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ORIGINAL ARTICLE

INVESTIGATION OF MACROFUNGI IN THE MIDDLE OF IRAQ

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ABSTRACT

The study aimed to survey mushroom species from fields among herbs, palm trunks, and trees in central Iraq and to identify them on the basis of morphological and molecular characteristics. As a molecular identification result with polymerase chain reaction six species were recorded (with eight isolates): *Agaricus bitorquis* (Quéllt) Saccardo. 1887 (SHA14); *Candolleomyces candolleanus* (Fr.) D. Wächter & Melzer, 2020 (SHA15); *Cyclocybe cylindracea* (D.C.) Vizzini & Angelini, 2014 (SHA13); *Leucoagaricus hesperius* Vellinga, 2010 (SHA16); *Volvariella* sp. (SHA17), and *Volvopluteus gloiocephalus* (D. C.) Vizzini, Contu & Justo, 2011 (SHA10, SHA101 and SHA12), belonging to four families of Basidiomycetes: Agaricaceae, Pluteaceae, Psathyrellaceae, and Strophariaceae within the order Agaricales. The nuclear sequences of these species have been deposited in GenBank under the accession numbers: PP576693, PP578967, PP576221, PP576960, PP580694, PP575891, PP580693, and PP576069, respectively. These species were recorded for the first time in these areas in the middle of Iraq.

Keywords: Agaricales, Agaricomycetes, Basidiomycetes, Macrofungi, Mushroom.

INTRODUCTION

Living organisms on earth are incredibly diverse, including fungi, plants, animals, and bacteria (Diaz and Malhi, 2022; Al-Joboury and Aliwy, 2023). Nowadays, researchers are focused on obtaining new sources useful to human food and health, with therapeutic effects on certain infectious diseases (Kumar *et al*., 2021; Al Kateeb *et al*., 2022). Fungi are considered a very important food source for many animals and humans, representing some of the largest and most varied organisms with diverse environmental functions and abilities (Kakon *et al*., 2012; Kumar *et al*., 2021). Mushrooms are a multipurpose food source and are frequently low in calories and sodium while rich in essential amino acids and potassium, making them useful for heart health and suitable for people with high blood pressure (Erbiai *et al*., 2021; Singh, 2023). Environmentally, fungi are decomposers of dead organic materials and recyclers of nutrients in forest ecosystems (Bahram and Netherway, 2021). Agaricomycetes are an important class of fungi, including mushroom forming fungi, reported in various ecosystems and associated with different habitats (Owaid *et al*., 2014; Sulaiman *et al*., 2024). They function in symbiotic relationships with plants, digest organic material, and on rare occasions become pathogens (Hibbett, 2014; Alshuwaili *et al*., 2023). Fungi belonging to this class show the highest level of multicellular diversity in fruiting bodies (Al-Khesraji and Suliaman, 2019).The order Agaricales is considered the largest order in this class and includes 23,000 species distributed globally (Ashraf *et al*., 2016). In Agaricales, species identification necessitates analyzing macroscopic and microscopic features; this is because phenotypic features are frequently absent or similar two species may have the same traits because of evolutionary convergence and close relationships (Schoch *et al*., 2012). The family is represented in almost every part of the world, and different botanists may recognize different numbers of genera. The boundaries between genera are frequently hard to establish (Al-Ashbal *et al*., 2025). Many researchers have estimated the actual biodiversity of fungi around the world. Still, only a small fraction of the fungi inhabiting a respective country has been studied, especially from a molecular point of view, to estimate the actual number of species of mushrooms in ecosystems.In Iraq, many Mushroom-forming members belonging to basidiomycetes have been recorded (Owaid *et al*., 2014; Sulaiman *et al*., 2024). For several reasons, fungi have become a desirable food source and a viable alternative to animal meat. They are highly nutritious, containing 20- 40% protein by dry weight, depending on the species (Kumar *et al*., 2021). Due to the increase in the world's population and the necessity to provide healthy food, the wild agricultural fungi available in Iraq have been of interest because of their high nutritional value (Alhusseini *et al*., 2021). Recent investigations on macrofungi in Iraq has led to the registration of new strains and detailed descriptions (Suliaman, 2023).

In the current study, a survey was conducted in some areas of central Iraq with the aim of identifying species of macrofungi that had not been previously reported in this region.

