Revue Agrobiologia
www.agrobiologia.net
ISSN (Print): 2170-1652
e-ISSN (Online): 2507-7627

FIRST RECORD, PHYLOGENETIC ANALYSIS, AND KEY TO *LACTARIUS MEDITERRANEENSIS* (RUSSULALES, BASIDIOMYCOTA) AND ITS RELATED SPECIES IN TLEMCEN'S NATIONAL PARK (ALGERIA)

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Reçu le 29/09/2020, Révisé le 12/12/2020, Accepté le 31/12/2020

Abstract

Description of the subject: The *Lactarius* genus has always attracted the interest of mycological researchers and has therefore occupied an important position in the world of mycology, from which comes the importance of exploring our little-known macrofungal diversity. This study was done in Tlemcen's National Park.

The goal of the study is to explore the national park of Tlemcen in order to discover the biodiversity of *Lactarius* genus, particularly those of socioeconomic and scientific interests.

Methods: Samples collected during the rainy season were identified based on their macroscopic and microscopic characteristics, then the results were confirmed by a molecular phylogenetic analysis based on the sequences of the ITS region.

Results: The use of molecular identification in this study allowed us to identify three species of *Lactarius*, which form ectomycorrhizal associations with pine trees (*Pinus halepensis*) or *Quercus* (*Q. ilex* and *Q. fagenia*). Among these, *L. sanguifluus* has already been reported previously in the central part of the country, but *L. mairie* and *L. mediterraneensis* are a new record.

Conclusion: This current study carried out in Tlemcen's National Park (HOT SPOT of Biodiversity), reports the presence of three species; of which two of them are rare. The rare species includes: *L. mairei* and *L. mediterraneensis* recorded for the first time; and *L. sanguifluus*, a common excellent edible mushroom, and widely appreciated for its high market value in Europe especially in Spain.

Keywords: *Lactarius*, Tlemcen's National Park, Algeria, molecular phylogenetic analysis, ITS.

PREMIER RECORE, ANALYSE PHYLOGÉNÉTIQUE ET CLÉ DE *LACTARIUS MEDITERRANEENSIS* (RUSSULALES, BASIDIOMYCOTA) ET DE SES ESPÈCES APPARENTÉES DANS LE PARC NATIONAL DE TLEMCEN (ALGÉRIE)

Résumé

Description du sujet : Comprenant plus de 600 espèces acceptées, le genre *Lactarius* a toujours intéressé les chercheurs en mycologie et a donc occupé une position importante dans le monde de la mycologie, d'où vient l'importance d'explorer notre diversité macrofongique peu connue. Cette étude a été réalisée dans le parc national de Tlemcen.

Les objectifs de l'étude sont d'explorer le parc national de Tlemcen afin de découvrir la biodiversité du genre *Lactarius*, à savoir celles d'intérêt socio-économique et scientifique.

Méthodes : Les échantillons collectés pendant la saison des pluies, ont été identifiés sur la base de leurs caractéristiques macroscopiques et microscopiques, puis les résultats ont été confirmés par une analyse phylogénétique moléculaire basée sur les séquences de la région ITS.

Résultats : L'utilisation de l'identification moléculaire dans cette étude nous a permis d'identifier trois espèces de *Lactarius*, qui forment des associations ectomycorhiziennes avec les pins (*Pinus halepensis*) ou *Quercus* (*Q. ilex* et *Q. fagenia*). Parmi ceux-ci, *L. sanguifluus* a déjà été signalé précédemment dans le centre du pays, mais *L. mairie* et *L. mediterraneensis* sont un nouveau record.

Conclusion : Cette originale étude, réalisée dans le parc national de Tlemcen (HOT SPOT d'une Biodiversité), a permis de signaler la présence de trois espèces ; deux entre elles sont rares : *L. mairei* et *L. mediterraneensis* enregistrées pour la première fois ; et *L. sanguifluus*, connu comme un excellent champignon comestible, et largement apprécié pour sa valeur marchande en Europe, en particulier en Espagne.

Mots-clés: *Lactarius*, Parc national de Tlemcen, Algérie, analyse moléculaire phylogénétique, ITS.

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INTRODUCTION

Lactarius is one of the largest known genera with more than 600 species accepted worldwide, but the actual number is estimated to be at least 800 species [1]. Lactarioids can be easily identified for a long time in the field by their particular character of exudation of latex (milk) from the basidiome when they are injured [2]. They survive under different forest formations, on different substrates, from sea level to high altitude [3]. Several hundred currently recognized, Lactarius taxa play an important role as late-stage colonizers of a variety of trees and shrubs in a wide range of ecosystems [4, 5]. Forty-three of the Lactarius species known so far had an ectomycorrhizal character [6]. The symbiosis of the genus Lactarius with conifers and deciduous trees has been widely studied [7]. Thus, Lactarius species are fungi with high commercial value, especially on local markets in Europe [8, 9, 10]. This genus includes 94 edible species reported from 39 countries [11]. Edible species of good quality belong to the Dapetes section, which includes orange, red-orange, purplish red latex milk. The *L. sanguifluus*, belonging to this section are among the most appreciated species in the Mediterranean countries [3]. Fifty-six different species are regularly harvested and consumed in 17 countries where the commercial harvesting of edible mushrooms is an important local activity [11]. In addition to its culinary quality, Lactarius is a potential source of bioactive compounds of medical or nutraceutical importance [12]. Mycological studies in Algeria are still rare. René Maire (1887-1949) [13], Georges Malençon (1898-1984) and Raymond Bertault (1905-1986) [14] were the pioneers of mycology in North Africa at the beginning of the 20th century. Their research was mainly concentrated in Morocco, "Flora of the higher mushrooms of Morocco» the outcome of their work. Although, in recent years, some Algerian scientists have developed interest in wild mushrooms Youcef Khodja [15], Djelloul & Samraoui [16], Mesfek [17], Benazza-Bouregba [18], macromycetes are still insufficiently studied. In Algeria, only two species of Lactarius have been reported, *L. sanguifluus* and *L. deliciosus*, collected in (1972) in Cheria's mountains in central Algeria [19].The species *L. deliciosus* was associated to holm oak in Relizane's forest (west of Algeria) [17] and *L. sanguifluus* associated to cork oak in M'sila's forest (Oran, western Algeria) [18]. However, no molecular phylogenetic study has been carried out for these species. In this study, we provide morphological descriptions, focusing on the three species of Lactarius (*L. mediterraneensis, L. mairei* and *L. sanguifluus*), collected from the forests of Tlemcen's National Park, located in the Betico-Rifain complex, one of the Mediterranean HOT SPOTS of Biodiversity [20]. We supported our work by a molecular phylogenetic analysis based on the sequences of the ITS region (Internal Transcript Spacer) of nuclear ribosomal DNA, by comparing the Algerian species of Lactarius with those reported in other Mediterranean countries.

