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Original article

First report on morphological and molecular characteristics of eight *Agaricomycotina* mushrooms in Saudi Arabia

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ABSTRACT

Some fruiting body fungi, such as mushrooms, have valuable nutritional and medicinal properties. The present study aimed to report some mushroom species for the first time in several regions of Saudi Arabia: Abha, Al-Baha, Al-Taif, and Jeddah. Morphological and microscopic features, the internal transcribed spacer (ITS) region, and a Basic Local Alignment Search Tool (BLAST) analysis were studied to identify *Crinipellis scabella, Parasola conopilea, Agrocybe pediades, Pyrofomes demidoffii,* and *Tulostoma fimbriatum* in Al-Baha, and *Conocybe macrospora*, and *Chlorophyllum palaeotropicum* throughout Saudi Arabia. All mushrooms identified in this present work were *Agaricomycotina* mushrooms recorded for the first time in Saudi Arabia.

1. Introduction

Recently, there has been a significant increase in research and food production of edible mushrooms worldwide, as they have a great medicinal and nutritional value (Adeniyi et al., 2018). It has been reported that some fruiting bodies of edible mushrooms are considered important natural ingredients in many medicinal agents such as anti-inflammatories, trehalose, oleic acid, gallic acid, melatonin, biotin, lycopene, and zinc can be extracted from *Agaricus bisporus* and *Lentinus edodes* (Muszyńska et al., 2018). Many scientific reports have confirmed that numerous fruiting bodies of mushrooms (such as *Lentinus* spp, *Auricularia* spp, *Hericium* spp, *Grifola* spp, *Flammulina* spp, *Pleurotus* spp, and *Tremella* spp) have immunomodulation, anti-obesity, hepato-prophylactic, antifungal, antibacterial, antiviral, antinociceptive, antidiabetic, antitumor, anticancer, and antioxidant agents properties (Ganesan and Xu, 2018).

The fruiting bodies of mushrooms are generally considered among the most biodiverse organisms on earth, for this reason, much research has been undertaken to investigate these microbes. The fungal kingdom includes more than 5 million species worldwide that play essential environmental roles and can colonize nearly all natural environments. They also have several nutritional and medicinal benefits (Martins, 2017). Global estimates indicate that there are more than 2300 and 2100 species of wild fruiting fungi that are edible and medicinal fungal strains, respectively (Martins, 2017).

Hibbett (2006) has reported that almost one-third of the identified fungal species belong to the *Agaricomycotina*, also called the hymenomycetes. *Agaricomycotina* is a subdivision belonging to the division Basidiomycota. The subdivision *Agaricomycotina* contains most of the described mushrooms which can produce fruiting bodies with hymenial layers. This subdivision consists of four classes "Agaricomycetes, Dacrymycetes, Walleomycetes and Tremellomycetes" (Lestari et al., 2019).

Biodiversity studies on mushrooms have been done in local environments in many countries, including India (Lakhanpal, 2014), China (Zhang et al., 2021), USA (Martins, 2017), Nigeria (Vyas et al., 2014), Indonesia (Siahaan et al., 2019), Egypt (Soliman and El-Sayed, 2021), Saudi Arabia (Abou-Zeid and Altalhi, 2006), and Iraq (Toma et al., 2013).

Obtaining maximum benefit from mushrooms depends on local survey studies to localize the cultivation of beneficial strains, then small- and large-scale production of bioactive compounds can be established. Hence, the present work aimed to study the biodiversity of wild mushrooms in four regions (Al-Taif, Al-Baha, Abha, and Jeddah) in Saudi Arabia.

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2. Materials and methods

2.1. Study regions and design

The study regions included four governorates, Al-Taif, Al-Baha, Abha, and Jeddah, with GPS coordinates 21.2841°N, 40.4248°E; 20.0217°N, 41.4713°E; 18.2465°N, 42.5117°E; and 21.4858°N, 39.1925°E, respectively. Each region was divided into four large squares characterized by the presence of vegetation cover and humidity using a completely randomized design.

2.2. Collection of samples

The fruiting body fungi samples were collected from the above regions between November 1, 2020, and January 30, 2021. The dust and dirt were removed from the samples using a gentle air stream and soft brushes. The samples were kept in sterile plastic boxes then transferred to the Microbiology Laboratory, Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia.

2.3. Macroscopic and microscopic characteristics

The structure of the fruiting body and their spores (spore color, attachment, symmetry, size, ornamentation, shape) were recorded according to Largent et al., (1977).

2.4. Preparation of spore suspension

For each sample, a section of the gill of the mushroom was mixed well with sterile normal saline (0.089 % NaCl), and the spore suspension was collected by centrifugation at 3000×*g* for 15 min at 25 °C. The total count of spores was calculated using direct microscopic count by hemocytometer then the serial dilution was applied to obtain 1 mL of sterile normal saline solution containing approximately 5 ± 2 spores. The microscopic characteristics of the spores were studied using digital light microscopy (Motic Plus 2.0 ML, China); after that, the spore suspensions were stored in sterile glycerol solution (30 % v/v) at –80 °C till further analysis.

2.5. DNA extraction, amplification internal transcribed spacer (ITS) region, and sequencing PCR products

The DNA was extracted from the collected mushrooms using DNeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol. The pure DNA product was preserved at –80 °C for future investigations. Amplification of ITS region was done using forward primer (5′-TCCGTAGGTGAACCTGCGG-3′) and reverse primer (5′-GCTGCGTTCTTCATCGATGC-3′) (Usyk et al, 2017). The program of PCR was designed using Optimase ProtocolWrtiter™ in a simple multiple-steps PCR protocol.

