A new record of *Agaricus litoralis*, a rare edible macro-fungus from Eastern Algeria

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Abstract

Agaricus is one of the most important genera of saprobic fungi in the worldwide with more than 500 species. The objective of this study was to investigate a rare species of edible macrofungus of the phylum Basidiomycota belonging to the genus *Agaricus* (Agaricales, Agaricomycetes) in the forested areas of Algeria. This macro-fungus was identified as *Agaricus litoralis* Wakef. & Pearson using morphological and molecular data. *A. litoralis* was characterized by a white annulus membranous and its whitish context compact, pileus hemispherical then plano-convex, surface whitish, with bitter odor. The identified *A. litoralis* was interacted with the initiator and matched its counterparts by 99% in Genbank mushroom record with serial number MW165560 of the International Centre for Biotechnology Information (NCBI). It is the first registration of this type of Algeria mushroom at the World Genetic Bank as shown in the registration information to study the convergence and similarity of registered fungi.

Keywords: Agaricomycetes, *Agaricus litoralis*, Algeria, Phylogeny, Taxonomy.

Introduction

Agaricus is a genus of macro-fungi within the order Agaricales (Basidiomycota). It has including more than 500 species in all over world such as Asian countries, North America, Europe as well as in some part of Africa (Chang and Miles, 1989; Gui *et al*., 2015; Zhao *et al*., 2017; Chen *et al*., 2019). These species are common in different places as forests, pastorale and herbage land (Callac and Chen, 2018). The *Agaricus* species are characterized by a stipe detachable from the pileus. The stipe provided with one or several annuli, the basidiome produce brown spore called basidiospore. Typically, the odor faint or fungoid similare anise or phenol, the cheilocystidia often absent or hardly different from immature basidia (Parra, 2008; Zhao *et al*., 2011). Nevertheless, the taxonomy of *Agaricus* has been well developed using DNA-based phylogenetic methods (Zhao *et al*., 2017; He *et al*. 2018; Parra *et al*., 2018). The taxonomic classification of *Agaricus* genus includes twentyfour sections and six subgenera (Chen *et al*., 2017; Callac and Chen, 2018; He *et al*., 2018; Parra *et al*., 2018).

Due to climatic conditions and biodiversity of Algeria, the forest of this country is rich with higher macrofungi diversity especially the edible mushrooms (Ait-Hamadouche *et al*., 2021; Zatout *et al*., 2023). Despite of incredible popularity, little work has been carried out on the collection and identification of the wild edible macro-fungi in Algeria (Khodja, *et al*., 2020; Mesfek, *et al*., 2021). In particully, Djebel el Ouahech's forests of Constantine region (Zatout *et al*., 2021a). Thus, the main aim of this work was to identify and edible wild macro-fungus (*Agaricus* sp.) that are the most abundant in Djebel el Ouahech's forests, (Constantine, Algeria).

Materials and Methods

Studied area and specimen sampling

The harvests were carried out during two periods of the yaear; December 2017 and May 2018 from Djebel el Ouahch's forest (Constantine, East of Algeria), which covers 66,535 ha (Fig. 1). It lies between 36°14'20,19 "and 36°33'55,81" North latitude and between 6°38'0,82 "and 6°58'37,65" East longitude.

Morphological studies

The morphological identification of macro-fungi was carried out at the Laboratory of Mycology, Biotechnology and Microbial Activity (LaMyBAM), Department of Applied Biology, University of the Mentouri Brothers, Constantine 25000, Algeria. The

macro-fungi were photographed dirctly in the same palce of their natural habitat. Collecting data including locality, date, habitat, odor and colour of the basidiomata were recorded in the field. The description of tese macro-fungi was made from macrocharacters such as shape, size and color of pileus, lamellae, annulus, stipe and context (Zhao *et al.*, 2011; Courtecuisse and Duhem, 2013). A voucher specimen was deposited at the Herbarium of Université de Montpellier 2, Institut de Botanique, France.

Molecular studies

Molecular identification was carried out at ALVALAB, Oviedo 33006, Spain. Total DNA was extracted from dry specimens employing a modified protocol based on Murray and Thompson (1980). A piece of tissue (about 0.5 cm) was blended in 600 µL CTAB 2X buffer with the aid of a micropestle and incubated at 65 ºC for 10 min. 550 µL of chloroform were gently mixed with the sample and the mixture centrifugated at 13000 rpm for 10 minutes. The supernatant was transferred to a new tube and mixed with 450 µL of cold isopropanol. Samples were then centrifugated again for 15 min at the same speed, and the supernatant discarded. The pellet was washed in 300 µL of cold 70% ethanol and centrifugated again for 2 min at the same speed. Supernatant was discarded and the pellet dried at room temperature. Finally, it was resuspended in 200 µL of dd H_2O and freezed.

