained verification by using the ITS region of nuclear ribosomal DNA, which includes ITS1, ITS2, and ITS4 (Adeniyi *et al.,* 2018). The PCR amplification by using ITS1 and ITS4 primers for 10 mushroom accessions yielded products ranging from 680 to 800 bp (Figure 2). The mushroom accessions, E3, B9, I3A, F1, B2, E4, and C2, produced an amplified band of about 800 bp, while the accessions I1 and I6 had a 700 bp band, and the accession B16 yielded an amplified band of approximately 680 bp. The presented results contradicted the findings of Alrubayae *et al.* (2022), who reported PCR product size between 700 and 1000 bp. De-Leon *et al.* (2013) demonstrated that analysis of the ITS sequence of seven wild edible mushrooms has a PCR product of about 740 bp. These variations could be due to differences in the size of the ITS across the different fungal species, as well as variations in the DNA quality (Lorenz, 2012).

The precise genetic code of the mushrooms resulted from the PCR results undergoing a process for nucleotide sequencing. These DNA sequences continued subsequent comparisons to known sequences in the GenBank databases. Analysis using the BLAST program revealed the isolates shared sequence similarities ranging from 93.8% to 100% with entries in the NCBI database. Ten mushrooms' recognition used the aligned sequences (Table 1). Among these, I3A and F3 exhibited 100% identity with the *Volvariella bombycina* (PP921334.10) and *Collybia nuda* (PP921333.1), respectively. The accessions, E3, I6, B2, E4, and C2, have the 99% identity belonging to *Melanoleuca leucopoda* (PP921330.1), *Amanita crocea* (PP921336.1), *Melanoleuca rasilis* (PP921332.1), *Stropharia coronilla* (PP921331.1), and *Melanoleuca leucopoda* (PP921329.1), respectively. Each of *Amanita lividopallescens* (PP410315.1), *Macrolepiota orientiexcoriata* (PP410313.1), and *Phallus hadriani* (PP410312.1) appeared with different identities (98.3%, 93.85%, and 99.3%). Past studies enunciated that macrofungi totaling 180 gained discovery through the ITS region, exhibiting identity between 98% and 100%, with 47% categorized as macrofungi species *Trametes* and *Ganoderma*. However, although this study took place in a neighboring country, Iran, none of the identified species was identical to those found in our study (Alimadadi *et al.*, 2019). The ITS region used to identify three accessions of the genus *Ganoderma* collected in Mosul, Iraq (Abdul-Hadi *et al.*, 2022), revealed their findings indicated *Ganoderma resinaceum* had 99.85% identity, *Ganoderma applanatum* had 100% identity, and *Ganoderma sp.* had 99.84% identity. None of their findings agreed with our study.

Figure 1. Images for different mushroom species. A = *Amanita lividopallescens,* B = *Melanoleuca leucopoda,* C = *Volvariella bombycina,* D = *Stropharia coronilla,* E = *Amanita crocea,* F = *Phallus hadriani,* G = *Macrolepiota orientiexcoriata,* H = *Collybia nuda,* I = *Melanoleuca rasilis,* and J = *Melanoleuca leucopoda.*

Figure 2. Agarose gel electrophoresis of amplified DNA sequences. M = DNA ladder (100 bp); lanes (1 = E3, 2 = I1, 3 = B9, 4 = B16, 5 = I6, 6 = I3A, 7 = F1, 8 = B2, 9 = E4, and 10 = C2).

Table 1. Blast results of the identified mushroom species.