PCR product length was 600 bp. Purification of PCR products was done using a montage PCR clean-up kit (EMD Millipore, Burlington, MA, USA) to remove the PCR primers and deoxynucleotide triphosphates that were not included as part of the product. BigDye™ Terminator Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) and Applied Biosystems model 3730XL automated DNA sequencing system were used to sequence the pure PCR products.

2.6. Identification and BLAST analysis

The sequenced PCR products were edited using Bioedit software (BioEdit Sequence Alignment Editor), then the nucleotide basic local alignment search tool (nucleotide – nucleotide BLAST) analysis was done in GenBank (https://www.ncbi.nlm.nih.gov/genbank/). The identification was performed using the morphological and spore features, together with BLAS analysis. Evolutionary analysis of identified samples was done using the maximum likelihood test in MEGA-X v10.1.8 software according to Hasegawa et al., (1985) and Kumar et al. (2018).

3. Results

BLAST analysis, morphological and spore features of query-63801 sample collected from Al-Baha is a mushroom strain belonging to *C. scabella* (Figs. 1 and 2). For details of the analysis (the max score, total score, query cover, E value, percent identity, and accession length for all BLAST analysis), see tables in the supplementary section. Figs. 1 and 2 show the macroscopic and microscopic characteristics as well as the BLAST analysis of samples (query-35787) collected from Al-Baha, Saudi Arabia. The results confirmed that samples were *P. conopilea* (Table 2 in the supplementary section). The data obtained from Figs. 1 and 3 confirm that the mushroom sample (query-45217) collected from Al Baha were strains of *A. pediades* (Table 3 in the supplementary section).

The mushroom samples (query-46765) collected from Al-Taif were investigated using BLAST analysis and their morphological characteristics (Figs. 1 and 4). The data showed the samples belong to *C. macrospora* (Table 4 in the supplementary section). Figs. 5 and 6 show the BLAST analysis, macroscopic characteristics, and spores of the samples (query-57701) collected from Al-Baha. The data reported that the samples belong to *Agrocybe pusiola* strains (Table 5 in the supplementary section).

The morphological features and BLAST analysis of the samples (query-53867) collected from Al-Baha indicated the mushroom samples were *Pyroformes demidofii* strains (Figs. 5 and 7). The identification parameters of BLAST were listed in Table 6 in the supplementary section.

The BLAST analysis, morphologic characteristics, and spore features of *C. palaeotropicum* collected from Jeddah (query-106927) are arranged in Figs. 5 and 8 and the details of BLAST analysis was arranged in Table 7 in the supplementary section.

Figs. 5 and 9 show the BLAST analysis, morphologic characteristics, and spores features of *T. fimbriatum* collected from Al-Baha (query-36233). The identification parameters of BLAST analysis was arranged in Table 8 in the supplementary section.

Phylogenetic analyses and models of DNA sequence evolution (substitution model) of the identified mushroom samples were done using the maximum likelihood method and Hasegawa-Kishino-Yano model. The relative rates of various changes among all samples are shown in Fig. 10. The results showed that *C. palaeotropicum* and *P. demidofii* originated (derived) from ancestral strains of the tested mushroom samples with the 56 certainty degree (it represents the value of certainty the fungal strains originated (derived) from ancestral strains). *C. macrospora*, *T. fimbriatum* and *C. scabella* originated from ancestral fungal strains with 82 certainty degree. The certainty degree of *A. pediades*, *A. pusiola* and *P. conopilea* was 56. The taxonomy analysis showed that the mushroom samples comprised eight species belonging to five families (Maramiaceae, Psathyrellaceae, Bolbitiaceae, Agaricacea, and Polyporaceae) in two orders (Agaricales and Polyporales) and in one class (*Agaricomycotina*) (Figure 1 in the supplementary section).

4. Discussion

In the present study, as far as we know, we were able to collect and identify-eight mushroom species for the first time in Saudi Arabia. On the other hand, we could not collect (and include in

