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The Morphological and Molecular Identification of Macro Fungi (Agaricales: Basidiomycota) from Southern Iraq

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Mushrooms are critical fungi esteemed for their nutritional and medicinal properties and their wide array of genera and species. However, the phenotypic and molecular traits of mushrooms can intersect, making it vital to distinguish between beneficial and detrimental types. This study honed in on the phenotypic and molecular identification of select mushroom species discovered in the Basrah Governorate, southern Iraq, throughout the rainy season from late November to early March 2022. Five macro fungi species (Basidiomycetes, Agaricales) – *Agaricus bitorquis*, *Conocybe velutipes*, *Coprinellus radians*, *Gymnopilus purpureosquamulosus*, and *Psathyrella trinitatensis* – were collected and categorised. Three of these, *C. velutipes*, *G. purpureosquamulosus*, and *P. trinitatensis*, emerged as new records for Iraq's mycoflora. The phylogenetic relationships among these species and other closely related species were analysed and recorded in GenBank.

Keywords: Basidiomycota; Agaricales; morphological characteristic; ITS and LSU analysis; taxonomy

I. INTRODUCTION

Macro fungi produce fruit bodies that are visible to the naked eye, or they form microscopic fruit bodies (Mueller *et al.*, 2007). These fungi can be saprotrophic or symbiotic, and in some cases, they can be pathogenic to plants (Devi & Shrivastava, 2016). They also serve as sources of food and are important for the production of drugs and pharmaceutical compounds, making them vital to both agricultural and natural ecosystems (Redhead, 1997; Suliaman *et al.*, 2017).

These fungi are significant living organisms on Earth (Mueller & Beals, 2004). It is estimated that macro fungi constitute only 7.3% of the world's total fungi, approximately 110,000 species. Mueller *et al.* (2017) suggested that most macro fungi belong to the Basidiomycota and Ascomycota groups. An example of macro fungi is mushrooms, which can be found throughout the world. These heterotrophic organisms have specific nutritional and environmental requirements. Mushrooms can be found in various parts of the world and can be introduced to new locations through airborne spores or other means (Farid *et al.*, 2013).

Iraq is home to a diverse range of mushrooms, with numerous studies conducted to isolate and classify them (Al-Khesraji, 2016; Muhsin *et al.*, 2011; 2012; Suliaman *et al.*, 2017). Agaricales, the largest group of fungi responsible for producing mushrooms, comprises more than half of the homobasidiomycetes species, encompassing around 9,000 species and 350 genera (Hibbett *et al.*, 1997; Hibbett & Thorne, 2001; Kirk *et al.*, 2001).

Previously, the classification of mushroom fungi relied on phenotypic characteristics. However, variables such as phenotype variation, geographical and environmental differences, and the developmental stages of the basal base influence the morphological features, making it challenging to distinguish between species (Challen *et al.*

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2003; Kerrigan, 1986; Singer, 1986). DNA-based molecular techniques are effective in determining species relevant to a genus (Chen *et al.*, 2015; Kerrigan *et al.*, 2008; Thongklang *et al.*, 2016; Zhao *et al.*, 2011), leading to the identification of new species. With these considerations in mind, this study aspires to identify mushroom species in the Basrah Governorate in southern Iraq using both phenotypic and molecular approaches.

II. MATERIALS AND METHOD

The specimens for examination were collected from Basrah Province in southern Iraq between December 2021 and February 2022. The morphological features of the basidiocarps were recorded, while the micromorphological features (shape, size, and colour of spores, basidia, and cheilocystidia) were observed under a light microscope. The material was mounted using 5× KOH, lactic acid, and blue lactophenol pigment. For specimen identification, the following references were used: Hoiland (1998), Prydiuk (2007), Kaur *et al.* (2011), Al-Khesraji *et al.* (2018), and Altaef *et al.* (2021).

A. Molecular Studies

The Ez-10 Spin Column Fungal Genomic DNA Miniprep kit (BioBasic, Canada) was used to extract DNA from fresh specimens, adhering to the manufacturer's instructions. The DNA amplification of the internal transcribed spacer (ITS) region was executed using the primers ITS5 and ITS4 (White *et al.*, 1990), while primer LR5/LR0R was used for the nuclear large ribosomal subunit LSU (Vilgalys & Hester, 1990). All PCR products were subsequently purified and dispatched to a Korean company for sequencing. The genetic analysis was conducted using the MEGA X program.