Accession code	Scientific name	Identity (%)	Accession numbers	Compared sequence
E3	*Melanoleuca leucopoda*	99	ID: PP921330.1	ID: OR364588.1
I1	*Amanita lividopallescens*	98.3	ID: PP410315.1	ID: NR_173158.1
B9	*Macrolepiota orientiexcoriata*	93.8	ID: PP410313.1	ID: NR_119833.1
B16	*Phallus hadriani*	99.3	ID: PP410312.1	ID: NR_119579.1
I6	*Amanita crocea*	99	ID: PP921336.1	ID: MF278768.1
I3A	*Volvariella bombycina*	100	ID: PP921334.1	ID: HM562212.1
F1	*Collybia nuda*	100	ID: PP921333.1	ID: MT644922.1
B2	*Melanoleuca rasilis*	99	ID: PP921332.1	ID: LT594154.1
E4	*Stropharia coronilla*	99	ID: PP921331.1	ID: PP085448.1
C2	*Melanoleuca leucopoda*	99	ID: PP921329.1	ID: OR364588.1

In this study, identifying 10 mushroom species constitutes a new record, as Iraq has no previous literature documenting such mushroom identification. This finding shows the region's biological variety and suggests the possibility of identifying new mushroom species in Sulaimani Province and Iraq. Several environmental factors, including humidity, temperature, and soil type, are crucial for the optimal growth of macrofungi, which typically prevail in forested areas (De-Leon *et al.,* 2013). The climate of Sulaimani Province closely aligns with the Irano-Turanian type, characterized by cold, snowy winters and long, warm, and dry summers, with brief autumn and spring seasons. The region receives an average annual rainfall ranging from 600 to 1000 mm. Sulaimani has a predominant cover of oak forests with good stocking density, mostly unexploited, and interspersed with *Pinus brutia* in some areas (Khwakaram, 2009). The interplay of wet seasons, diverse tree species, and organic-rich soils creates an ideal environment for numerous mycorrhizal and saprophytic mushrooms. Sulaimani Province has the highest mushroom diversity, with several scientific studies on the identification of macrofungi having reached publication. However, most of these investigations depended on morphological characterization (Suliaman *et al.,* 2017; Toma *et al.,* 2018).

The evolutionary relationship revealed a close association between *Amanita crocea* from Iraq and the same species from the database (99.8%) (Figure 3). Environmental factors, such as nutrition availability, temperature, and light, can influence mushroom development and distribution; nonetheless, their genetic makeup is typically consistent throughout places (Sher *et al.,* 2010). This species is a newly recorded one in the fungal flora of Iraq, with a known occurrence in North America, Europe, and Morocco (Haimed *et al.,* 2021). Similarly, the species *Melanoleuca leucopoda* in Iraq showed 99.55% identity with the species *Melanoleuca leucopoda* GenBank sequences and clustered with it. *Melanoleuca leucopoda* is a globally rare mushroom species, previously recorded only in China (Shurong *et al.,* 2022). Its identification is a significant finding for the new taxa of Iraq. Its discovery in this region is a remarkable range of expansion, implying that the species' distribution could be more widespread than previously thought. This discovery advances our understanding of mushroom biodiversity in Iraq. Species forming a distinct cluster on the phylogenetic tree, such as *Phallus hadriani,* formed a cluster with matched sequences, with a bootstrap value of 99.62% (Figure 3). The same results were also outcomes recorded by Melanda *et al.* (2021).

Similarly, the species *Volvariella bombycina* in Iraq clustered with the species *Volvariella bombycina* from Spain, with a bootstrap value of 100%, indicating a significant relationship among these mushroom accessions. However, *Volvariella bombycina* is a new record species in Iraq, although it has a global distribution, with previous recordings in Morocco, as noted by Ajana *et al.* (2017). Species *Stropharia coronilla* emerged quite different from the reference sequence in the GenBank data, although the identity among them was 99%. However, the said isolate did