MATERIALS AND METHODS

1. Description of the study area

Mushrooms were collected from five sites of Tlemcen's National Park, in the west of (Algeria). It is one of the Mediterranean HOT SPOT of Biodiversity [20]. (Fig.1).

1.1. Three in the Aleppo pine forests

-The first two sites are the forest of (Lalla Setti) and the forest of (Attar), resulting from an artificial plantation of *Pinus halepensis*, which was carried out in 1890, stretches to the northwest, sitting on a fersialitic soil, at an altitude between 1039 m and 1198 m.

-The third site is a reforestation carried out during sixties; it is a dense forest stretching to the east. Its altitude varies between 1009 m and 1040 m [21].

1.2. Two sites in Hafir's cork oak stand

The cork oak forest of Hafir is one of the very few natural relics of *Quercus suber* from western Algeria [19]. It is located in the southwest of the city of (Tlemcen). Its altitude is about 1158 meters above sea level. This forest covers 1653 ha and belongs to the communes of Ain Ghoraba and Sabra. The most important species that inhabit this forest are the cork oak (*Quercus suber)*, the holm oak (*Quercus rotundifolia)* and the Zeen oak (*Quercus faginea sp tlemceniensis)*. These forests are in a sub-humid bioclimatic atmosphere with a maximum rainfall of 600 mm which occurs from September to February [20].

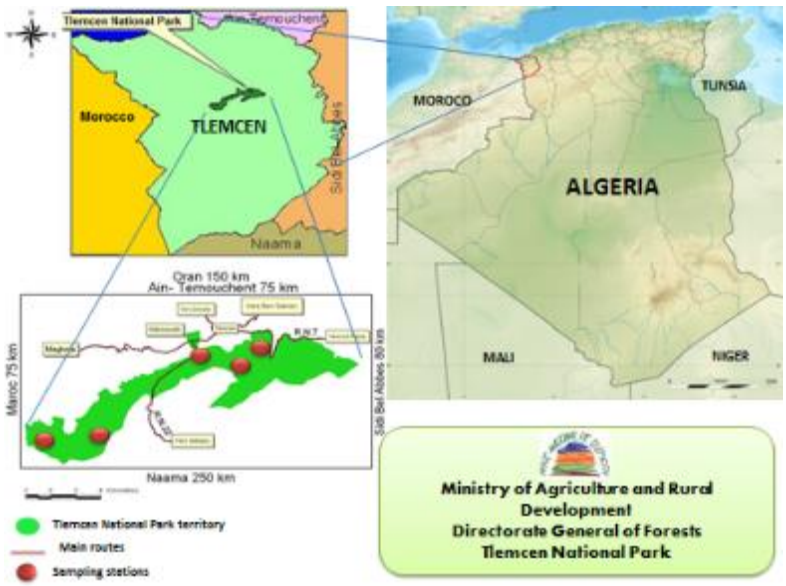

Figure 1: Geographic situation of Tlemcen's National Park and sampling sites [21].

2. Collection of Basidiocarps

Sampling sites were visited regularly after each favourable weather in spring and autumn during 2014-2017. *Lactarius* species were harvested especially after the first autumn rains. All mushroom's information related to their harvesting site which encompasses; the locality, geographical coordinates and altitude (using a GPS) as well as ecological data (type of vegetation, substrate, nature of the soil, proximity of a watercourse, exposure and so on) were noted. The specimens were photographed on the ground to keep the details of the fresh state. The carpophores were packed in polythene bags to avoid evaporation and crumbling. This was carefully brought to the laboratory. Each sample isolated was given an individual collection number. The macroscopic descriptions concerned the shape, colour, appearance and other peculiarities as the cap and the stipe. This study was supplemented by a microscopic description of some sections: hymenium, cuticle, flesh, stipe and veil. The dimensions of spores, cystids, and basids were also noted. Microscopic study using a compound microscope (Leica DM 1000, Leica Microsystems Ltd, Germany) was carried out on freshly collected mushrooms. A thin sliver using a sharp razorblade was cut off and placed on a slide, stained with Lactophenol cotton blue and Melzer's reagent then covered with a cover slip. Any excess stain was removed with an absorbent tissue. Observations were done after few minutes [55]. About the morphological identification, the authors used the published taxonomic keys of Galli [3], Kranzlin [23], Basso [24], Courtecuisse & Duhem [25], Bon [26], and Roux [27]. Collected sporocarps were dried and deposited at the personal fungarium at Tlemcen's University.

3. Molecular Identification

Mushroom material (approx. 20 mg) was dried in a freeze-dryer for 24h and genomic DNA was obtained by using the DNeasy® Plant Mini Kit (Qiagen GmbH, Hilden,Germany) following the manufacturer's instructions. Fungal specific primers ITS1/ITS2 [28], ITS-1F/ITS-4 [29; 30] were used to amplify the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. The negative control (no DNA) was included in each set of amplification. The following profile was used for PCR, initial denaturation step at 95°C for 2 min followed by 35 cycles, each consisting of 95°C for 40s, annealing temperature 53°C for 30s and 72°C for 40s, with a final extension at 72°C for 5 min. PCR products were loaded in a 1% agarose gel immersed in 1 x TAE buffer and electrophoresis was run for half an hour at 70V. The gel was stained with ethidium bromide (0.5 µg mL^{-1}) and photographed on a UV transilluminator with a Polaroid camera. PCR product was thereafter purified according to user's manual of PCR clean-up gel extraction Macherey-Nagel Gmbh &co. KG. Germany and then sequenced in both directions using the same pair of primers (ITS1/ITS2 and ITS-1F/ITS-4).

4. Sequence Data Analysis

Initially, the nrITS sequences of Algerian *Lactarius spp*. were analysed and compared in the GenBank through Basic Local Alignment Search Tool (BLAST) network services using National Center for Biotechnology Information (NCBI), USA database. The closely related sequences were retrieved from the GenBank and aligned by ClustalW in Molecular Evolutionary Genetics Analysis (MEGA7) software [31]. Then, Neighbor-Joining (NJ) analysis was performed in the MEGA software using Kimura 2-parameter nucleotide substitution model. The nucleotide sequences of *L. sanguifluus* and *L. mediterraneensis* sequences generated in this study were deposited in GenBank (accession numbers MH160427-MH160437) (Tab. 1).