B. Ethical Approval

This paper was approved by the Internal Ethical Committee Board of the Department of Biology, College of Education for Pure Sciences, at the University of Basrah (No. 6/2022).

III. RESULT

A. Morphological Identification

Five species of basidiomycete macro fungi were collected and identified, all belonging to the order Agaricales: *Agaricus bitorquis, Conocybe velutipes, Coprinellus radians, Gymnopilus purpureosquamulosus* and *Psathyrella trinitatensis*.

B. Agaricus bitorquis (Quelet) Sacc.

Sample 5: GenBank accession numbers – ITS (OQ346260) and LSU (OQ390103). The pileus is 30–75 mm wide and has a white to creamy colour, a convex shape, and a smooth surface with spots near the margin. The lamellae are free, crowded, and white, spanning 20–50 mm wide; they turn from brown to dark brown upon maturity. The stipe is 30–50 mm, white in colour, with a wooden texture, centrally positioned with a thick, solid, cylindrical shape, and a creamy annulus. The basidiospores are circular to oval, smooth, with a thick cell wall and dark brown, measuring 6–7.5 × 4.8–6 µm. Basidia measure 18–25 × 6.5–8 µm, they are clavate, hyaline, and have thin cell walls. Cheilocystidia are 10–19.5 × 3.5–5 µm and are clavate, smooth, hyaline, and cylindrical. The habitat is within the desert soil in Safwan, Basrah, Iraq (Figure 1).

C. Conocybe velutipes (Velen.), Hauskn and Svrcek in Hauskn, 1999

Sample 1; GenBank accession numbers: ITS (OQ346256), LSU (OQ390100). The pileus measures 10–25 mm in width and 2–6 mm in height, showcasing a convex to campanulate shape, rounded or oval. It presents a cinnamon-bark brown colour, a smooth texture, hydrophanous properties, and an irregular margin. The lamellae are equal, adnate, crowded, and yellow. The stipe exhibits sizes of 20–60 × 2–4 mm, and is hollow, maturing from a pale brown to white colour. The basidiospores, at 8–11 × 6–9 µm, are oval, mimicking an elongated ellipse and range from yellow-gold to light brown. They are also thick with a smooth wall. The basidia, at 15–22 × 6–9 µm, are hyaline and clavate. The cheilocystidia come in at 9–22 × 6–12.5 µm, with a head measurement of 2–5 µm in breadth (Figure 2). This

species was discovered in Iraq, specifically in the agricultural area of Shatt al-Arab, Aljazeera, Basrah, marking a new record for the region.

D. Coprinellus radians (Desm.) (Vilgalys et al., 2001)

Sample 4: GenBank accession numbers – ITS (OQ346259) and LSU (unregistered). The pileus measures 10–20 mm in width, with a bell shape that extends from a closed state to the stipe. Its smooth texture ranges in colour from white to light brown or yellow. The stipe, cylindrical and white, is swollen at the base, hollow, and measures between 10–30 × 7–15 mm (Figure 3). The habitat and locality of the sample were found on soil in the Aljazeera district of Shatt al-Arab, Basrah, Iraq. The sample was damaged.

E. Gymnopilus purpureosquamulosus, (Hoil, 1999)

Sample 3; GenBank Accession Numbers: ITS (OQ346258) and LSU (OQ390102). The pileus measures between 40 and 100 mm, featuring a flat profile with concave edges and a central depression. The surface boasts a coarse texture and smooth scales, ranging in colour from bright yellow to orange. The lamellae are quite numerous, 5–7 mm wide, decurrent and close, sporting a yellowish-brown hue. The yellow stripe measures 50–130 × 6–13 mm, characterised by a solid, fibrous texture which is often striated or curved and branched off at its base. The basidiospores measure 7.5–9.6 × 3.6–4.8 µm and are yellowish-golden, defined by a scar on their tapering side, and an ellipsoid to ovoid shape with rough walls. The basidia range from 18 to 33 × 5.5–8 µm, hyaline and clavate. The cheilocystidia measure 15–32 × 4.2–9 µm, showcasing a hyaline and rostrate-rounded appearance. The caulocystidia, on the other hand, measure 24.4–60 × 4–15 µm, bearing a hyaline, cylindrical form with thin walls (Figure 4). This specimen was discovered on a date palm tree located in Shatt al-Arab (Aljazeera district), Basrah, Iraq.