MATERIALS AND METHODS

Specimens' collection: In the current study, sixty specimens were collected from various regions in central Iraq, including: Zawiyat Al Zaraa Village, located 11.5 km western of Al-Aziziyah District; Western Shadif Village, located 14km Southern of Aziziyah in Wasit Province; Bab AL-Muadham, located in central Baghdad; and Al- Madain District, located 22 km south of Baghdad, across six geographical locations (Map 1, Tab. 1). Their fruiting bodies were present on palm trunks and among grasses on the ground and were collected from multiple areas in central Iraq between January and March 2024. The valid names and synonyms of the species mentioned in this study were adopted from (GBIF Secretariat, 2023), and the distribution data for the species mentioned in this study were adopted according to the method of Al-Joboury and Zurgany (2024). The temperatures at the time of specimens collection ranged between 20-22°C. Using a brush, dust and dirt were removed from the fruiting bodies, in situ, and the ecological data of the fungi, along with the collection date and location, were recorded. Photographs of all specimens were taken in their natural habitats (palm trunks, grasses), as well as in the laboratory.

Map (1): Specimens' collection regions (marked with the symbol ▲).

Table (1): Stations with the coordinates in the study areas.

No	Coordinate of Stations
1	32°53'01.0"N 45°05'31.1"E
2	32°52'16.4"N 45°09'13.4"E
3	32°51'37.3"N 45°03'10.4"E
4	32°51'31.0"N 45°02'09.7"E
5	33°21'15.0"N 44°23'33.9"E
6	33°06'13.8"N 44°35'07.5"E

Morphological identification of mushrooms: The species were identified morphologically depending on characteristics such as fruiting body color, stipe length, presence or absence of a ring and volva, cap diameter, the presence or absence of volva, and scales. Using illustrated field guidebooks, monographs and photographs in addition and previously published studies, macro-fungi were characterized. as well as databases were consulted (Tibuhwa and Kivaisi 2010; Tibuhwa, 2012; Owaid *et al*., 2014; Chelela *et al*., 2015; Muslat *et al*., 2020), macro-fungi have been characterized. Each specimen was documented with photographs showing its growth habit, whether solitary, overlapping, or clustered.

Identification of macro-fungi using polymerase chain reaction (PCR) technology

DNA extraction and amplification: The DNA of all sample species was extracted at Jisr Al-Musayyab Company using a DNA extraction kit (Geneaid Biotech Company with catalog No. GP100 South Korea). DNA amplification was performed using a primer pair that targets the specific sequence of ITS: the universal primers ITS1and ITS4 (White *et al*., 1990). All samples were investigated using polymerase chain reaction (PCR). The PCR protocol included an initial denaturation at 95ºC for 5 min, followed by 35 cycles consisting of secondary denaturation at 95ºC for 30 sec; annealing at 55ºC for 30 sec; and elongation at 72ºC for 1 min, with a final elongation step at 72ºC for 10 min. The PCR products were stored at 4ºC (Sulaiman, 2023).

Sequence analysis of amplified DNA from the macrofungal species: The PCR products of the mushroom species (SHA10, SHA101, SHA12, SHA13, SHA14, SHA15, SHA16, and SHA17) were sent to Macrogene Company in South Koreafor sequencing. Using Mega 11 software, phylogenetic tree was constructed for all of the species *Volvopluteus gloiocephalus* (SHA10, SHA101, SHA12) *Cyclocybe cylindracea* (SHA13), *Agaricus bitorquis* (SHA14), *Candolleomyces candolleanus* (SHA15), *Leucoagaricus hesperius* (SHA16), and *Volvariella* sp. (SHA17) and compared with reference sequences in the GenBank (Tab. 2).

RESULTS

The findings presented in the current study of macrofungi were collected from central Iraq and recorded eight specimens belonging to Agaricomycetes. A total of sixty fungal specimens were were collected in the current study belong to eight isolates and six species, belonged to six genera from four families: Agaricaceae, Psathyrellaceae, Pluteaceae, and Strophariaceae within the order Agaricales (Tab. 3). These species were identified depending on fruiting body morphology. They were observed in different seasons, depending on weather conditions, soil composition, and habitat. The geographical areas from which the samples were collected had a climate with 74-79% humidity and temperatures ranging from 19–32 °C at the time of sample collection. The following details were presented on the classification and characteristics of all species and genera of fungi collected from different areas in the middle of Iraq.

Table (2): The species were reported in the Middle of Iraq for the first time.