Table 1: Accession numbers of Algerian *Lactarius* specimens.

Species	Tlemcen's University herbarium numbers	Collection Date / GPS/ locality	GenBank accession numbers
Lactarius sanguifluus	uabtAl3	January 2015- November 2016. 34°51'32.5"N/ 1°18'52.3"W *Pinus halepensis* forest	MH160427
Lactarius sanguifluus	uabtAl4	January 2015- November 2016. 34°51'22.0"N /1°20'17.4"W *Pinus halepensis* forest	MH160428
Lactarius sanguifluus	uabtAl13	January 2015- November 2016. 34°51'10.0"N /1°21'25.8"W *Pinus halepensis* forest	MH160429
Lactarius sanguifluus	uabtL6	January 2015- November 2016. 34°51'10.9"N /1°21'16.8"W *Pinus halepensis* forest	MH160430
Lactarius sanguifluus	uabtL16	January 2015- November 2016. 34°51'08.7"N/1°21'05.3"W *Pinus halepensis* forest	MH160431
Lactarius sanguifluus	uabtL61	January 2015- November 2016. 34°51'40.0"N /1°18'34.9"W *Pinus halepensis* forest	MH160432
Lactarius sanguifluus	uabtL116	January 2015- November 2016. 34°51'08.5"N /1°20'02.6"W *Pinus halepensis* forest	MH160433
Lactarius mairei	uabtL32	November 2015 34°45'33.5"N /1°27'37.5"W Mixt forest of *Quercus ilex* and *Q. fagenia*	- -
Lactarius mediterraneensis	uabtT11	November 2015 34°45'33.5"N/ 1°27'37.5"W Mixt forest of *Quercus ilex* and *Q. fagenia*	MH160434
Lactarius mediterraneensis	uabtT11A	November 2015 34°45'42.1"N/ 1°29'18.6"W Mixt forest of *Quercus ilex* and *Q. fagenia*	MH160435
Lactarius mediterraneensis	uabtT11C	November 2015 34°45'32.6"N /1°28'41.2"W Mixt forest of *Quercus ilex* and *Q. fagenia*	MH160436
Lactarius mediterraneensis	uabtT11C1	November 2015 34°45'35.1"N/ 1°27'54.5"W Mixt forest of *Quercus ilex* and *Q. fagenia*	MH160437

*uabt: Tlemcen's University fungarium

RESULTS

1. Description

1.1. Lactarius mediterraneensis Llistosella & Bellù 1996 [32].

The studied specimens (uabtT11, uabtT11A, uabtT11B, and uabtT11C) were harvested on November 2015.

Ecology: growing in gregarious form, on limestone soil, under a mix of green oak (*Quercus ilex*) and zeen oak (*Quercus fagenia*).

Cap: is about 3.5-14 cm in diameter. The hat depressed, fleshy, pinched by the navel, precocious and concave in the middle, at the end of the funnel, lumpy, lobed; thin margin, first rolled in, then down, smooth, full. Cuticle of the filamentous hat formed by a thick layer of interspersed hyphae elastic, viscous, shiny and very separated by gelation, with rounded and non-capitated emergent terminations. Creamy, yellow-fleshed, cream-yellowish, light yellow-orange colour, with mostly marginal, irregular and concentric, darker, ocher-pink or brown-dyed dimples.

Flesh: is very hard, medium thickness and firm, then stony, whitish, with a yellowish cut, then creamy. With potash gives a typical yellow reaction of chromium, which disappears after a while then intense and immediate to Guayacol blue, with slight odour of honey and taste very spicy. Fruity and weak aroma, bitter taste. The hymenophore gills slightly spaced, from the adnate to the subdecoliform, discrete, not elastic.

Gills: arched, sometimes forked and venous, joined to the stem, cream, yellowish cream, pale ocher, ocher brown in the lesions. Pointed rule, whole and colour.

Stem: is about 2.5-5 × 1.5-3 cm, short and squat, cylindrical thinned towards the base, or conical trunk, also compressed, smooth with ice, slightly eviscerated downwards; full, then bony, fragile and finally hollow; dry, opaque and bloomy, white or cream and with few depressed circular spots scatte, attenuated or moniliform at the tip, similar macropos red towards the base, tinged with red ocher in old specimens.

Spore Print: Light ochre in bulk.

Milk: is fluid, white, immediately turns yellow by oxidation when isolated and on flesh and gills (Fig. 2A).

Odour and Taste: Acre and bitter this is why the species is not edible.

Microscopic Features: Basidiospores are ellipsoidal, medium-sized sub-globular from 10-12 x 8-9.5 µm (Fig. 3A). Spores are covered with fine amyloid ridges which form a very complete cross-linked, with shallow, forming essentially complete networks; trivial basidian, claved, tetrasporic. Macrocosilocystid are almost lanceolate to fusiform (Fig. 3A), more abundant in the edges, and well dispersed in the laminae, narrow and terminated in a long tip of average size 31-49 × 5-7 µm, attenuated or moniliform at the tip. Slightly larger spores exist 49-71 × 7-9 µm in some bulges and terminal strangulations (Fig. 3A).

1.2. Lactarius mairei Pearson A. 1950 [3]

Ecology: The studied specimen (uabt L32) was harvested on November 2015, on limestone soil, under a mix of green oak (*Quercus ilex*) and zeen oak (*Quercus fagenia*).

Cap: is from 3-6.5 cm in diameter. The hat is little fleshy, clearly funnel or depressed. The entire surface appears velvety. The coating is thin, sticky by wet weather, smooth in the centre, woolly elsewhere, hairy more and more towards the edge (Fig. 2).With scales grouped arranged in a concentric way. The colour is uniform, yellow to yellow-ocher, but became darker with age, yellow-orange to red ocher (Fig. 2B).

Flesh: spongy at the beginning of the stem, pink white at the cut.

Gills: fine and tight, decurrent, curved, arched forked, with many anastomosing sheets. Coloured undertones from cream-ocher to ocher-yellow.

Stem: is short, cylindrical, attenuated at the base, hard, lapidary at first, and finally cord, dry, smooth or slightly bloomy with cream colour to yellow.