F. Psathyrella trinitatensis (Baker & Dale, 1951)

Sample 2; GenBank accession numbers: ITS (OQ346257) and LSU (OQ390101). The pileus measures 18–38 mm and is convex, and flat, with a pale brown to white colour and a brown centre. It has an irregular margin and is moist. The lamellae are crowded, brown to yellow, adnate, and narrow. The stipe is 20–35 mm, hollow, white, and smooth. The basidiospores measure 5–7 × 4.8–5.7 µm and are smooth with a thick wall. They are globose to elongate, clavate, and yellow. The basidia measure 8–15.4 × 4–5 µm and are hyaline and clavate. The cheilocystidia measure 17–25 × 7–9 µm, have a thick wall, and are lageniform (Figure 5). The habitat and locality of this species are found on the soil in Shatt al-Arab, Aljazeera, Basrah, Iraq.

G. Phylogenetic Analyses

The ITS and LSU datasets comprised 104 sequences, including 94 from GenBank and ten new sequences from this study. The phylogenetic tree (Figure 6) was divided into two main clades: clade I, which encompasses the LSU region, and clade II, which encompasses the ITS region. In the ITS region, *A. bitorquis* (Sample 5) displayed a 97.76% identity with isolate KY401334.1, whereas, in the LSU region, it had a 99.5% identity with isolate MH874006.1. The phylogenetic analysis of the ITS region of *C. velutipes* (Sample 1) associated it with the *C. leptospora* isolate MK412344.1, showing a 95.97% identity, while in the LSU region, it was associated with *C. velutipes* JX968346.1, featuring a 99.12% identity. The *C. radians* (Sample 4) in the ITS region showed a perfect match with isolate OU989345.1, with a 100% identity, whereas in the LSU region, it had a higher match with *C. aff. radians* isolate FJ185160.1, demonstrating a 99.78% identity. The *G. purpureosquamulosus* (Sample 3) in the ITS region showed a high match with isolate MK088248.1, indicating a 99.24% identity, while in the LSU region, it had a high match with isolate MK278099, with a 99.77% identity. The Psathyrella (Sample 2) was associated with two species in the ITS region, *P. candolleana* MW915603.1 and *P. roguelana* NR173216.1, and with *P. trinitatensis* and isolate KC992882.1, both exhibiting a 99.77% identity, in the LSU region. *Aspergillus montoensis* was used as an outgroup.

The Iraqi environment boasts a rich variety of fungi, with many new species previously recorded as additions to the Iraqi mycoflora (Abdulla, 2021; 2023; Abdulla & Abass, 2022). This study describes five species of fungi from the Agaricales order, identified through classical morphology and molecular analysis of the ITS and LSU positions.

Despite several studies on Iraqi fungi, many species have not been definitively confirmed with molecular data. In the Basrah province in southern Iraq, morphological and molecular identification revealed three new species: *Conocybe velutipes*, *Gymnopilus purpureosquamulosus*, and *Psathyrella trinitatensis*. Two previously identified species, *Agaricus bitorquis* and *Coprinellus radians*, were also studied. Several studies on mushroom fungi in northern and central Iraq rely on morphological taxonomy. Given the morphological similarities between many species, molecular methods are necessary to corroborate morphological classifications.

The genus *Agaricus* represents a large genus of mushrooms, comprising more than 500 species found across numerous countries. This genus holds both economic and therapeutic importance, significantly impacting human life. *Agaricus bitorquis* (Sample 5), which belongs to the family Agaricaceae, was collected from the Safwan district in the Basrah province of southern Iraq. These species appear in winter and spring, corresponding to the rainy season, the timing of which varies annually.