Sr. No.	Family	Species
1	**Agaricaceae**	*Agaricus bitorquis* (Quéllt) Saccardo, 1887 (SHA14)
		Leucoagaricus hesperius Vellinga, 1990 (SHA16)
2	**Psathyrellaceae**	*Candolleomyces candolleanus* Wächter and Melzer, 2020 (SHA15)
3	**Strophariaceae**	*Cyclocybe cylindracea* Vizzini and Angelini, 2014 (SHA13)
4	**Pluteaceae**	*Volvopluteus gloiocephalus* Vizzini, Contu and Justo, 2011 (SHA10), (SHA101) and (SHA12)
		Volvariella sp. (SHA17)

Table (3): Molecular similarity ratios for the local macrofungi relative to the global reference sequences received into the NCBI

Sr. no	Species	NCBI Registration No.	Country NCBI Registration No.		Percentage of sequence similarity (%)
1	*A. bitorquis* (Quéllt) Saccardo, 1887 (SHA14)	PP576693	Chine	OQ571893	99.01
2	*C. cylindracea* Vellinga, 1990 (SHA13)	PP576221	Algeria	MT553101	99.78
3	*C. candolleanus* (SHA15)	PP576696	USA	PQ111719	95.19
	L. hesperius Wächter and Melzer 2020 (SHA16)	PP576960	USA	GU139790	96.00
4	*Volvariella* sp. Schäffer 1898 (SHA17)	PP580694	Italy	HM246485	98.00
	V. gloiocephalus Vizzini, Contu and Justo, 2011 (SHA10)	PP575891	Denmark	MT644914	98.95
	V. gloiocephalus Vizzini, Contu and Justo 2011 (SHA101)	PP580693	New Zealand	MN738634	96.36
	V. gloiocephalus Vizzini, Contu and Justo, 2011 (SHA12)	PP576069	Denmark	MT644914	97.79

Morphological identification of mushrooms

Phylum: Basidiomycota

Class: Agaricomycetes

Order: Agaricales

1. Family: Agaricaceae

Agaricus bitorquis (Quél.) Saccardo, 1887 (SHA14)

Habitat: The results showed (Pl. 1 A, B) that this mushroom is found among grasses and near tree trunks. Cap: It is 3.6-1.20 cm broad, white when young, becoming yellowish; convex, and smooth gills; Pink when young, turning dark reddish or brown. Stem with 4.2 cm length, volva absent.

Leucoagaricu hesperius Vellinga, 2010 (SHA16)
This species with a thick litter layer from decomposing tree remains; cap with 7.1-7.3 cm in diameter, round in shape, and becoming convex with age, colors ranges from light brown to beige, covered with fine scales; gills; free and spaced, white initially, then becoming pink with age, stem; 2.5 cm length, cylindrical and somewhat thin, white colored with a thin ring (Pl. 1 C, D).

Plate (1): *A. bitorquis* (SHA14); (A1-A2) fruiting bodies and collection sites near a tree trunk, (B1-B2) fruiting bodies in the laboratory, (C1-C2, D1-D2) fruiting bodies and collection sites of *L. hesperius*. (SHA16) between dry grasses and in the laboratory, respectively.

2. Family: Psathyrellaceae

Candolleomyces candolleanus Wächter & Melzer, 2020 (SHA15)
The results are shown in Plate (2). This mushroom is found among grasses and in woodlands and often grows around stumps and tree roots in lawns and pastures, commonly on decaying wood or nutrient-rich soil. Cap with 7.4-7.9 cm in diameter, light brown when young, starts conical in shape and then becomes rounded with reversed margins at maturity. Gills free, color varies depending on the age. Stipe with 4.8 cm in diameter, cylindrical and similar in color to cap or slightly lighter.

Psathyrella candolleana was the initial name given to this mushroom. For a long time, it was known within the genus *Psathyrella*. It was first recorded in Iraq by Al-Habib *et al*. (2014). However, recent recent investigation, especially genetic analysis, showed that this fungus doesn't fully belong to *Psathyrella* genus. It had been transferred to the genus *Candolleomyces* (Wächter and Melzer, 2020).

Plate (2): *Candolleomyces candolleanus* **(**SHA15**)** on decaying wood or rich soil.