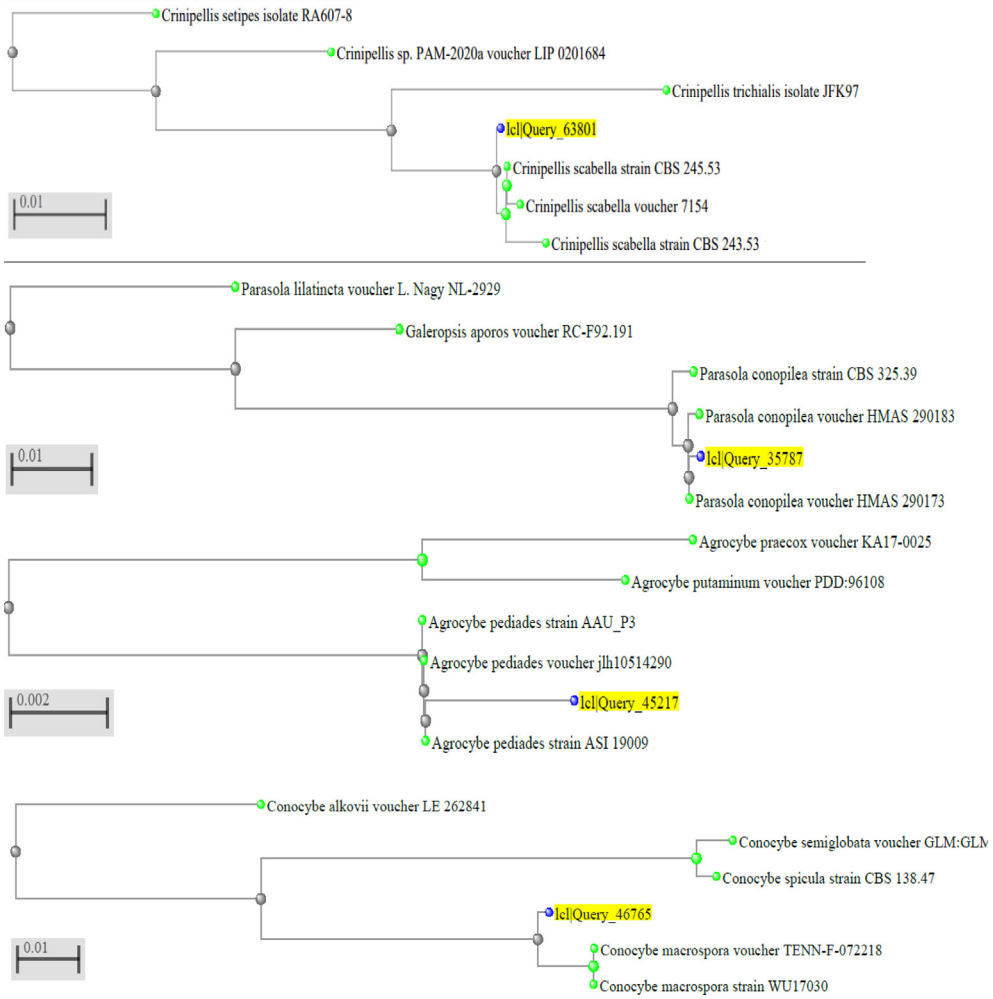

Fig. 1. BLAST analysis of mushroom ITS sequence for query-63801 (*C. scabella*), query-35787 (*P. conopilea*), query-45217 (*A. pediades*) and query-46765 (*C. macrospora*).

our work) some mushroom species identified by previous studies of the same regions. In 2019, Manzelat published results reporting that *Morchella* spp., *Agaricus* spp., *Agrocybe* spp., *Amanita* spp., *Boletus* spp., *Coprinus* spp., *Lepiota* spp, *Pleurotus* spp, and *Podaxis* spp could be collected from the Abha and Jizan regions (Manzelat, 2019). Before that, in 2006, Abou-Zeid and Altalhi collected mushrooms samples from some regions of Al-Taif; these were identified according to their morphological characteristics as *A. arvensis*, *A. bisporus*, *A. augustus*, *Lepiota procera*, *Lepiota rhacodes*, *Lepiota cristata*, *Pleurotus ostreatus*, *Pleurotus cornucopiae*, *Coprinus comatus*, *A. cylindracea*, *Pleurotus pistillaris*, *Inocybe splendens*, *Phaelepiota aurea*, and *Boletus edulis* (Abou-Zeid and Altalhi, 2006).

We could not collect many mushrooms species like collected previously in the above studies such as *Morchella* spp., *Amanita* spp., *Boletus* spp., *Coprinus* spp., *Lepiota* spp, *Pleurotus* spp, *Podaxis* spp, *Inocybe* spp., and *Phaelepiota* spp. In fact, in our present study, all mushroom species (*C. scabella*, *P. conopilea*, *A. pediades*, *C. macrospora*, *P. demidofii*, *C. palaeotropicum*, and *T. fimbriatum*) that were collected and identified were new mushroom species.

In 2010, Antonín and Noordeloos described *C. scabella* as having a small fruiting body (basidiocarp) with orange-brown to red-brown cap (pileus) hairs on a pale cream white background, and its basidiospores were wide with differently fashioned cheilocystidia (large cells on the edge of the gill) (Antonín and Noordeloos, 2010). Adamczyk reported that *C. scabella* grows on grasses that are considered special substrates for this mushroom (Adamczyk, 2016). In our present study, we collected *C. scabella* from one of the deep valleys of Al-Baha containing perennial trees, hardwood, shade, water, and grass. This valley is characterized by little human activity. Adamczyk has reported that more than 40 mushroom species (including *C. scabella*) showed biodiversity related to abandoned lands and the development stage of those lands (Adamczyk, 2016). According to https://www.gbif.org/species/2538113 (8/20/2021), *C. scabella* has recorded 2369 occurrences (not including Saudi Arabia) worldwide, and there are 4 infraspecies of this fungus. There are only 17 nucleotides of *C. scabella* strains that have been identified and their data have been deposited in NCBI, and according to BLAST tree analysis, there were only 7 strains of *C. scabella* (data not shown).

P. conopilea (formerly named *Psathyrella conopilea*) was collected from the same valley as *C. scabella*. *P. conopilea* features a breakable main body, dark basidiospores, and a hygrophanous brown pileus surface. In addition, it can grow well in groups of 2–4 fungi (but not in crowded groups) in cultivated regions or hardwood forests on wood debris or manure (dung) (Kuo, 2011). There are 38 nucleotides of *P. conopilea* strains that have been analyzed in NCBI (data not shown). According to https://www.gbif.org/species/10364983 (8/20/2021), *P conopilea* has recorded 2343 occurrences (not including Saudi Arabia) worldwide. *P. conopilea* grows well in spring and fall and has been collected from several regions in Hungary and USA (Ganga and Manimohan, 2019). The presence of these mushrooms in the local environment encourages