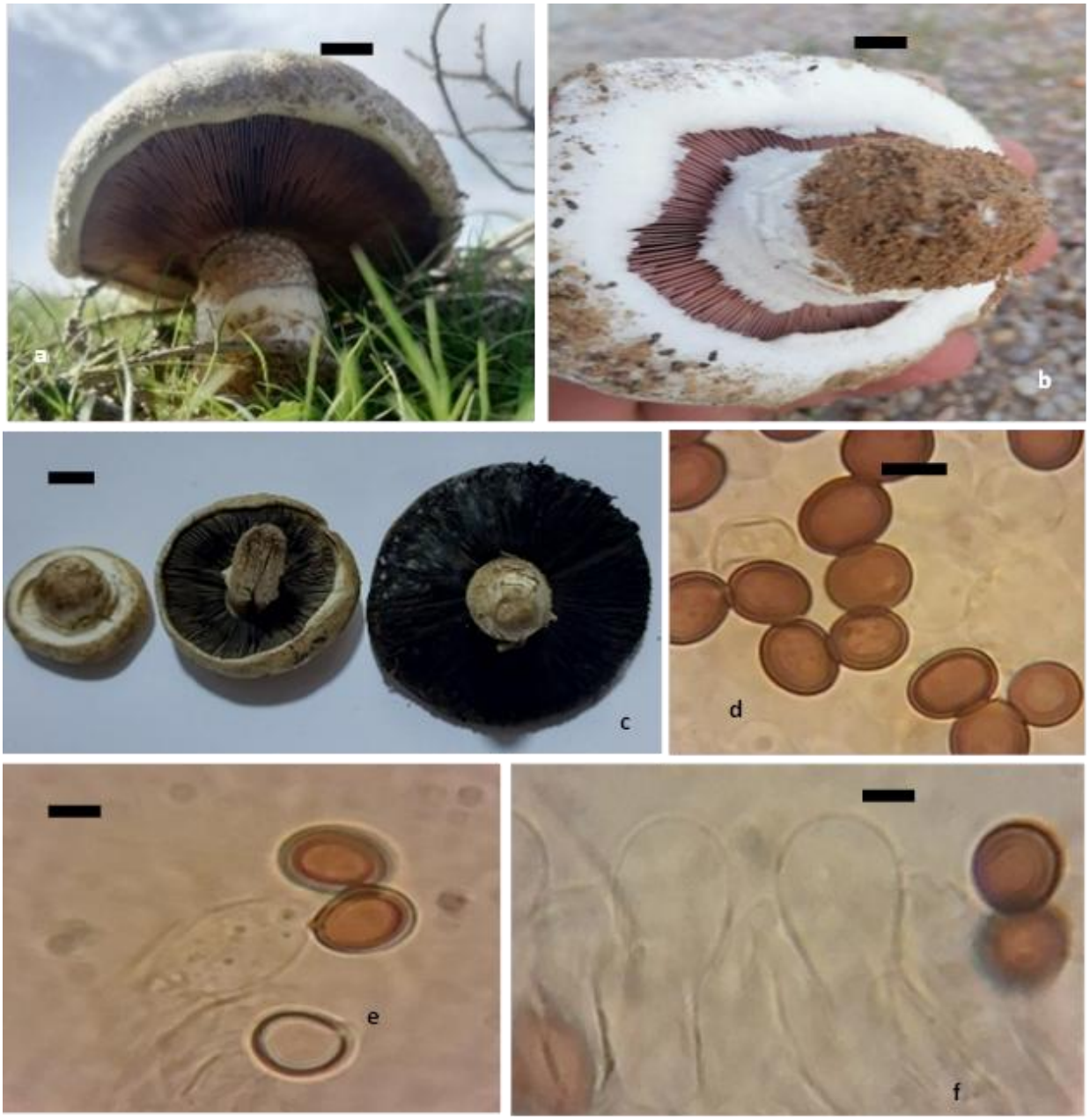

Figure 1. *Agaricus bitorquis* (sample 5), a–c. Basidiomata displaying pileus and lamellae. d. Basidiospores. e. Basidia. f. Cheilocystidia. Scale bar a–c = 10 mm, d–f = 5 µm.