3. Family: Strophariaceae

Cyclocybe cylindracea Vizzini & Angelini, 2014 (SHA13)

Habitat: it found on tree trunks, present as clusters; diameter of the cap 6.5 to 6.7 cm across when fully expanded; shape: they are initially hemispherical, becoming broadly convex or planar and undulating marginally in ordinary conditions; pale buff but flushed mid-brown from the center outwards with age, gills; minimal decurrent or adnate; firstly creamy, becoming grey-brown and then mid-brown colored, stipe; 3 cm long, creamy, becoming brown with age, ring present (Pl. 3). The genus *Cyclocybe* is a source of dietary fiber, amino acids, minerals, and bioactive substances such as vitamins, antioxidants, and β-glucans (Koutrotsios *et al*., 2018). Before molecular studies, *Cyclocybe* was a subgenus of *Agrocybe*. However, according to molecular phylogenetic analysis, *Agrocybe* is polyphyletic, which is why the genus *Cyclocybe* has been revived (e.g., Vizzini *et al*., 2014).

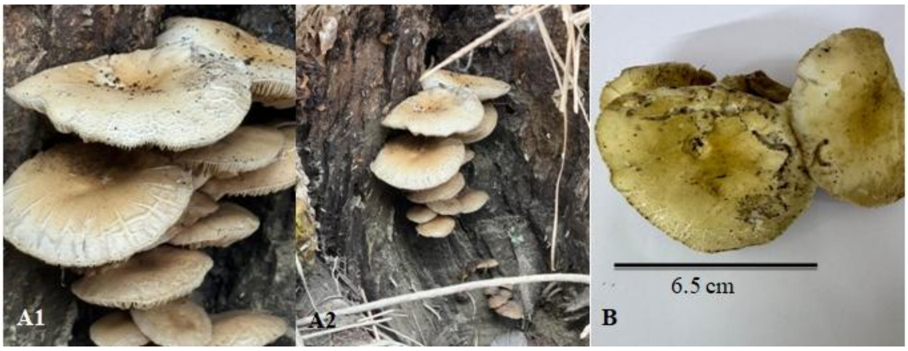

Plate (3): *Cyclocybe cylindracea* (SHA13); (A1-A2) Fruiting bodies on palm trunk, (B) In the lab.

4. Family: Pluteaceae

Volvopluteus gloiocephalus Vizzin, Contu and Justo, 2011 (SHA10, SHA101 and SHA12)
Habitat: Specimens were collected from among grasses (SHA10), woodchip piles (SHA101), and near palm trunks/dry grasses, and leaves (SHA12) (Pl. 4).

Three species, all belonging to the same genus, differ in phenotypic and genetic traits, resulted in their NCBI registration as shown in (Table 1). *V. gloiocephalus* (SHA10), Cap; 11.4-11.6 cm broad, smooth no scale, from yellowish to brown, plano-convex in age and is shown to be sticky and shiny when moist and fresh, randomly streaked; gills; pink to pinkish-brown colored, broad, crowded and thick; stem 6 cm long, short and broad, cylindrical shape, the ring is absent, white colored sometimes yellowish, and has membranous volva, cyst-like appearance at the base. The morphological characteristics are similar to the ones described by Justo *et al*. (2011a). *V. gloiocephalus* (SHA101) Cap; 10.2-10.3 cm broad, convex hat shape, fleshy, creamy to brown colored, streaking with gray to brown radial lines, gills; crowded and thick, whitish to creamy, stem; white broad-stem 4 cm long, cylindrical decreasing slightly to upper, granulated, presence of the ring, volva; white colored at the base. *V. gloiocephalus* (SHA12) cap; 6.2- 6.4 cm long, conical or oval shape, white to greyish, smooth, stem when moist turning to shiny when dry, gills; broad, whitish, close, stem; 3.7 cm long, 1.7 cm thick, volva; membranous, veil absent.

The genus *Volvopluteus* was created to consist of species previously classified as *Volvariella*, characterized by a gelatinous pileipellis and an average basidiospore typically exceeding 11 μm in length (Justo *et al*., 2011b).

Plate (4): *Volvopluteus gloiocephalus*; (A & B) Fruiting bodies and collection sites between grasses and in the lab. of *V. gloiocephalus* SHA10, (C) Fruiting bodies and collection sites between grasses and leaves near the palm trunk and (D1-D2) in the lab. Of *V. gloiocephalus* SHA101, and (E1) fruiting bodies and collection sites between dry grasses and leaves, (E2) in the lab. of *V. gloiocephalus* SHA12.

Volvariella sp. (SHA 17)

Habitat: present among grasses and leaves. Cap; diameter6.4-6.6 cm (not fully spread out), centrally located, densely and finely scaly, creamy to light brown in colour, the surface becomes increasingly loose and finely scaly toward the edge, with a continuous transition from the center of the cap to the edge, gills; broad and thick, creamy to pink colored, stem; 4 cm long, cylinder, has a seen at the base of the stem, volva; universal veil saccate is present at the base and absence of a ring made it difficult to distinguish it from *Amanita* spp. (Adewoyinn *et al*., 2017) (Pl. 5).