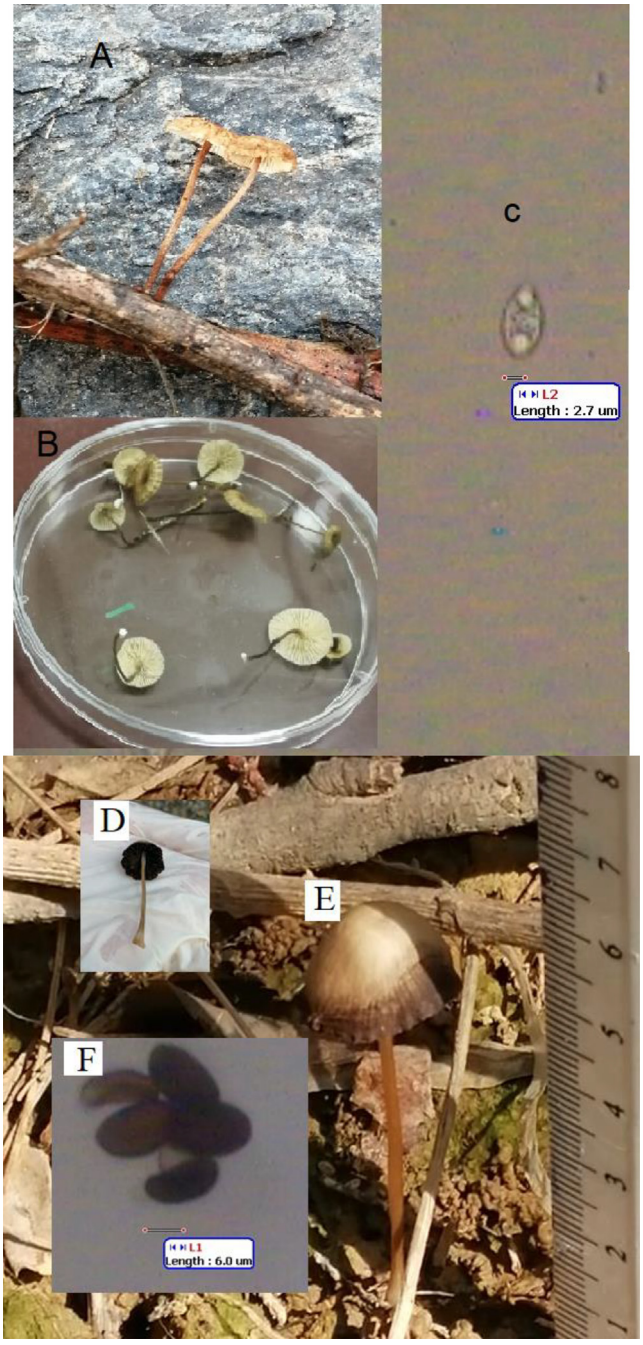

Fig. 2. Morphologic and spore characteristics of *C. scabella* and *P. conopilea* collected from Al Baha, Saudi Arabia, in the field (A), in the laboratory (B) and image of single spore (C) for *C. scabella*, in the laboratory (D), in the field (E), and image of spores (F) for *P. conopilea*.

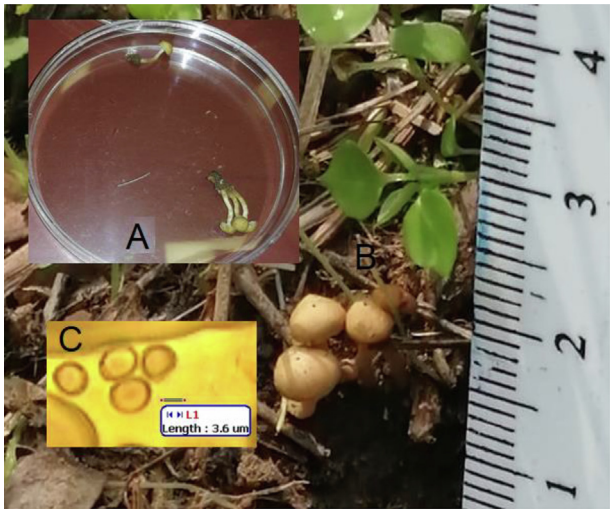

Fig. 3. Morphologic and spore characteristics of *A. pediades* collected from Al Baha, Saudi Arabia in the laboratory (A), in the field (B) and image of spores (C).

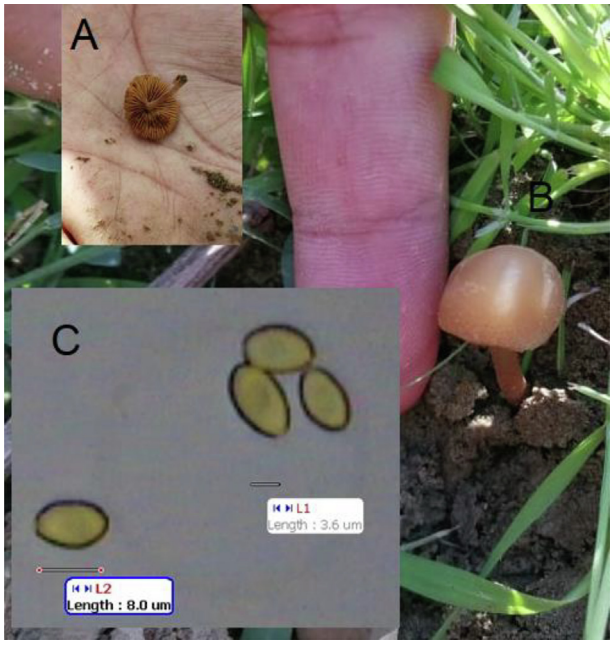

Fig. 4. Morphologic and spore characteristics of *C. macrospora* collected from Al-Taif, Saudi Arabia. In the field (A and B), and image of spores (C).

their use for the production of bioactive compounds; for example, *P. conopilea* is a natural resource of drosophilid A (Riquelme et al., 2020).