PCR reactions included 35 cycles with an annealing temperature of 54 ºC (Mullis and Faloona 1987). The forward primer ITS1F (5'-TCC GTA GGT GAA CCT GCG G-3') (Gardes and Bruns, 1993), and reverse primer ITS-4 (5'-TCC TCC GCT TAT TGATAT GC3') were employed to amplify the ITS rDNA region (White *et al*., 1990). PCR products were checked in 1% agarose gels, and amplicons were sequenced with PCR primers. The sequence was deposited in GenBank.

Phylogenetic analyses

The Maximum Likelihood method and Tamura-Nei model were using to construted the phylogenetic tree (Tamura and Nei 1993). This tree, with the highest log likelihood (-1743.10) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. The tree of *Agaricus* species is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 19 nucleotide sequences. There were a total of 650 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

Results and Discussion

Taxonomy

The harvested edible macro-fungus was:
Agaricus litoralis **(**Wakef. & Pearson), Pilat (1952), *Klíc Kurc. Hrib. Bedl.* (Praha): 403 – Fig. 2.

Synonyms

Psalliota litoralis Wakef. & A. Pearson, Brit. Mycol. Soc. 29(4): 205 (1946)
Psalliota spissa F.H. Møller, Friesia 4(1-2): 43 (1950) [1949-50]
Agaricus spissus (F.H. Møller) Pilát, Acta Mus. Nat. Prag. 7B (1): 5 (1951)
Agaricus spissicaulis FH Møller, Friesia 4(3): 203 (1952) *Agaricus maskae* Pilát, Ceská Mykologie 8(4): 165 (1954)
Agaricus maskae var. *imrehii* Bohus, Annls hist.-nat. Mus. natn. hung. 66: 83 (1974).

Macroscopic characteristics

The macro-fungus revealed several macroscopic characteristics shown in Fig. 2. Pileus 7–15 cm in diam., hemispherical then plano-convex, surface whitish, then grey-ochraceous-brownish, sometimes light brown, when mature the disc broken into brown scales, margin first involuted then appendiculate, entire, distinctly exceeding the lamellae when young. Lamellae free, crowded, pale, then flesh-coloured, finally dark brown at maturity. Stipe short, robust, ventricose, almost smooth, with the base usually prolongated by one or more rhizoids. Annulus membranous, white, fragile, persistent, rather ample, upper side striate, lower side smooth, often torn and appendiculate at pileus margin. Context compact, thick, whitish, slightly reddening when cut especially towards stipe base, 32 of bitter almonds. Taste mild, fungoid. Spore prints brownish.

Material examined

Agaricus litoralis, ZRDO25 (MW165560), Roukia Zatout: Algeria. Djebel el Ouahch forest's Constantine, May 2018, five mature and seven thalli (immature or incomplete) at various stages of development have been observed.

Habitat

Scattered in the grass- and herb-rich dry grazed meadows and steppe.

Distribution

This species, recorded for the first time in Algeria, was originally described from Britain. It has been also recorded from Morocco and from several European countries. It seems to be quite common locally in Oland and Gotland islands in Denmark. *A. litoralis* is a rare species in Algeria, and this mushroom was growing on grass- and herb-rich dry meadows. Also, on grazed, steppe dry meadows with

plenty of juniper bushes. Wakefield and Pearson first described this species as *Psalliota litoralis* (Pearson, 1946) in Britain. It has mostly been reported from Europe, especially Scandinavia and Great Britain, but also under other names (*Agaricus spissicaulis* F.H. Møller from Denmark, *Agaricus maskae* from the Czech Republic (Møller, 1952; Pilát, 1954). While it may be quite common locally, such as on Öland and Gotland islands, it is rarely found in most areas except southern Europe. Because the genus *Agaricus* is significantly diversified in Europe and poorly documented so far in the Mediterranean regions, it is likely that *A. litoralis*, morphologically like other species such as *Agaricus arvensis*, *Agaricus crocodilinus*, has been already collected but misidentified in North Africa. (Malencon and Bertault, 1970; El Kholfy *et al.*, 2011) reported it from Morocco as *Agaricus spissicaulis*.