Plate (5): *Volvariella* sp. (SHA 17) Fruiting bodies and collection sites between grasses (A) and in the lab (B).

Identification of macrofungal species using polymerase chain reaction (PCR) technology

The results of the polymerase chain reaction (PCR) showed the amplification of the DNA sequence from eight species of (SHA10**,** SHA101, SHA12, SHA17**,** SHA13**,** SHA14**,** SHA16 and SHA15) using primers ITS1 and ITS4. The electrophoresis of the PCR products was demonstrated using an agarose gel with the appearance of DNA fragments. The DNA gel electrophoresis of eight species showed a molecular weight of 700-750 base pairs.

Nucleotide sequence analysis and registration in GenBank

The nucleotide sequences of the DNA bands that were duplicated for the ITS region of the eight mushroom species were determined and it was found that they belong to the mushroom species *A. bitorquis* (SHA14), *Candolleomyces candolleanus* (SHA15), *C. cylindracea* (SHA13), *Leucoagaricus hesperius* (SHA16); *Volvariella* sp. (SHA17) and *Volvopluteus gloiocephalus* (SHA10, SHA101, and SHA12). The sequences of the eight species were deposited on the National Center Biotechnology Information GenBank (NCBI) website (http://www.ncbi.nlm.nih.gov/blast) and belong to many genera in Agaricales under the accession numbers PP576693, PP576221, PP575891, PP576960, PP576696, PP580693, PP576069 and PP580694, respectively as shown in Table (2). For the registration was for the first time in the middle of Iraq.

Phylogenetic tree

The phylogenetic tree of mushroom species was plotted in fixed scales where the lengths of branches were of the same units using evolutionary distances (Diag. 1). The Maximum Composite Likelihood Method for calculating evolutionary distances was used to calculate the nucleotide sequences of the ITS region of the respective species (SHA10, SHA101, SHA12, SHA12, SHA17, SHA13, SHA14, SHA16 and SHA15). The sequences generated through the sequencing method were analyzed using Mega program, version 11. These sequences were aligned with the sequences of nucleotides information obtained from locally isolated fungi.

Designed via evolutionary distances, the phylogenetic tree—including the regional fungal species has evolved between selected scales with the lengths of branches set by the same units. The Maximum Composite Likelihood Method (MCLM) was used to measure the evolutionary distance between species. The process involved base modifications at every position of the target gene sequence. The modified sequenced regions ranged in length from 219 to 674 base pairs.

The genera *Agaricus*, *Candolleomyces*, *Cyclocybe*, *Leucoagaricus*, *Volvariella* sp. and *Volvopluteus* could be classified according to the species level based on their sequences. Eight species were previously unrecorded in central Iraq were identified, and their placement in the phylogenic tree revealed their evolutionary relationships , confirming their identity were considered as distinct species.

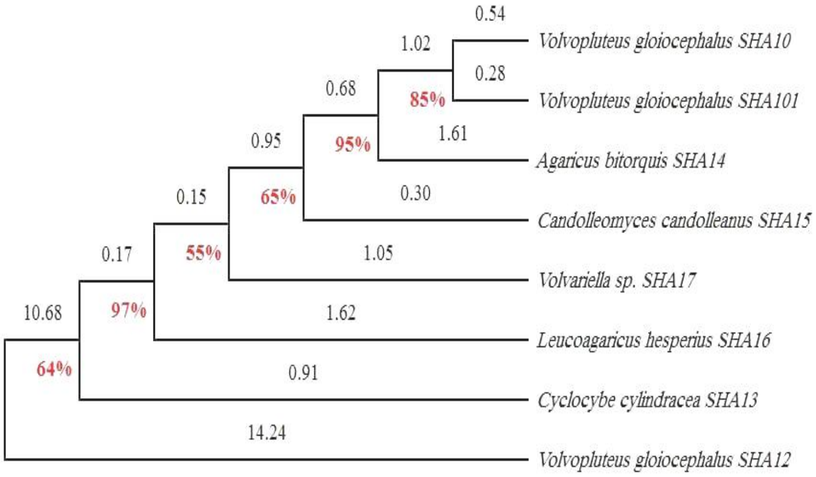

Diagram (1): Phylogenetic tree illustrating the phylogeny of eight genera belonging to Agaricales based on the combined sequence dataset of ITS + ITS4. The codes (e.g., SHA10, SHA101) refer to specific collected of the fungi, and the numbers represent genetic distances, sequence similarities.