Milk: is in the beginning sweet, then slowly pungent which makes the species inedible.

Odour and Taste: Its odour is fruity, pungent taste.

Microscopic Features: Ellipsoidal to subglobose spores (Fig. 3B) are of average size 6-8 × 5.4-6.1 µm (5.5), with walls decorated with ridges and amyloid warts, others thick and thin joined in a discontinuous network;

basid trivial, subclavated, tetrasporic. Macrocheilocistid are numerous (Fig. 3B), subfusiform, acuminate, mucronated or moniliform at the top, with about 30-60 × 5-8 µm. In our microscopic observations, some macropleurocistids appear larger 40-90 × 7-12 µm. Intermediary pileipellis are between ixocute and ixotricoderma, slightly gelled consisting of sub-cylindrical, irregular hyphae thick and medium-large, 58µm in wide, collated and emerging tufts in particular towards the fleece margin. Cystids on the faces and edges of very large and emergent leaves, fusiform and ending in a point. Basal claviform or a little paunchy and tetrasporic.

1.3. Lactarius sanguifluus (Paulet) Fries1838 [14]

Ecology: Species represented by (uabt Al13, uabt Al3, uabt Al4, uabt L6, uabt L61, uabt L16, uabt L116) harvested on January 2015 and autumn 2016 under a dense stand of *Pinus halepensis* and grows gregarious, on calcareous soil in north exposure.

Cap: is from medium to large size, 7-16.5 cm. It's fleshy, at the beginning convex flat, but immediately spread and depressed in the centre, at the extremity in the form of a funnel, regular, but lobed and undulated; with the straight edges, long contoured, then curved down, wavy, lobed, finely felted overall (Fig. 2C).

The cuticle: is thin, elastic, slightly viscose in wet weather, dries quickly, shiny and matte appearance especially at the dry moment, no or little zoned. Creamy-orange with concentric bands reddish or vinous and in the old specimens scattered greenish spots that come to invade the entire surface (Fig. 2C), constituted by cylindrical hyphae, broad on average 2-5 µm, intertwined and branched.

Flesh: is firm and thick, slightly lapidary and could be detached from the stem, whitish in the inner areas, dark red or purplish red in the outer areas and in particular above the hymenophore.

Stem: is cylindrical, short and thick of about 2.5-7 × 1.2-2.7 cm. Attenuated towards the base, smooth surface with veined veins, with small irregular dimples downwards; firm and full at first, hollow and cavernous, as if eaten by worms; dry and dull, pruinose in white at the top, on a reddish winey background; adorned with darker small dimples, usually pink to reddish; greenish spots in the lesions.

Hymenophore: is thick gills, adnate under decurrent, thin, with many small gills, arc-shaped, fork-shaped at the stem, venous, attached from the root to the stem, lilac-pink colour, vinous pink, washed with lilac. With injury or with age they become purplish-red then greenish.

Spore Print: Spores are creamy.

Milk: latex mostly sweet low, medium, dark red, purplish red. It becomes green on the blades or if isolated.

Odour and Taste: Odour more or less fruity, sweet or slightly bitter flavour.

Microscopic Features: Basidiospores are ovoid-elliptic (Fig. 3C), of about 7.9-10 × 6.6-11 µm; egrets, with little thick and multiple edges thin connections to form incomplete lattices; basidio trivial, subclavati, tetrasporic. Macrocheilocistid are numerous, almost spindle-shaped, of average size of 30-50 x 7-9 µm but some of them are longer up to 60 µm, attenuated or moniliform above (Fig. 3C).

Figure 2: Morphological aspects of Algerian *Lactarius* species
L. mediterraneensis (A), *L. mairei* (B) and *L. sanguifluus* (C)

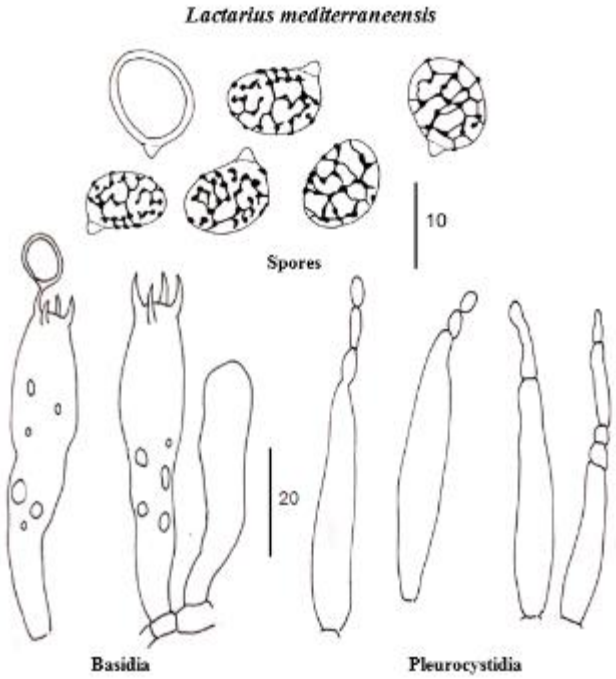

Figure 3A: Microscopic characters: *L. mediterraneensis*.
1. Spores: small, globose. Subreticulate with crest, almost zebra, 2. Basides: tetrasporic.
3. Pleurocystides: pointed spindle-shaped

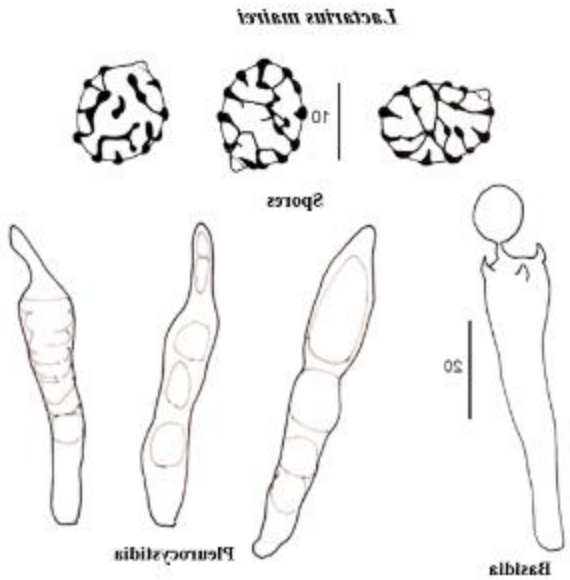

Figure 3B: Microscopic characters: *L. mairei*.
1. Spores: oval. Cross-linked to full network, 2. Basidia: tetrasporic. Length equivalent to 5 times the size of the spore
3. Pleurocystidia : rare and condensed in certain places. Few emerging, the same size or smaller than the basides. Spindles with pointed and pluri-strangled apex, 4. Epicutis: slightly gelled trichoderma (presence of spores stuck to the hairy coating)