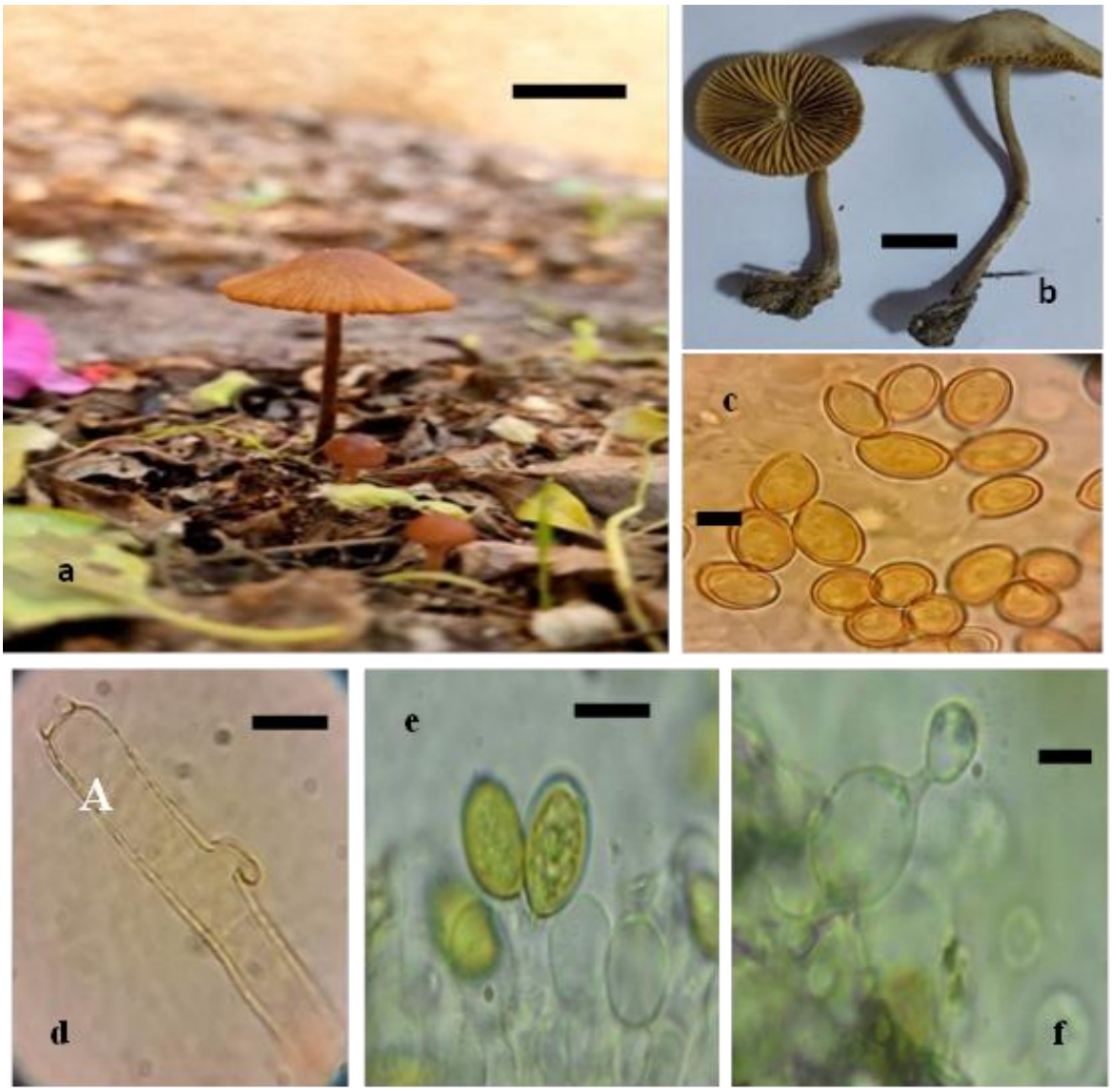

Figure 2. *Conocybe velutipes* (Sample 1), a–b. Basidiomata, c. Basidiospores, d. Clamp connections, e. Basidia, f. Cheilocystidia. Scale bar: a, b = 10 mm, c–f = 5 µm.

Figure 3. *Coprinellus radians* (Sample 4), Basidiomata Scale bar = 10 mm.

Figure 4. *Gymnopilus purpureosquamulosus* (sample 3), a, b. Basidiomata showing pileus scales, c. Lamellae, d. Basidiospores, e. Cheilocystidia. Scale bar: a–c = 20 mm, d, e = 5 µm.

Figure 5. *Psathyrella trinitatensis* (Sample 2), a–c. Basidiomata showing pileus and lamellae; d, e. Basidiospores, f. Cheilocystidia. Scale bar: a–c = 10 mm; d–f = 5 µm.