The analysis results show that the evolutionary tree is divided into two main branches. Firstly, branch 1 exhibits several distinct clades of closely related fungi. A tight clade formed by species *V. gloiocephalus* (SHA10 and SHA101), with accession numbers (PP576069.1 and PP580693.1) in the GenBank, indicates a recent common ancestor. Their branch length is 0.28, which is relatively short, revealing minimal genetic distance, referring to a recent separation. A bootstrap score of 95%, demonstrates strong support with an elevated level of reliability. Further certainty for the inferred relationships is supported by other relatively high bootstrap values of other nodes, such as the node grouping SHA10 and SHA101, which have a bootstrap value of 85%.

L. hesperius (SHA16) and *C. cylindracea* (SHA13) show the longest branch measurements in the evolutionary tree they constructed, which indicates that the two species have a wide genetic separation from other species. Likewise, the 14.24 measurement shows genetic distance that SHA13 and SHA16 are indeed distantly related to each other.

Candolleomyce candolleanus (SHA15) is more closely related to *Volvariella*sp. (SHA17*)* than to *A. bitorquus* (SHA14). The branch length between SHA15 and SHA17 is of intermediate value, indicating a moderate genetic distance between the two. The branch length between the two previous genera is 1.05.

As shown in Table (4), the species recorded in central Iraq exhibit varying matching rates with species recorded from different regions.

*V***.** *gloiocephalus* **(**SHA10 and SHA12): These species shows a matching rate with *V. gloiocephalus* DM1042 strain fungus reported in Denmark by (Leerhoei and Lang Kjaer, 2020). This genus is found solitary or scattered among grasses. *V. gloiocephalus* is classified as a sister species to all other members of the *Volvopluteus* genus (Kaygusuz *et al*., 2021). The *V. gloiocephalus* (SHA101) species shows similarities to *V. gloiocephalus*10832, a strain fungus reported in New Zealand by Cooper (2019).

Morphologically, this strain is similar to another strain collected from Northern Iraq, with minor differences in cap shape, color, and stem length. (Sulaiman *et al*., 2017). The presence of this species has been recorded across an array, including Iraqi Kurdistan and Sable Island, off the coast of Nova Scotia, in Europe and North America (Suliaman *et al*., 2017; William and Lucas, 2021; He *et al*., 2019). This species was reported in Turkey (Atila and Kaya, 2008; Kaya, 2015) and Iran (Fadavi and Abbasi, 2015). Known commonly as the Stubble Rose Gill, this species was reclassified as *Volvopluteus gloiocephalus* by Italian mycologists (Vizzini *et al*., 2011), becoming the type species of the newly created genus *Volvopluteus*.

Volvariella sp. (SHA 17); shows Similarity to *Volvariella* sp. AV140 strain reported in Italy by Vizzini. The Iraqi strain SHA17 shares similar morphological features with the Italian strain, though with minor differences in shape, color, and stem length (Vizzini *et al*., 2011). *C. cylindracea* (SHA13) shows a matching with *C. cylindracea* MEST42 strain fungus reported in Algeria by Toumi *et al*. (2020). Further, *L. hesperius. (*SHA16) is similar to the species recorded, California, USA by Vellinga (2012), while C*andolleomyces candolleanus* **(**SHA15**)** shows a matching with C*andolleomyces candolleanus* CAFUNDIS fungus strain, reported in the USA by Rozanoff *et al*. (2024).

DISCUSSION

In the current study, eight species of macrofungi were collected from Al-Zawiya and Shadif villages in the Al_ Aziziyah District, and from Bab Al_Muadham, Al_Madain District in Baghdad, Iraq. These species were identified using phenotypic and genetic methods. The presented species are the first to be collected from these locations mentioned above in central Iraq. These species belong to six genera across four families: *Agaricaceae, Pluteaceae, Psathyrellaceae,* and *Strophariaceae,* within the order Agaricales.

The unrecorded species were found more frequently in *Agaricus*, *Candolleomyces*, *Cyclocybe, Leucoagaricus* and *Volvariella* sp. (one species for each genus) and *Volvopluteus* (three species).