Molecular identification

This study represents an edible macro-fungus *A. litoralis* that was recorded for the first time in Algeria (Zatout *et al.*, 2021b; Zatout and Chaouche, 2023). The samples identification was based on morphological observation and DNA sequence analyses of the ITS region. Use of ITS marker for identification of fungi has been reported in many recent studies (Khan and Javaid, 2020, 2021, 2022). The BLAST result was obtained by comparing partial nucleotide sequences to the GenBank database (Table 1). The sequences matched those of *A. litoralis* from Spain (one substitution gagacG/Aacttc difference compared whith KT951327 (identity 99.85%) and from France (one gap and two substitutions differences compared to JF727867 (identity 99.72%). The phylogenetic analysis presented in Fig. 3, confirms the monophyly of the clade encompassing sequence MW165560.1 from Algerian sample and sequences JF727867 from sample identified as *A. litoralis* (Zhao *et al.*, 2016). The holotype of *A. litoralis* has not been sequenced. Three markers (LSU, ITS (KT951327) and Tef1-α) have been used to characterize sample LAPAG420 (Zhao *et al.*, 2016). ITS from specimen voucher CA829, well-known mycologist, have been sequenced (JF727867) and cluster in the same ITS clade as KT951327. Then we will consider this clade as corresponding to *A. litoralis* until proven otherwise. AJ884642, AJ884641 and AJ884640 belong to the same clade. The first one was named *Agaricus maskae* in GenBank, but this is a synonym of *A. litoralis* (cf. Index Fungorum). The two others are named as *A. romagnesii* but are misidentified as this name is a synonym of *A. bresadolanus* (cf. Index Fungorum) and *A. bresadolanus* correspond to another clade represented in Zhao *et al.* (2016) by ITS sequence DQ185570 (sample CA177). These two sequences correspond to *A. litoralis* too. AJ887993 and MK156341 sequences have more differences and belong to *A. litoralis* complex. This is the first published record of *A. litoralis* in Djebel el Ouahch forest (Constantine, East Algeria).

Conclusion

The identification of the harvested wild edible mushroom was based on macroscopic and microscopic characterization completed with a molecular characterization. The study of the effect of physicochemical factors (temperature, pH, relative humidity and light) on the mycelial growth of the isolated strain of mushroom indicated that there is always a value allowing the optimal growth. The results showed the nutritional value of cultivated edible mushrooms from isolated strain and their importance. It is interesting to note that they are very rich and sources of nutrients such as carbohydrates, proteins and low-fat content.

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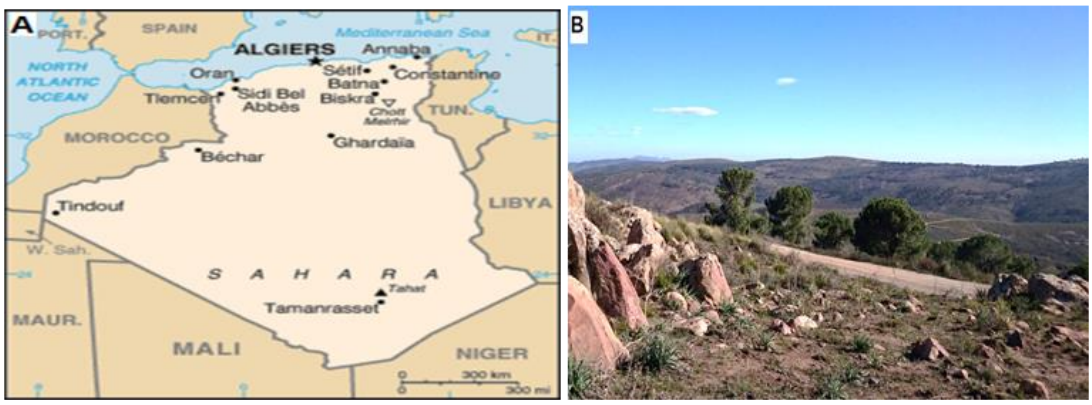

Fig. 1: The studied area. **A:** The map of the study area (in triangle). **B:** The Djebel el Ouahch forest.