In the present study, *A. pediades* and *A. pusiola* were also collected from the same location in Al-Baha. There are species of *Agrocybe* (but not *A. pediades* and *A. pusiola*) that have been collected in Saudi Arabia in previous studies from the Al-Taif, Abha, and Jizan regions (Abou-Zeid and Altalhi, 2006; Manzelat, 2019). *Agrocybe* is distinguished by basidiospores with a reduced to broad germpore, collybioid to tricholomatoid basidiomata (spore-producing structure with a fleshy club shape) with rusty to dark sporeprint, and a hymeniform (spore-bearing surface) pileipellis (the superior layer of hyphae in the cap). *A. pediades* have basidia (sporangium or spore-producing structure) with four sterigmata (the apical extension of the basidium) and pileus (caps) with appendiculate annular remnants residues (Niveiro et al., 2020). *A. pusiola* contains numerous nuclei when stained with acetocarmine, and has a veil coated with gelatin (Arnolods, 2006). The nucleotides deposited NCBI related to *A. pediades* reached 1504 whereas there were not more than 2 nucleotides for *A. pusiola* according to research using https://www.ncbi.nlm.nih.gov/taxonomy (8/20/2021). According to research using https://www.gbif.org/species/search (8/20/2021), there are more than 20,500 occurrences of *Agrocybe* including 127 species (although they have a global widespread, they have not yet been recorded in Saudi Arabia) where there has been 4826 and 150 recorded occurrences of *A.*

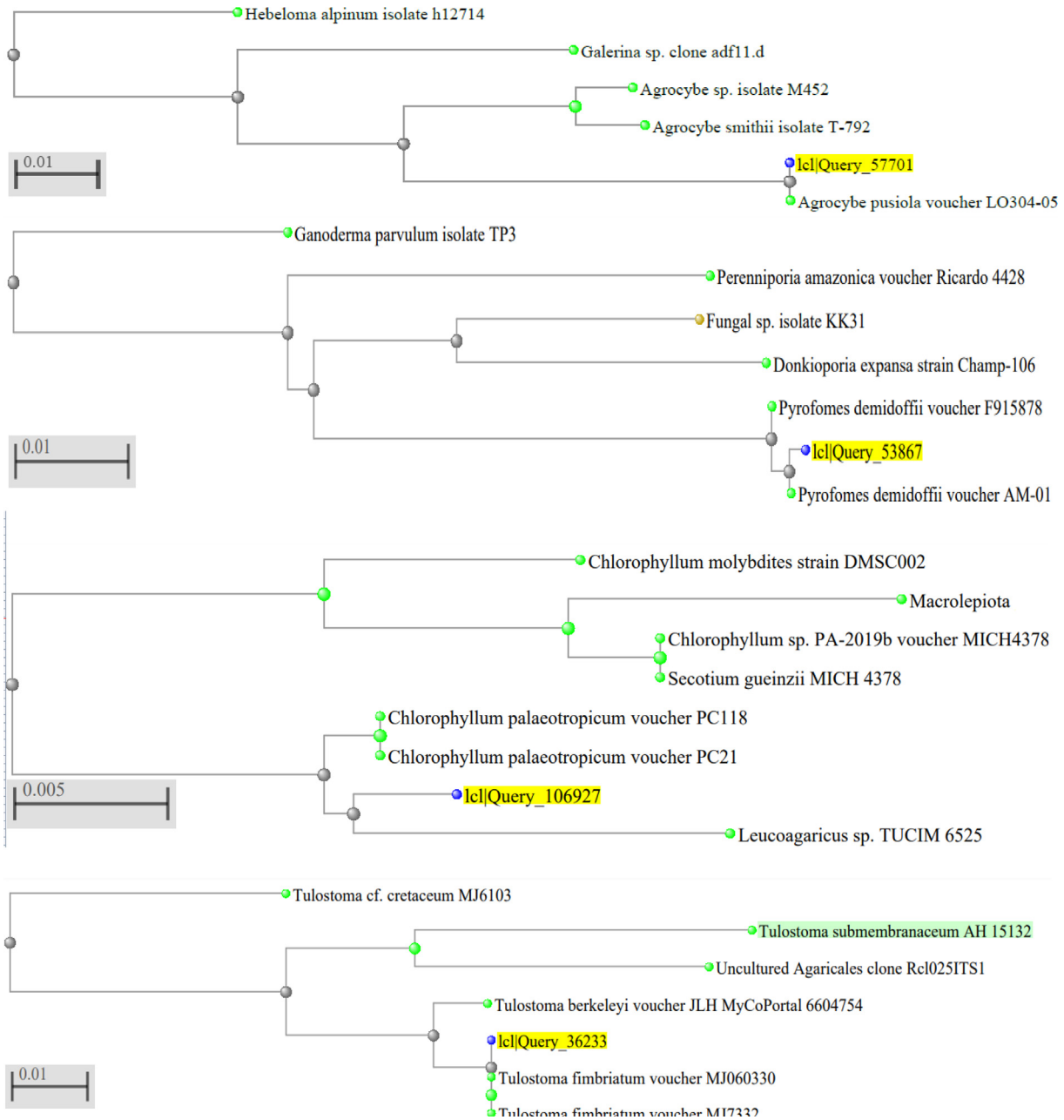

Fig. 5. BLAST analysis of mushroom ITS sequence for query-57701 (*A. pusiola*), query-53867 (*P. demidofii*), query-106927 (*C. palaeotropicum*), and query-36233 (*T. fimbriatum*).

pediades and *A. pusiola*, respectively, among all occurrences of *Agrocybe*.

The only mushroom species we could get from Al-Taif was *C. macrospora*. This does not mean that Al-Taif is free of other mushrooms, but as time and location are crucial factors in detecting the mushrooms, the mushrooms you may find today may not be found tomorrow in the same location. They can grow singly or in groups on manured soil, burnt land, forests, and grasslands. Its front view is ellipsoid to somewhat lemon-shaped; its side view is ellipsoid, slightly spread ventrally or slightly amygdaliform, germ-pore central, about 3.0 μm wide; and its wall is yellow to brown, thick in water becoming reddish-brown in alkaline conditions (Prydiuk, 2014). The worldwide distribution of *C. macrospora* is 158 occurrences, most of them are in Europe, and there are not any occurrences in Saudi Arabia (https://www.gbif.org/species/2529858 (8/20/2021). According to https://www.ncbi.nlm.nih.gov/Taxonomy, the nucleotides deposited NCBI related to *C. macrospora* are only 2 nucleotides refer to 3 strains of *C. macrospora*.