Generally, these fungi were identified in the field based on their specific morphological characteristics. Nevertheless, it proved challenging to determine these fungi at the event of species depending only upon the shape of the fruiting bodies. Accurate identification requires detailed observation of microscopic features and expertise in fungal taxonomy (Jeewon, 2016). Polymorphism among fungi complicates identification, by consistently recognizing specific morphological characteristics (Thines, 2018), as well as many groups of fungi that appear morphologically similar but are genetically distinct species (Gautam *et al*., 2022). In such instances, the evolutionary study of ITS sequences in combination with microscopic features may enhance the ability to identify through the species level. Generally, the largest order in the fungal kingdom is Agaricales, involving nearly 20,000 reported species according to the Catalogue of Life22 (Roskov *et al*., 2019). The ITS region is considered a genetic indicator for the classification of fungal species-level across several other DNA markers due to a region can be easily amplified and provides sufficient accuracy to differentiate among species; it provides a large amount of information available in an open database (Chechan *et al*., 2020).

Volvariella sp. is an edible mushroom and a polyphyletic member that can grow terrestrially, adhering to the soil's litter layer or decomposing vegetable matter, or attached directly on wood as mycoparasites. (Liu *et al*., 2024). The phylogenetic tree revealed tight clustering of *Volvopluteus gloiocephalus* samples, which are closely related to each other in an evolutionary sense in terms of the genes used for analysis. Previous studies have identified *V. gloiocephalus* from different regions, including Portugal, New Zealand, Spain, Turkey and the USA, using molecular data such as ITS and LSU sequences. These studies have shown that *V. gloiocephalus* , confirming its status as a distinct clade within the genus *Volvopluteus* (Justo *et al*., 2011b; Kalinina and Malysheva, 2023). The presence of multiple *Volvopluteus gloiocephalus* specimens shows intraspecific diversity within this species. *V. gloiocephalus* (SHA12) according to its appearance in a distinct clade; this specimen was related to certain species than others in the phylogeny tree. The phylogenetic tree exhibited that *V. gloiocephalus* (SHA12) is not as closely related to other species of the same species (SHA10 and SHA101). The genetic variance of *V. gloiocephalus* strains exhibits an intraspecific variation, which may correspond to genetic diversity or ecological adaptation within populations.

More distantly related species include among others *A. bitorquis* and *Candolleomyces candolleanus*, which all share a common ancestor with the *Volvoplateus* group. Branches of the species that are longer represent more distantly related species, which suggests that the species diverged earlier from the common ancestor (ASIF *et al*., 2022; Karalti *et al*., 2022). These relationships are supported by bootstrap support value, as higher percentages indicate more reliable groupings.

One of the important reasons is genetic diversity (85%) within the same species. Genetic variability significantly within species may result from mutations and adaptation to different environments and can display different features as a result of local adaptation and geographic isolation (Amend *et al*., 2012), as seen in *V. gloiocephulus* (SHA12). *V. gloiocephulus* (SHA12) may indicate a genetically separate strain from *V. gloiocephulus*. Additionally, this strain is collected from a different location than (SHA10 and SHA101), so it may be a significant genetic variation that has occurred gradually due to local adaptation. Other reasons that led to the separation of *V. gloiocephulus* SHA12 from other strains, e.g.; might be a hybrid or have undergone introgression with another strain, leading to genetic differences that place it further from other *V. gloiocephalus* species, and maybe sample-contaminated during sequencing.

A. bitorquis (SHA14) is moderately related to the *Volvopluteus* group , with a branch length of 0.68 and a the bootstrap support of 85%, suggesting a solid location in the tree. Depending upon their intermediate length of the branches and bootstrap support (e.g., 85) *A. bitorquis* (SHA14) and *Candolleomyces candolleanus* (SHA15) exhibit a slightly moderate relationship. While sharing a common ancestor, they separated earlier than the strains of *Volvopluteus*.

The evolutionary relationship is demonstrated through clustering: the fact that these species (*V. gloiocephulus* SHA12, *Volvariella* sp. SHA17 and *C. cylindracea* SHA13) cluster together in the phylogenetic tree strengthens the hypothesis that they share a more recent evolutionary history (Justo *et al*., 2011b; Frings, 2020). In fungi, the universal veil represents a temporary membrane that completely encases immature fruiting bodies of some mushrooms as a protective layer that covers the developing mushroom before it fully expands, like a volva or patches (Varga *et al*., 2020).