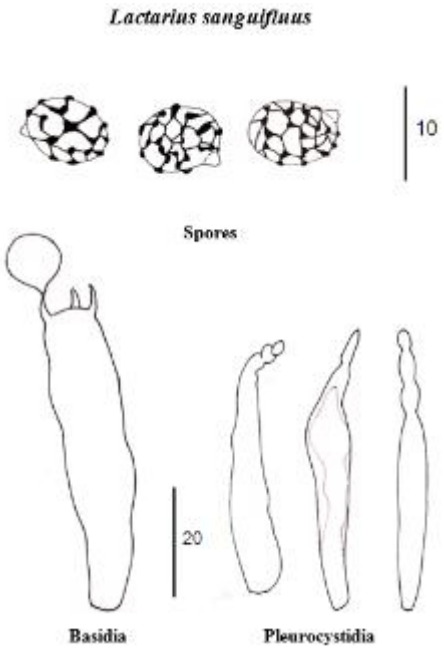

Figure 3C: Microscopic characters: *L. sanguifluus*.
1. Spores: subglobular. Crested, cross-linked with an almost complete network, 2. Basidia: tetrasporic. Length equivalent to 6 times the size of the spore, 3. Pleurocystidia: larger than the basides. Spindle-shaped with a long tapered, multi-constricted end, 4. Epicutis: erect trichoderma.

Figure 3: Microscopic characters: A. *L. mediterraneensis*, B. *L. mairei* and C. *L. sanguifluus*.
1: Spores, 2: Basidia, 3: Pleurocystidia, 4. Epicutis (Drawing by Hayat NADOUR)

2. Phylogeny

The phylogenetic analysis of 11 sequences of Algerian *Lactarius* species and other references ones from GenBank are presented in Figure 4.

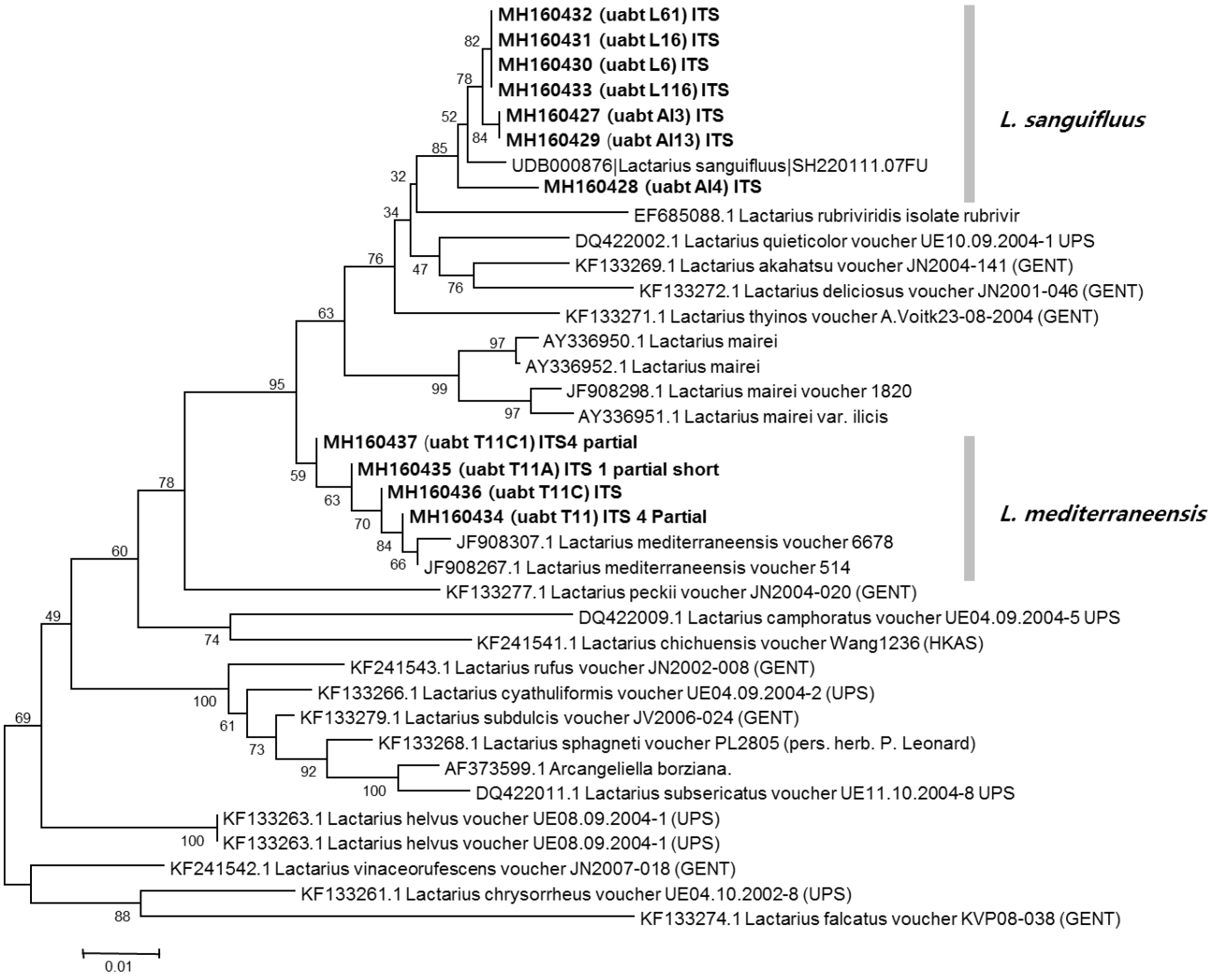

Figure 4. The Neighbor-joining tree of Algerian *Lactarius* spp. based on ITS rDNA nucleotide sequences. The bootstrap values are indicated above the corresponding branches. The sequences generated in this study are shown in bold. The scale bar indicates the proportion of sites changing along each branch.