P. demidoffii is an important and dangerous parasitic fungal species on several juniper species. There is no case report in Saudi Arabia about fruiting body fungus. However, there are many international reports of *P. demindoffii* parasites on many plant species such as *Juniperus excelsa* and *Juniperus foetidissima*. (Dogan and Karadelev, 2006). In China, Su and colleagues developed a method to produce the mycelial biomass of *P. demidoffii* as a resource of medicinal and therapeutic products (Su et al., 2010). *Pyrofomes* spp. are characterized by their slightly red to reddish-brown basidiocarps that have no cystidia (large cell on the basidiospore-carps) and setae with thick-walled, amputate, variable pseudoamyloid (dextrinoid), cyanophilous basidiospores.

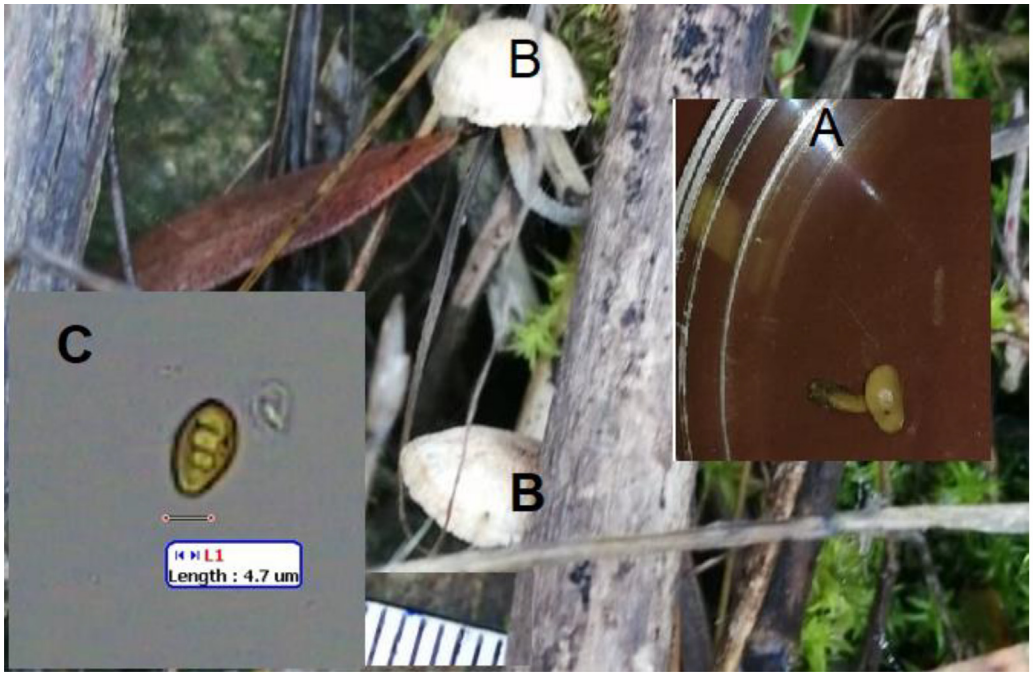

Fig. 6. Morphologic and spore characteristics of *A. pusiola* collected from Al Baha, Saudi Arabia. In the laboratory (A), in the field (B) and image of single spore.