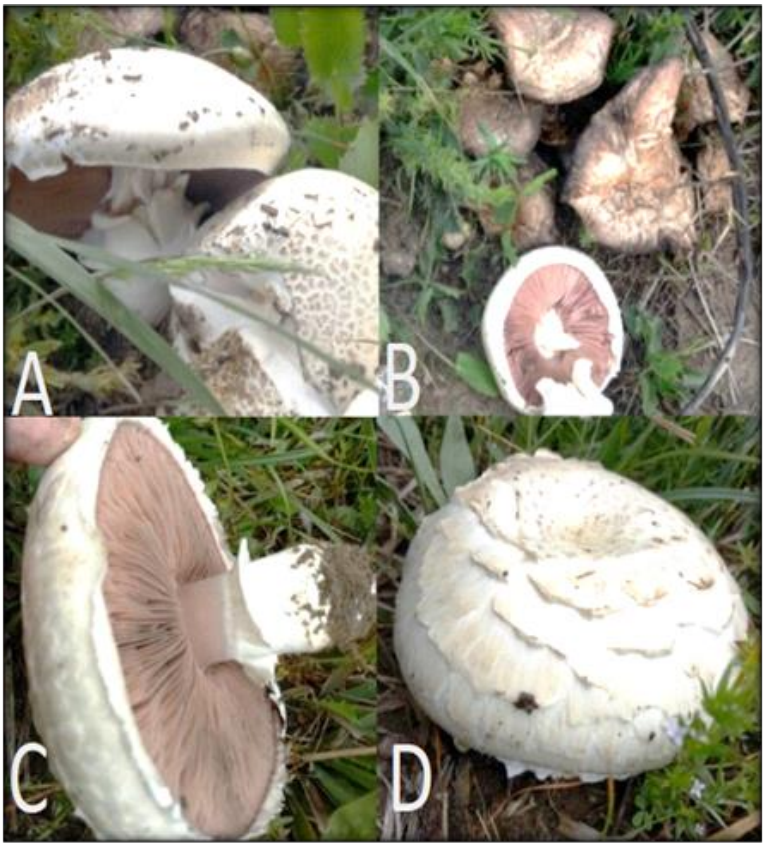

Fig. 2: *Agaricus litoralis*. **A:** Basidiomata in the field. **B:** Mature basidiomata. **C:** Lamellae and stipe with annulus. **D:** Cap surface.

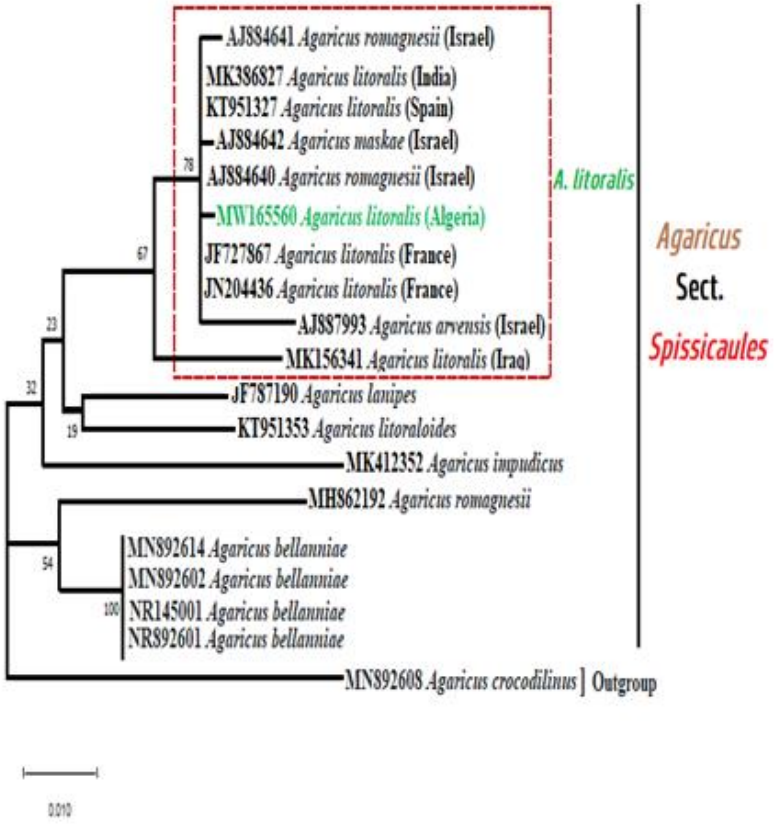

Fig. 3: Phylogenetic maximum likelihood (ML) tree of *Agaricus litoralis*.

Table 1: List of used specimens in molecular phylogenetic studies and their GenBank accession numbers. The strain from the current study is in bold.

Species name	Specimen or strain Id	GenBank accession No	Origin	References
Agaricus sp.	792	AJ884641	Israel	Didukh *et al.* (2005)
Agaricus litoralis	YM-19	MK386827	India	unpublished
Agaricus litoralis	LAPAG420	KT951327	Spain	Zhao *et al.* (2016)
Agaricus sp.	816	AJ884642	Israel	Didukh *et al.* (2005)
Agaricus sp.	791	AJ884640	Israel	Didukh *et al.* (2005)
Agaricus litoralis	**ZRDO25**	**MW165560**	**Algeria**	**This study**
Agaricus litoralis	CA829	JF727867	France	Zhao (2016)
Agaricus litoralis	CA120	JN204436	France	Zhao *et al.* (2012)
Agaricus sp.	15	AJ887993	Israel	Didukh *et al.* (2005)
Agaricus litoralis complex	RA44	MK156341	Iraq	Unpublished
Agaricus lanipes	CA406	JF797190	France	Zhao (2016)
Agaricus litoraloides	ZRL2011249	KT951353	China	Zhao *et al.* (2016)
Agaricus impudicus	GLM: GL M-F39352	MK412352	Germany	unpublished
Agaricus sp.	CBS 623.89	MH862192	India	Vu *et al.* (2019)
Agaricus bellanniae	S.D. Russell MycoMap #1591	MN892614	USA	Unpublished
Agaricus bellanniae	S.D. Russell MycoMap #964	MN892602	USA	Unpublished

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