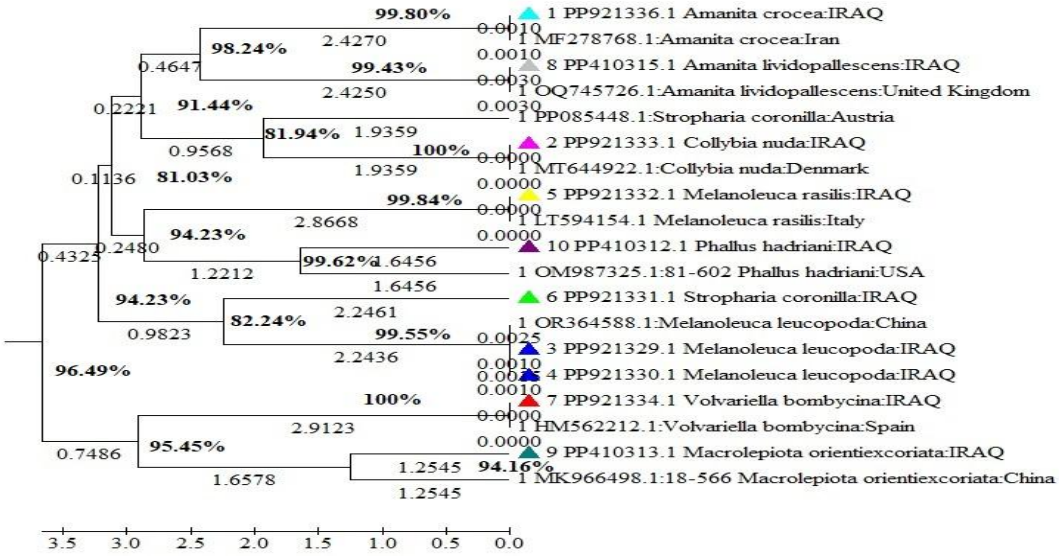

Figure 3. Phylogenetic tree showing the relationship among the wild mushroom species.

not cluster with the reference taxa from the GenBank. This could refer to the distinct nucleotide signature and may be due to geographical distribution, environmental factors, and historical events (Adeniyi *et al.,* 2018). Overall, the presented results provided a close relationship among the identified mushroom species with the global reference taxa. Additionally, their evolutionary relationship provides further exploration for studying the active biochemical compounds.

CONCLUSIONS

Molecular identification of wild mushrooms is a reliable method for overcoming the limitations of morphological identification, providing accurate species recognition and insights into the evolutionary relationships among various species. Using the ITS region helped identify 10 mushroom species in Sulaimani Province, representing new records for the region and Iraq. It contributed to the country's discovery of novel taxa and enhanced knowledge of mushroom biodiversity. Phylogenetic analysis improves our understanding by uncovering the crucial relationships between mushroom species and their evolutionary histories. Continued research on mushrooms is vital in developing a comprehensive checklist of macrofungi in Iraq.

ACKNOWLEDGMENTS

The authors highly thank the University of Tikrit's College of Science-Biology Department and Sulaimani University's College of Medicine-Biology Laboratory for providing all the available facilities to perform this study.

REFERENCES

Abdul-Hadi SY, Owaid MN, Gergees RN, Al-Bayyar AH (2022). Molecular identification of three novel species of *Ganoderma* from different habitats in Mosul, Iraq. *Songklanakarin J. Sci. Technol.* 44(3): 794–802 10.14456/sjst-psu.2022.106.

Adeniyi M, Titilawo Y, Oluduro A, Odeyemi O, Nakin M, Okoh AI (2018). Molecular identification of some wild Nigerian mushrooms using internal transcribed spacer: Polymerase chain reaction. *AMB Expr.* 20 (8):148. https://doi.org/10.1186/s13568-018-0661-9.

Ajana M, Ouabbou A, El-kholfy S, Nmichi A, Ouazzani TA, Benkirane R, Douira A (2017). Some new observations on the *Volvariella* genus Speg. 1898. *Int. J. Environ. Agric.*

Biotechnol. 2(2): 940–945. http://dx.doi.org/ 10.22161/ijeab/2.2.48.

Alimadadi N, Pourianfar HR, Amoozegar MA, Zabihi SS, Mahdizadeh V, Shahzadeh FSA (2019). Molecular identification of some wild medicinal macrofungi from Northern Iran. *Stud. Fungi* 4(1): 26–36. https://doi.org/10.5943/sif/4/1/4.

Al-Khesraji TO, Suliaman SQ, Hassan AA (2018). First record of fourteen basidiomycetous macrofungi (Agaricomycetes) from Iraq. *Int. J. Curr. Res. Biosci. Plant Biol.* 5(6): 25–44. https://doi.org/10.20546/ijcrbp.2018.506.003.