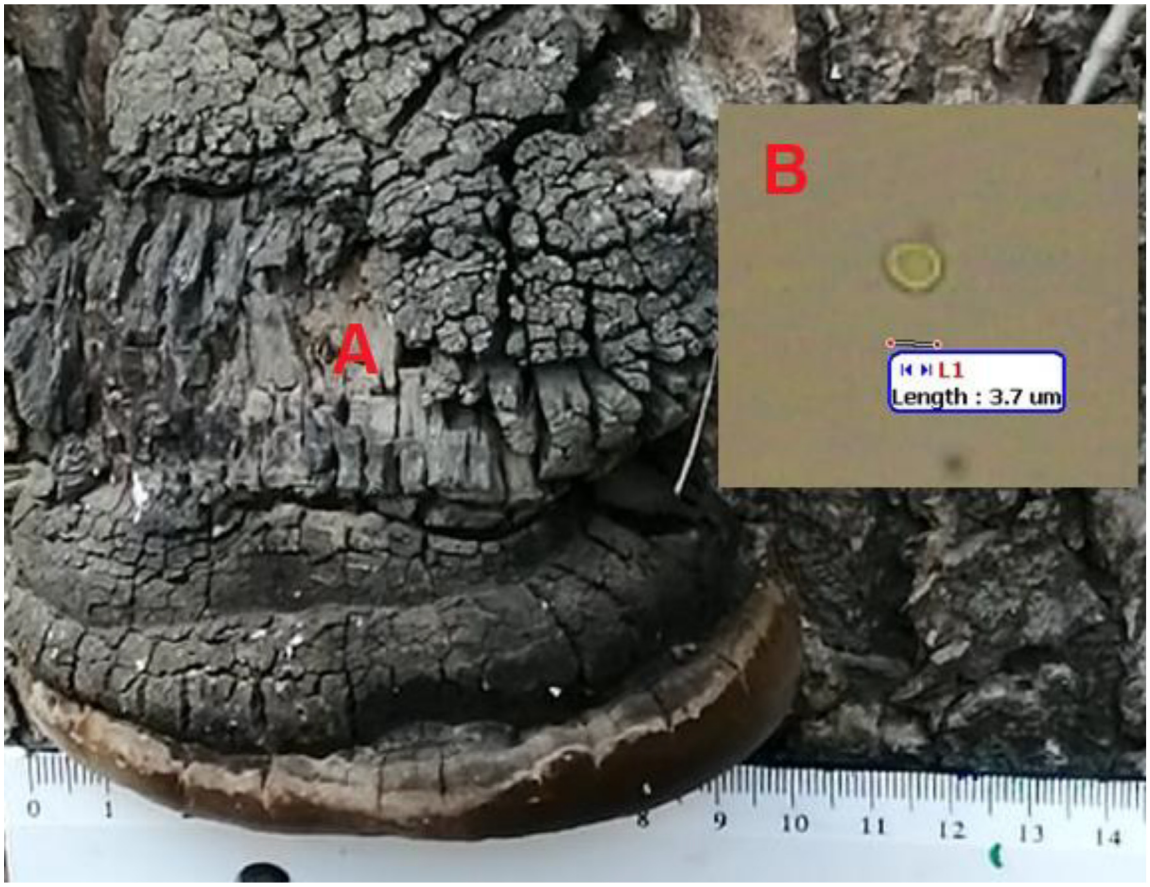

Fig. 7. Morphologic and spore characteristics of *P. demidoffii* collected from Al-Baha, Saudi Arabia. In the field (A) and image of single spore (B).

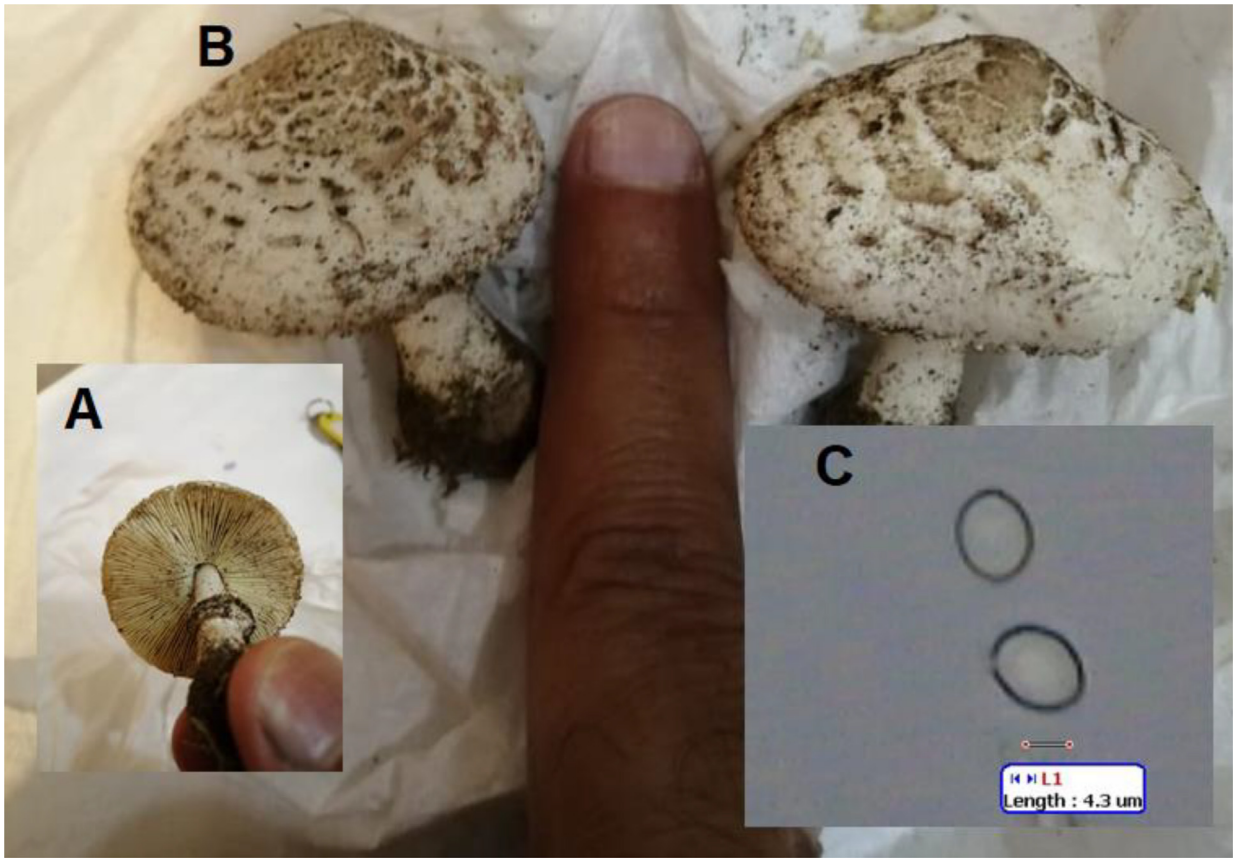

Fig. 8. Morphologic and spore characteristics of *C. palaeotropicum* collected from Jeddah, Saudi Arabia. In the laboratory (A and B) and image of spores (C).

Fig. 9. Morphologic and spore characteristics of *T. fimbriatum* collected from Al-Baha, Saudi Arabia. In the laboratory (A), in the field (B) and image of spores (C).