DISCUSSION

Seven sequences from the uabt specimens which were morphologically identified as *L. sanguifluus* formed a robust clade with the reference sequence of *L. sanguifluus* (UDB000876). Four sequences of Algerian specimens were clustered with *L. mediterraneensis* with a weak bootstrap support (53%). However, excluding the shortly sequenced ones, uabt T11A and T11C1, the *L. mediterraneensis* clade received a moderate support (70%). In this study, 12 samples of *Lactarius* species from Tlemcen's National park in the North West of Algeria were collected. They are identified morphologically as *L. sanguifluus*, *L. mediterraneensis* and *L. mairei*. The identification of the two first species was confirmed by molecular analysis using ITS region.

Lactarius mediterraneensis was recently discovered [32]. It is one of the most rare and little-known Lactaries in the Mediterranean area [33]. It has been reported in Greece [34], Catalonia [35], the Balearic Islands [32], Provence and Costa Azzurro, Sardinia, Liguria, Tuscany, Sicily [3], and even in Pakistan [36] very recently. However, it has never been reported in North Africa before this work. The collected species correspond to the description macroscopic and microscopic of Llistosella & Bellù [32], Galli [3] and Basso [24]. Carpophores of *L. mediterraneensis* persist until late autumn. It is a thermophilic and mycorrhizal species. It was growing in gregarious form, on limestone soil, under a mix of green oak (*Quercus ilex*) and zeen oak (*Quercus fagenia*) in Hafir's forest, while its presence has been reported in association with *Quercus ilex* only [3; 24].

Lactarius mairei is a rare species; it was harvested for the first time in Algeria and North Africa. Under a mixture of holm oak (*Quercus ilex*) and zeen oak (*Quercus fagenia*). It is mentioned in the Red List of Romanian Macrofongus Species as almost threatened species (NT) [37], and as endangered species in the Red List of threatened species in Switzerland [38]. It is a characteristic species of the Mediterranean environment, mycorrhizal of holm oak (*Quercus ilex*) [3]. Its presence has also been reported under *Cistus monspeliensis* and *C. salviaefolius* [24]. It forms mycorrhizae with rockrose (*Cistus ladanifer*) and other rockrose [39].

Lactarius sanguifluus occupies a large area of distribution. It belongs to the *Lactarius* sect. *Deliciosi*, which groups together ectomycorrhizal fungi (CEM) that produce valuable edible mushrooms [7].

Generally, they form ectomycorrhizae with Pines (*Pinus* spp) [40]. In China it is associated with *P. yunnanensis* or *P. radiata* [7], in Turqui with *P. pistachio*, *Cantharellus cibarius* and *Picoa lefebvrei* [41], in Spain [14 ; 39] with *P. halepensis* and *P. pinaster*, In Italy to *Pinus nigra* and *Pinus sylvestris* [3] in Belgium to *P. sylvestris* [42], in Tunisia to *P. pinea* [43], in Morocco to *P. maritima* [44 ; 40] and even in Algeria [19] to *P. halepensis*. However, *L. sanguifluus* is declared a potentially threatened species in Switzerland because of its decline in these numbers [23].

Our study signals the presence of *Lactarius sanguifluus* for the first time, in Tlemcen's National Park. It grows in autumn and early winter, associated with *Pinus halepensis* on calcareous soil. *Lactarius deliciosus* and probably other close species, such as *L. sanguifluus*, are food species, with a millennial transition, already known since ancient Rome [3]. Several species of the genus *Lactarius: L. deliciosus*, *L. sanguifluus*, and *L. semisanguifluus* are valued and consumed worldwide [45]. *L. deliciosus* and *L. sanguifluus* have been marketed in Europe, Asia and North Africa [8]. Indeed, it is sold in rural markets [46], Italy and Turkey [47]. In Cyprus, *L. sanguifluus* is widely collected by the locals, but considered inferior to the saffron milk cap (*L. deliciosus*) [48]. In Spain; the city of Barcelona, where the mushroom is considered a culinary specialty in Catalan cuisine, they are abundantly sold fresh on the markets (including the very famous and ancient "la Boqueria") and packaged in stores [3]. Some locals consider it to have a better flavour than its more well-known relative [49]. In Asia, the mushroom is appreciated in Yunnan Province, China [50]. In Algeria, the locals do not know this mushroom, they are afraid of consuming a poisonous one. Some old people from the region of Tizi Ouzou in the central part of the country and in Tlemcen's Mountains confirmed that they collected some *Lactarius* species to sell to French during the colonial period. They learned how to recognise them by French colonizers themselves and take these mushrooms as their source of life. According to their description, it was *L. sanguifluus* and *L. deliciosus*.

In addition to its culinary interest, the pharmacologically important properties of the genus *Lactarius* are also incredible [51]. According to a Turkish study, *L. sanguifluus* is classified as a mushroom with strong antioxidant activity [41]. Another study has confirmed the antimicrobial activity of methanol from extracts of several lactates, including *L. sanguifluus* and *L. deliciosus* [52]. Considering their culinary and pharmaceutical interests, their export trade is stimulated by strong and growing demand, which cannot be sustained by sustainable logging [53; 9]. Utilization of *L. sanguifluus* offers two interests for forestry in Algeria. On one hand, as ectomycorrhizal partners, this species have a potential positive effect on the growth and physiology of host pines [54]. On the other hand, managing mycorrhizal inoculations with edible *Lactarius* to increase forest productivity can be a promising alternative for many Mediterranean forest areas with limited resources [7].

CONCLUSION

This work is the first contribution to the study of Lactaries in Tlemcen's National Park (HOT SPOT), using molecular phylogenetic analyzes. The presence of three species of the genus *Lactarius* has been reported; two of them are rare and registered for the first time in Algeria. These are: *L. mairie* and *L. mediterraneensis*; the third species is *L. sanguifluus*, first reported from Tlemcen's National Park. *L. sanguifluus* is recognized as an excellent edible, and widely appreciated for its market value in Europe, in particular in Spain. These lactaries form mycorrhizal associations with pines (*Pinus halepensis*) or Quercus (*Q. ilex and Q. fagenia*). These non-wood forest products can contribute to wealth creation and the development of a local economy, but also to the preservation of the fragile forest ecosystem. As the demand increases and rural communities struggle to find new sources of income. There is a growing need for better information on existing trade so that informed decisions can be made that both protect natural resources and allow their sustainable harvest. Edible mushroom stimulation would be a significant economic diversification in Mediterranean zones where the environmental conditions greatly limit timber production. Collaboration between research organizations and investors can foster an appropriate climate for innovation and the creation of ecological start-ups.