Alrubayae IMN, Maktoof SA, Mansour NA (2022). Molecular and morphological identification of wild mushrooms isolated in Basrah Province with the global recording of new strains. *J. Ichthyol*. 1: 320–333. http://www.ijichthyol.org.

Ambhore JP, Adhao VS, Rafique SS, Telgote AA, Dhoran RS, Shende BA (2024). A concise review: Edible mushroom and their medicinal significance. *Expl. Food Foodomics.* 2(3): 183–194. https://doi.org/10.37349/ eff.2024.00033.

Appiah T, Agyare C, Luo Y (2017). Molecular identification of some Ghanaian mushrooms using internal transcribed spacer regions. *Mol. Biol.* 06(03): 191. https://doi.org/ 10.4172/2168-9547.1000191.

Aziz FH, Toma FM (2012). First observations on the mushroom in mountain area of Iraqi Kurdistan region. *Adv. in BioSci*. 3(4): 302–312.www.sospublication.co.in.

Carrasco J, Zied DC, Pardo JE, Preston GM, Pardo-Giménez A (2018). Supplementation in mushroom crops and its impact on yield and quality. *AMB Expr.* 8:146. https://doi.org/ 10.1186/s13568-018-0678-0.

Çayan F, Tel-Çayan G, Deveci E, Duru ME (2021). A comprehensive study on phenolic compounds and bioactive properties of five mushroom species via chemometric approach. *J. Food Process Preserv.* 45(9): 15695–15706, https://doi.org/10.1111/ jfpp.15695.

De-Leon A, Luangsa-ard J, Karunarathna S, Hyde K, Reyes R, Dela CT (2013). Species listing, distribution, and molecular identification of macrofungi in six Aeta tribal communities in Central Luzon, Philippines. *Mycosphere* 4(3): 478–494. https://doi.org/10.5943/ mycosphere/4/3/4.

Dulla EL, Kathera C, Gurijala HK, Mallakuntla TR, Srinivasan P, Prasad V, Mopati RD, Jasti PK (2016). Highlights of DNA barcoding in identification of salient microorganisms like fungi. *J. Mycol. Med.* 26(4): 291–297. https://doi.org/10.1016/j.mycmed.2016.05.006.

Haimed M, Elmadhi Y, Attrassi K (2021). Contribution to a monograph of *Amanita* in Morocco. *Int. J. Recent Sci. Res.* 12 (7): 42227–42231. doi: http://dx.doi.org/10.24327/ijrsr.2021.1207.6051.

Kaewgrajang T, Kaewjunsri S, Jannual N, Nipitwattanaphon M (2020). Morphological and molecular identification of some *Lactarius* and *Russula* species. *Genomics Genet*. 13(2&3): 44–58. https://doi.org/10.14456/gag.2020.6.

Kakraliya SS (2020). Economic importance of mushroom and their uses. *Agriculture* 1(3). www.justagriculture.in.

Khwakaram AI (2009). Phytoremediation of wastewater using some of aquatic macrophytes as biological purifiers for irrigation purposes. Ph.D. Dissertation, Univ. of Sulaimani, Iraq.

Lorenz TC (2012). Polymerase chain reaction: Basic protocol plus troubleshooting and optimization strategies*. J. Vis. Exp.* (63): e3998. https://doi.org/10.3791/3998.

Lücking R, Aime MC, Robbertse B, Miller AN, Ariyawansa HA, Aoki T, Cardinali G, Crous PW, Druzhinina IS, Geiser DM, Hawksworth DL, Hyde KD, Irinyi L, Jeewon R, Johnston PR, Kirk PM, Elaine M, May TW, Meyer W, Maarja O, Vincent R, Stadler M, Thines M, Vu D, Yurkov AM, Zhang N, Schoch CL (2020). Unambiguous identification of fungi: Where do we stand and how accurate and precise is fungal DNA barcoding? *IMA Fungus* 11:14. https://doi.org/10.1186/ s43008-020-00033-z.

Mala A, Kamal S, Attri BL (2023). A Review: Health benefits and quality value of mushroom. *IJFMR*. 5 (4). https://doi.org/10.36948/ ijfmr.2023.v05i04.4841.