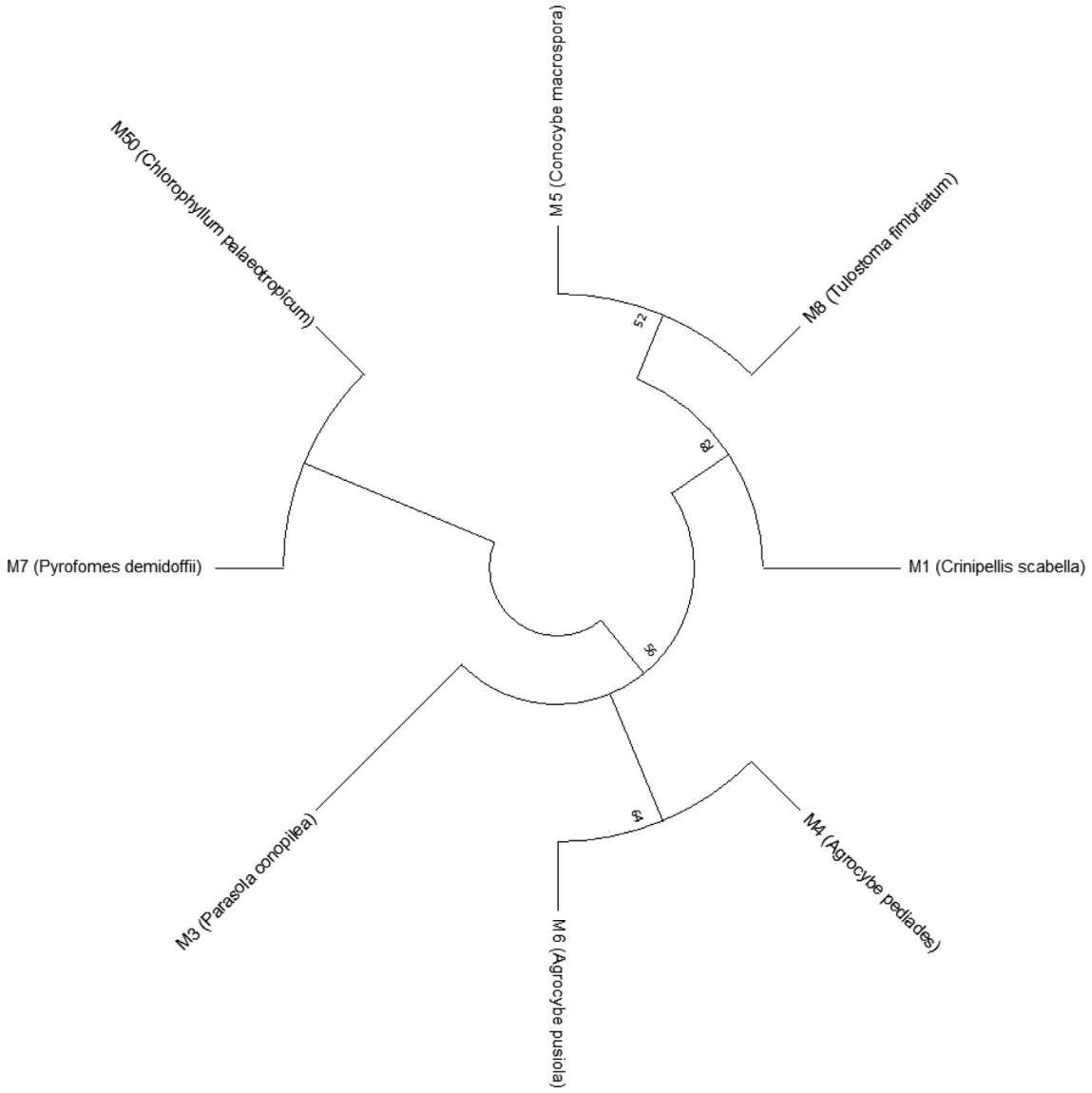

Fig. 10. The evolutionary history using the maximum likelihood method and Hasegawa-Kishino-Yano model.

P. demidoffii is characterized by the brightly rusty red color of the context tissue (Assefa et al., 2015). According to https://www.gbif.org/species/2546121 (8/20/2021) there were 139 occurrences of *P. demidoffii* globally, but not one record in Saudi Arabia. This fungus is frequently collected from many regions in Asia and North America. A total of 33 nucleotides that have been deposited in NCBI related to *P. demidoffii* (https://www.ncbi.nlm.nih.gov/taxonomy (8/20/2021).

Research effort, location, and time are important factors for recording new mushroom species in any country. For example, Hobart reported that it is surprising that *T. fimbriatum* was not recorded in the United Kingdom before 2012, although it is one of the most frequently recorded species of *Tulostoma* in Europe (Hobart, 2012). *T. fimbriatum* reportedly grows in open lands, forests, and cultivated lands on sandy soil (Karadelev and Rusevska, 2009).

In our work, all *T. fimbriatum* samples were collected from the surfaces of sandy soils in Al-Baha. *T. fimbriatum* is one of the most commonly distributed *Tulostoma* species worldwide and it has distinguishable features including fimbriate (having a narrow border) stoma, hyphal exoperidium (a protective layer that encloses spores), and its spores have verrucose and decoration of subreticulate (Esqueda et al., 2004).

There are 1934 occurrences of *T. fimbriatum* Fr. worldwide according to https://www.gbif.org/species/5243305 (8/20/2021), but no case has been recorded yet in the Middle East and Africa. According to https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=1305709 (8/20/2021), there are five subspecies of *T. fimbriatum*: var. *campestre, egranulosum, heterosporum, punctatum, and tuberculatum*, and the total number of nucleotides deposited was 67.

In the present study, we did not find any fruiting body fungi from the Abha region during the above collection period, but it does not mean that the mushrooms cannot grow in the natural environment in the Abha region. We believe that more researches and surveillance are required during different seasons, along with surveys in several valleys where human activity is low.

5. Conclusion

The findings obtained from the present work reported the existence of eight species of *Agaricomycotina* recorded for the first time in Saudi Arabia. *C. scabella, P. conopilea, A. pediades, P. demidoffii,* and *T. fimbriatum* were collected from Al-Baha while *C. macrospora*, and *C. palaeotropicum* were collected from Al-Taif and Jeddah, respectively.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2022.102345.

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