These micro-enterprises, by exploiting innovative techniques, can contribute to the fight against poverty and unemployment, and ensure sustainable development, while preserving biological resources.

Acknowledgements

This paper is the result of the convention on 'edible mushrooms in Algeria' between the University of Tlemcen, Algeria and RDA, South Korea represented by Kopia Algeria. The authors wish to acknowledge Dr Jaeung Kim, Director of Kopia Algeria, Dr. Sanggu Park, Dr. Pae Do Ham (former directors of Kopia Algeria). Responsible of RDA Korea and those of University Abou Bekr Belkaid Tlemcen. We also thank all researchers in Mushroom Research Division, National Institute of Horticultural and Herbal Science, Eumseong, Republic of Korea". Finally, we want to thank Mr. Bright Danso, PhD student in the department of Forest resources, Tlemcen's university, for proofread and English corrections.

REFERENCES

[1]. Verbeken A. and Nuytinck J. (2013). Not every milkcap is a Lactarius. Scripta Botanica Belgica, 51: 162-168.

[2]. Maba D.L. (2015). Diversity, Molecular phylogeny, Ecology and Distribution of the genera Lactifluus and Lactarius (Russulales, Basidiomycota) in West Africa, Dissertation from the Faculty of Biology the Ludwig Maximilians, University of Munich, Munich, 11 p.

[3]. Galli R. (2006). I Lattari, Edition dalla Natura, Milano: 3-167.

[4]. Trapagese JM. (1962). Fungus associates of ectotrophic mycorrhizae. Botanical Review 28: 538-606.

[5]. Hutchison L.J. (1999). *Lactarius*. In: Cairney JWG, Chambers SM (eds) Ectomycorrhizal fungi. Key genera in profile. Springer, Berlin, 285 p.

[6]. De Roman M., Claveria V. and De Miguel A.M. (2005). A revision of the descriptions of ectomycorrhizas published since 1961. Mycological Research 109, 1063–1104.

[7].Wang R., Guerin-Laguette A., Huang L. L., Wang X.H., Butler R., Wang Y. and Yu F.Q. (2019). Mycorrhizal syntheses between Lactarius spages, section Deliciosi and Pinus spages, and the effects of grazing insects in Yunnan, China. Canadian Journal of Forest Research, (2019), Vol. 49, N° 6: 616-627.

[8]. Singer R. (1986). The Agaricales in Modern Taxonomy, vol. 1, Koenigstein Königstein im Taunus, Germany: Koeltz Scientific Books, 4ème édtion 981, 73 p.

[9]. FAO. (2004). Wild edible fungi, a global overview of their use and importance to people, by BoaE, Non-Wood Forest Products No. 17, Rome, 145 p.

[10]. Hortal S., Pera J., Galipienso L. and Parlade J. (2006). Molecular identification of the edible ectomycorrhizal fungus *Lactarius deliciosus* in the symbiotic and extra radical mycelium stages. Journal of Biotechnology 126 : 123–134.

[11]. FAO. (2006). Champignons comestibles sauvages vue d'ensemble sur leurs utilisations et leur importance pour les populations, par Boa E, Produits forestiers non ligneux N°17, Organisation des Nations Unies pour l'alimentation et l'agriculture, Rome, 156 p.

[12].Lazarević J. and Menkis A. (2018). Fungi inhabiting fine roots of Pinus heldreichii in the Montenegrin montane forests. Symbiosis, 74: 189–197.

[13]. Maire R. (2017). The mycologist and botanist René Maire (1878-1949). The course of a great naturalist in the context of colonial France. Environmental Dynamics, International Journal of Geoscience and the Environment. 39-40 : 202-239.

[14]. Malençon G. and Bertault R. (1971). Mushrooms Of The Iberian Peninsula, Departament Of Botanic - Faculty Of Science, University Of Barcelon, Acta Phytotaxonomica Barcinonensia. - Volume. 8, 55 p.

[15]. Youcef-Khodja L. (2010). Contribution à l'inventaire et à la connaissance des macrochampignons dans le Parc National de Chréa; impacts des facteurs écologiques. repository.usthb.dz

[16]. Djelloul R. and Samraoui B.(2011). Distribution and ecology of the superior mushrooms of the Aulnaie of Ain Khiar (El Kala National Park, Northeastern Algeria). African Journal of Environmental Science and Technology Vol. 5(6), p. 448-456

[17]. Mesfek F. (2014). Etude écologique et taxonomique des champignons forestiers et morphologie des ectomycorhizes du chêne vert dans la wilaya de Relizane. Mémoire de Magister en Biotechnologie. Université d'Oran. Algérie. 160 p.

[18]. Benazza-Bouregba M. (2017). Inventaire et identification des Basidiomycetes de la forêt de M'sila, Oran, Thèse Doctorat en Biotechnologie, Option: Microbiologie, Université d'Oran, faculté des sciences de la nature et de la vie, Département de Biotechnologie, pp. 150-152.

[19]. Dorleans G. (1972). Etude mycologique région de Chréa. Annales de l'Institut national agronomique El Harrach. Volume 7, pp. 27-42.

[20]. Medjahdi B. (2010). Response of the Coastal Vegetation Oranese in the Disturbances: Case of the Mounts des Trara (Northwest Of Algeria), Doctoral Thesis In Forestry, University Aboubekr Belkaid -Tlemcen, 78. p.

[21]. Tlemcen National Park. (2010). Management Plan II, Directorate General of Forests, Ministry of Agriculture and Rural Development, People's Democratic Republic of Algeria, pp.7-8.

[22]. Letreuch-Belarouci A. (2010). Structural characterization of the cork oak forests of Tlemcen National Park, natural regeneration and sustainable management, doctoral thesis in forestry, Aboubekr Belkaid-Tlemcen University, pp. 34-38.

[23]. Kranzlin F. (2005). Champignons de Suisse-, Edition Mykologia Lucerne, Tome 06, 317 p.

[24]. Basso MT. (1999). *Lactarius* Pers. Fungi Europaei 7. Mykoflora, Alassio, 845 p.

[25]. Courtecuisse R. and Duhem B. (1994). Guide des champignons de France et d'Europe, Ed.Delachaux & Niestle, Paris. 480 p.

[26]. Bon M. (1980). Clé monographique du genre *Lactarius* (Pers. ex Fr.) S.F. Gray. Documents Mycologiques 10 : 1–85.

[27]. Roux P. (2006). Mille et un champignons. Editions Roux-Sainte Sigolène, France, 129p. France, 1224 p.