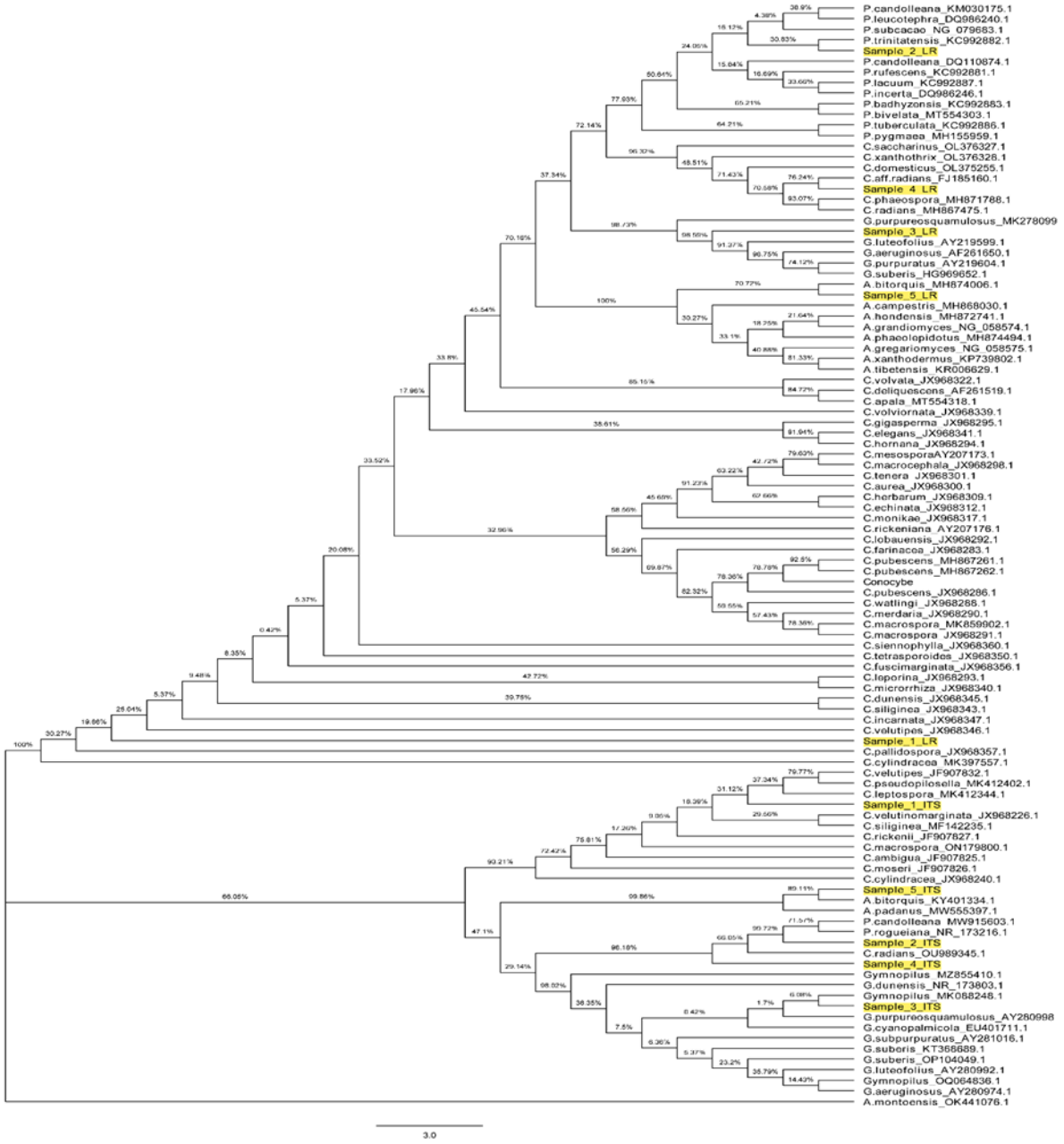

Figure 6. Phylogenetic tree based on ITS and LSU analysis; new sequences from this study (Sample 5: *Agaricus bitorquis*, Sample 1: *Conocybe velutipes*, Sample 4: *Coprinellus radians*, Sample 3: *Gymnopilus purpureosquamulosus* and Sample 2*: Psathyrella trinitatensis*) with *A. montoensis* used as an outgroup.

IV. DISCUSSION

A. bitorquis is a cosmopolitan species. Morphologically, it resembles *A. bernardi*, but the molecular data differs. *A. bitorquis* was once consumed because of its desirable size and taste. Al-khesraji *et al.* (2018) isolated this species from northeast Iraq and identified it morphologically, without a molecular study. In this research, *A. bitorquis* showed high similarity in the ITS and LSU regions. It was associated with *A. bitorquis* isolated from China.

Despite numerous studies, the genus Conocybe remains one of the least researched in the world. This lack of research may be attributed to factors such as limited taxonomic investigation, inadequate literature, and the notable similarity and diversity in the basidiocarp that render species differentiation a challenging task (Hausknech *et al.*, 2009). Conocybe is a genus in the family Bolbitiaceae, typically found growing in agricultural soil amid decayed grass and deciduous wood in forests. Its defining feature is the formation of lecythiform cheilocystidia (Amandeep & Munruchi, 2015).