Agaricus is a saprophytic fungus found globally in forests and grasslands. Its young fruiting bodies have white to pink free lamellae that turn dark brown at maturity, a stipe with an annulus, and creates brown spore prints (Jaichaliaw *et al*., 2021). As well as significant size variation is observed in *A. bitorquis*, distinguishing it from other *Agaricus* species (Nagy *et al*., 2013). This species was collected from Wasit Province, Aziziyah District, had a creamy white and convex cap, becoming more broadly convex With age. A slightly glistening surface is referred to as "white buttons". This species is similar to species previously identified in northern Iraq (Toma *et al*., 2018). It grows in parks, near trees, and in city gardens and offers adequate moisture and organic matter derived from the decomposition of plants and tree debris. According to the GenBank in NCBI site, *A. bitorquis* has been recorded under the identifier SHA and given the international code PP576693 as a new strain for the first time in from central Iraq, with a similarity rate of 99.01 % with those recorded in GenBank which had been collected from China.

C. cylindracea is a source of dietary fiber, amino acids, minerals, and bioactive substances such as vitamins, antioxidants and β-glucans (Koutrotsios *et al*., 2018). This species (synonym *Agrocybe cylindracea*) or “black poplar” is an edible mushroom that can be cultivated in different agricultural regions due its short life cycle. The fruiting bodies are excellent, high-quality mushrooms with a pleasant aroma and high nutritional value (Rezaeian *et al*., 2022).

L. hesperius (SHA16) and *C. cylindracea* (SHA13) exhibit an increased evolutionary separation, suggesting an earlier divergence from a shared ancestor. *L. hesperius*, typically found in tropical and subtropical forests (Ge *et al*., 2015), was collected in this study from a garden. This increased divergence in evolution reflects long-term adaptation to different ecological niches and environments (Sun *et al*., 2019). The development could result in distinct biological and phenotypic features that help them survive in the setting where the species inhabit. The discovery of certain evolutionary processes allows us to comprehend the variety of life or ecological significance found in these fungal species.

CONCLUSIONS

The current study recorded five macrofungal species from central Iraq. The phylogenetic tree provides valuable insights into the evolutionary relationships among the Agaricales strains studied. The phylogenetic tree indicates that while *Volvopluteus gloiocephalus* specimens (SHA10 and SHA101) are closely related, (SHA12) shows significant genetic divergence. This finding suggests intraspecific variation within the species, which more molecular and environmental research could help to investigate. Differences among strain-species variables may influence environmental roles, nutritional characteristics, or potential applications. This result aligns with global phylogenetic tree research that points *V. gloiocephalus* as a well-significant species within its genus depending on ITS and LSU sequence information. Results demonstrate that *Agaricus bitorquis* and *Candolleomyces candolleanus* separated earlier from a common ancestor species, such as *Volvopluteus gloiocephalus*. Their evolutionary structure, branch lengths, and genetic studies across multiple genera support and demonstrate this result.

CONFLICT OF INTEREST STATEMENT

The author declares that there are no conflicts of interest regarding the publication of this manuscript.

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التحري عن الفطريات الكبيرة وسط العراق

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الخلاصة

هدفت الدراسة إلى مسح أنواع الفطر من الحقول بين الأعشاب وجذوع النخيل والأشجار في وسط العراق، وتحديد صفاتها المورفولوجية والجزيئية. اظهرت نتائج التشخيص الجزيئي باستخدام تفاعل البلمرة المتسلسل تم تسجيل ست انواع لثمان عزلات شملت:

Agaricus bitorquis (Quéllt) Saccardo, 1887 (SHA14)
Candolleomyces candolleanus (Fr.) D. Wächter & Melzer, 2020 (SHA15)
Cyclocybe cylindracea (D.C.) Vizzini & Angelini, 2014 (SHA13)
Leucoagaricus hesperius Vellinga, 2010 (SHA16)
Volvariella sp. (SHA17)
Volvopluteus gloiocephalus (D. C.) Vizzini, Contu & Justo, 2011 (SHA10, SHA101 and SHA12),

تعود هذه العزلات الى ستة اجناس تنتمي الى اربع عائلات من الفطريات البازيدية: الغاريقيات Agaricaceae، البلوتيات Pluteaceae، الساثيريليسيا Psathyrellaceae و الستروفاراسيا Strophariaceae ضمن رتبة Agaricales . اودعت تسلسل النيوكلوتيدات في بنك الجينات بأرقام الدخول التالية: PP576693، PP578967، PP576221، PP576960، PP580694، PP575891، PP580693 و PP576069 على التوالي. سُجلت هذه العزلات لاول مرة من بعض مناطق وسط العراق.