Melanda GCS, Silva-Filho AGS, Lenz AR, Menolli N, de Lima AA, Ferreira RJ, Assis NM, Cabral TS, Martín MP, Baseia IG (2021). An overview of 24 years of molecular phylogenetic studies in *Phallales* (Basidiomycota) with notes on systematics, geographic distribution, lifestyle, and edibility. *Front. Microbiol.* 12: 689374. https://doi.org/10.3389/fmicb.2021.689374.

Nagy M, Semeniuc CA, Socaci SA, Pop CR, Rotar AM, Sălăgean CD, Tofana M (2017). Utilization of brewer's spent grain and mushrooms in fortification of smoked sausages. *Food Sci. Technol. Campinas* 37(2): 315–320. http://dx.doi.org/10.1590/1678-457X. 23816.

Raja HA, Miller AN, Pearce CJ, Oberlies NH (2017). Fungal identification using molecular tools: A primer for the natural products research community. *J. Nat. Prod.* 80(3): 756–770. https://doi.org/10.1021/acs.jnatprod.6b01085.

Ramadhani I, Idris, Masrukhin, Nurcahyanto D, Setiawan R, Ikhwani A, Elfirta R (2019). Isolation and identification of macrofungi from Gunung Halimun Salak National Park. *J. Mikol. Indones.* 3(2): 104–117. https://www.researchgate.net/publication/341657425.

Sher H, Al-Yemeni M, Bahkali AHA, Sher H (2010). Effect of environmental factors on the yield of selected mushroom species growing in two different agro ecological zones of Pakistan. *Saudi J Biol Sci.* 17(4): 321–326. https://doi.org/10.1016/j.sjbs.2010.06.004.

Shurong W, Shuqin LS, Junlong M, Mingchang C, Xiaodan Y (2022). Two new species of *Melanoleuca* from Taihang Mountains in Shanxi Province. *Mycosystema* 41(12): 1921–1931. https://doi.org/10.13346/j.mycosystema.220244.

Sulaiman SQ, al Anbagi RA, Alshuwaili FRH, Abdalrahman AS (2024). New records of three basidiomycetous species from Iraq using phenotypic and phylogenetic analyses. *Afr. J. Plant Sci.* 11(4): 1047–1054. https://doi.org/10.14719/pst.4416.

Suliaman SQ, AL-Khesraji TO, Hassan AA (2017). New records of basidiomycetous macrofungi from Kurdistan region - Northern Iraq. *Afr. J. Plant Sci.* 11(6): 209–219. https://doi.org/10.5897/ajps2017.1543.

Surawut S, Nak-eiam S, Kunsook C, Kamhaengkul L, Kanjanavas P, Yasawong M (2021). Diversity and the molecular identification of some ascomycetes macrofungi found in the para rubber plantation. *J. Biochem. Technol.* 12(4): 50–56. https://doi.org/10.51847/mqnb3fkvwb.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30(12): 2725–2729. doi: 10.1093/molbev/mst197.

Tekpinar AD, Kalmer A (2019). Utility of various molecular markers in fungal identification and phylogeny. *Nova Hedwigia* 109(1): 187–224. https://doi.org/10.1127/nova_hedwigia/2019/0528.

Toma FM, Ismael HM, Faqi Abdulla NQ (2018). Survey and identification of some new record mushrooms in Erbil governorate-Kurdistan region-Iraq. *Raf. J. Sci.* 27(4): 19–32. doi: 10.33899/rjs.2018.159365.

Yan Z, Liu H, Li J, Wang Y (2021). Application of identification and evaluation techniques for edible mushrooms: A review. *Crit. Rev. Anal. Chem.* 53(3): 634–654. https://doi.org/10.1080/10408347.2021.1969886.

Zeb M, Ullah A, Ullah F, Haq A, Ullah I, Badshah L, Haq MA (2023). Diversity and biological characteristics of macrofungi of district Bajaur, a remote area of Pakistan in the Hindu Kush range. *Heliyon* 9(7). https://doi.org/10.1016/j.heliyon.2023.e17818.