[28]. White TJ., Bruns T., Lee S. and Taylor J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ. (Eds.), PCR protocols. A guide to Methods and Apageslications. Academic Press, San. Diego, CA, pp. 315–322.

[29]. Gardes M. and Bruns TD. (1999). ITS primers with specificity for basidiomycetes: apageslication to the identification of mycorrhizae and rust. Molecular Ecology 2: 113–118.

[30]. Manter DK. and Vivanco J.M. (2007). Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. Journal of Microbiological Methods Volume 71: 7-14.

[31]. Kumar S., Stecher G. and Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0. Molecular Biology and Evolution. Mol. Biol. Evol. 33(7):1870–1874 doi:10.1093/molbev/msw054 Advance Access publication March 22.

[32]. Llistosella J. and Bellú F. (1996). Lactarius mediterraneensis, a new species from the Mediterranean region. Dep. Plant Biology (Botany), Univ. Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain, Journal article : Mycotaxon. ref.9. Vol.57 pages.175-186.

[33]. Basso M T. (2013). Interesting milkcaps, poorly known or new, of the mediterranean zone. Editors: Fraiture, A. Journal article; Conference paper : Scripta Botanica Belgica Vol.51: 73-78.

[34]. Kaounas V., Konstantinidis G., Sofronis D., Gkilas M. and Kottis L. (2016). Seven interesting basidiomycetes recorded in Greece for the first time. Mycologia Montenegrina. Vol. 19: 97-112. 16 p.

[35]. Tabarés M. and Rocabruna A. (1998). Contribution to the knowledge of the fungi of the Sierra de Collserola (Catalonia), III. Catalan Journal of Mycology. , vol. 21: 171 – 183.

[36]. Khan J. (2018). Morphological and Molecular Characterization of Selected Macrofungi in Northern Areas of Khyber Pakhtunkhwa (Pakistan), University of Swat, Swat, PhD Thesis. http://prr.hec.gov.pk/jspui/handle/123456789/10160

[37]. Tănase C. and Adriana P. (2005). Red List of Romanian Macrofungi Species, Bioplatform – Romanian National Platform for Biodiversity, Editura Academiei Române , Bucureşti, pp: 101-107.

[38]. Senn-Irlet B., Bieri G. and Egli S. (2007). Red list of endangered species in Switzerland, published by the Federal Office for the Environment FOEN and by the Federal Institute for Forest, Snow and Landscape Research WSLBerne, pp: 22-44.

[39]. Fajardo-Rodríguez J., Blanco-Sidera D. and Verde López A. (2003). The lactarius genre in the province of Albacete, ALBACETENSES Studies Magazine, Number 4: 5-35.

[40]. Ferrer J., Maire J.C., Moreau P.A., Robich G., Alesandri J. and Lavoise C. (2009). Compléments À La Flore des Champigions Supérieurs du Maroc de G. Malençon et R. Bertault , Confédération Européenne de Mycologie Méditerranéenne, Nice, 649 p.

[41]. Erdoğan S.S., Soylu M.K. and Can Başer K.H. (2017). Antioxidant Properties of Some Wild Mushrooms, Nevşehir Science and Technology Journal Volume 6 (ICAFOF 2017 Special Issue), pp: 254-260.

[42]. Nuytinck J. and Verbeken A. (2005). Morphology and taxonomy of the European species in Lactarius sect. Deliciosi (Russulales), MYCOTAXON, Vol. 92 : 125–168.

[43]. Ouali Z., Compagno R., Sbissi I., Gargano M L., Rhaiem A., Ben Naceur M., Venturella G. and Jaouani A. (2016). A preliminary check list of macromycetes in northern Tunisia, Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology, pp: 31-58

[44]. Bertault. (1978). Lactaires du Maroc, Bull. Soc. mycol. France 94 (3), pp : 273-288.

[45]. Suárez J. O., Pajares J.A. and Diez J.J. (2018). In vitro antagonism of edible ectomycorrhizal fungi against Fusarium oxysporum and Fusarium verticillioides, the meeting of IUFRO, Working Party: Foliage, Shoot and Stem, Niagara Falls, The ForesTry ChroNiCle, Ontario, Canada, Vol. 94, N° 2, 118 p.

[46]. Biswas S., Datta M. and Ngachan S.V. (2012). Mushrooms: A Manual for Cultivation. PHI learning, 7 p.

[47]. Dugan F.M. (2011). Conspectus of World Ethnomycology: Fungi in Ceremonies, Crafts, Diets, Medicines, and Myths. APS Press, St. Paul, 151 p.

[48]. Loizides M., Kyriakou T. and Tziakouris A. (2011). Edible & Toxic Fungi of Cyprus (in Greek and English). Published by the authors. pp.188–89. ISBN 978-9963-7380-0-7.

[49]. Colman A. (2005). Catalan Cuisine, Revised Edition: Vivid Flavors From Spain's Mediterranean Coast. Houghton Mifflin Harcourt. p. 178.

[50]. Wang X.H. (2000). "A taxonomic study on some commercial species in the genus *Lactarius* (Agaricales) from Yunnan Province, China". Acta Botanica Yunnanica. 22: 419–27.

[51]. Joshi S., Vishwakarma M.P., Mahar R. and Bhatt R.P. (2013). Medicinally important and edible species of genus Lactarius from Garhwal Himalaya, India. Mycosphere 4(4): 714–720.

[52]. Barros L., Baptista P., Estevinho L.M. and Ferreira I.C. (2007). Effect of fruiting body maturity stage on chemical composition and antimicrobial activity of Lactarius sp. Mushrooms. J. Agric. Food Chem, 55(21): 8766-8771.

[53]. Hall I.R. and Wang Y. (1998). Methods for cultivating edible ectomycorrhizal mushrooms. In: Varma A (ed) Mycorrhiza manual. Springer, Berlin Heidelberg New York, pp. 99–114.

[54]. Guerin-Laguette A., Plassard C. and Mousain D. (2000). Effects of experimental conditions on mycorrhizal relationships between *Pinus sylvestris* and *Lactarius deliciosus* and unprecedented fruit-body formation of the saffron milk cap under controlled soilless conditions. Canadian Journal of Microbiology, 46: 790–79.

[55]. Khaund P. and Joshi S.R. (2014). Micromorphological Characterization of Wild Edible Mushroom Spores Using Scanning Electron Microscopy. National Academy Science Letters 37(6). DOI: 10.1007/s40009-014-0272-1.