Particularly noteworthy is the species *Conocybe velutipes* (Sample 1), being encompassed in literature records for the first time from Iraq, with its isolate closely resembling descriptions in existing literature (Hausknech *et al.*, 2009; Amandeep & Munruchi, 2015; Cagli *et al.*, 2019). *C. velutipes* is akin to *C. siennophylla*, the distinction lying in its lentiform, large, and thick-walled basidiospores (Hausknech *et al.*, 2009). Notably, only two species from this genus have been recorded in Iraq, *C. watlingii* and *C. deliquescens*, however, there is no associated molecular data (Al-Khesraji, 2018; Suliaman, 2019). In a phylogenetic study, the ITS region of *C. velutipes* was identified to be related to *C. leptospora* (95.97% identity), while in LSU regions, it was associated with *C. velutipes* (99.12% identity). The latter was chosen based on its higher identity and morphological characteristics akin to *C. velutipes*.

Coprinellus radians (Sample 4) belongs to the Psathyrellaceae family, which includes three genera: Coprinellus, Coprinopsis, and Parasola (Hopple *et al.*, 1999). Many Coprinellus species feature a hair structure on the pileus along with a hymeniform pileipellis and a veil comprising approximately spherical cells (Nagy *et al.*, 2012). Altaef *et al.* (2021) isolated three species of Coprinellus from Salahadin province, Iraq: *C. disseminatus*, *C. flocculosus*, and *C. radians*. In a recent phylogenetic study, C. radians were associated with *C. radians* isolated from Belgium.

The genus Gymnopilus consists of many saprophyte species, which serve as a significant segment of global wood mycobiota (Holec, 2005; Kirk *et al.*, 2008). Kuhner (1980) initially classified the Gymnopilus genus as Strophariaceae. However, Singer (1986) observed basidiospore ornamentation and germ pore loss, leading him to classify Gymnopilus as Cortinariaceae. Currently, based on molecular studies, Gymnopilus is categorised as Hymenogastraceae (Kirk *et al.*, 2008). *G. purpureosquamulosus* was first collected by Hoiland (1998) in Zimbabwe and was later found in Italy, Panama, Switzerland, and Nigeria (Guzman-Davalos *et al.*, 2008). *G. purpureosquamulosus* (Sample 3) is now being reported for the first time in Basrah, Iraq. This species was isolated from a date palm tree, indicative of a lignicolous habit. This Iraqi isolate was characterised by a large fruit body, erect pileus scales, and large basidiospores compared to other isolates. In a phylogenetic study involving ITS and LSU regions, *G. purpureosquamulosus* was found to be related to *G. purpureosquamulosus* isolated from Zimbabwe.

P. trinitatensis (Sample 2) is a member of the Psathyrellaceae family, comprising 1100 species globally. Most of these species live as saprophytes on organic materials like dung, leaves, and wood (Kirk *et al.*, 2008; Vasutova *et al.*, 2008). The Psathyrella genus features a fragile, ephemeral basidiocarp and small to large dark brown basidiospores. Because of these qualities and molecular analysis studies, researchers suggest that the origin of this genus is unstable (Orstadius *et al.*, 2015). *P. candolleana*, *P. spadiceogrisea*, and *P. piluliformis* have all been documented in Iraq (Toma *et al.*, 2013; Suliaman *et al.*, 2017; Al-Khesraji, 2018). *P. trinitatensis*, a recent find in Iraq, is distinct in its morphology given its brown, rather than dark, basidiospores. In a phylogenetic study, *P. trinitatensis* in the ITS region was linked with two species – *P. candolleana* and *P. roguelana*. In the LSU region, however, it is associated with *P. trinitatensis* from Sweden. The decision to select this species was due to its higher identity (99.77%) and distinctive morphological characteristics.

V. CONCLUSION

The study has successfully isolated and identified five species of Agaricales. This process was conducted utilising both morphological and molecular techniques, focusing particularly on the ITS and LSU regions. The distinguished species include *Agaricus bitorquis*, *Conocybe velutipes*, *Coprinellus radians*, *Gymnopilus purpureosquamulosus*, and *Psathyrella trinitatensis*. Notably, three of these species have been newly recorded in Iraq.

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