

First Record of *Lepista sordida* (Schumach) Singer in Eastern North Africa

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A NEW record of *Lepista sordida* was collected from a lemon fruit farm in El-Sinania at Damietta District of North delta of Egypt in December, 2014. It was identified using morphological (macro and microscopic) and molecular techniques. Complete description was performed for the collected fresh fruiting bodies and isolated pure culture. Radial growth rate of culture was estimated on Potato dextrose and Malt extract agar media (8.5 & 7.8 mm/day, respectively). *Lepista sordida* and *L. nuda* are very close in morphological characters; hence, the identification was confirmed by DNA sequence analysis of the ribosomal 5.8S rRNA gene including the flanking internal transcribed spacers (ITS). Then, its taxonomic position among some genera of Tricholomataceae and its relation with some other *Lepista* species was discussed. The surrounding habitat was also observed and environmental conditions were recorded as Temperature degree (29.7°C), relative humidity (RH: 44.6) and soil moisture was (5.56). *Lepista sordida* was reported from South Africa and Nigeria (in the South), Algeria and Tunisia (in the North-west) and this is first record in North-East Africa.

Keywords: Tricholomataceae, *Lepista* sp., Edible mushroom, Morphological identification, Phylogenetic tree, ITS DNA sequencing.

Introduction

Lepista (Tricholomataceae) is a widespread genus, with many edible species such as *L. sordida*, *L. nuda* and *L. saeva* (Stott, 1998). Those mushroom species are relatively popular in Europe, America and Australia (Moser, 1978; Breitenbach & Kranzlin, 1991; Young, 1994 and Davis et al., 2012) but rare in Africa except Nigeria and South Africa (Ekwebelam, 1980 and Popich, 2014). *Lepista sordida* (Schumach) Singer was reported from Algeria and Tunisia (north-west Africa) in *Q. ilex* woods (Malençon & Bertault, 1970-1975). This species was also reported by Contu & Signorello (1999) from Sicily in *Eucalyptus* woods. There is no any record for *L. sordida* in north-east Africa.

Some of *Lepista* species have commercial potentials. *L. nuda* presents antioxidant properties (Murcia et al., 2002) and antibiotic activities against many bacteria (Dulger et al., 2002). Besides, *L. sordida* produces two diterpenes that induce differentiation in human leukemic cells (Mazur et al., 1996). Polysaccharides extracted from *L. sordida* possessed potent ant

proliferative effect on mice and human laryngeal carcinoma Hep-2 cells (Miao et al., 2013), also had antioxidant activity and retard aging effects (Zhong et al., 2013). Those medicinally important polysaccharides could be used as a potential natural antitumor drug and attenuate age-related diseases in humans.

Remarkable evolution has been made to affirm phylogenetic relationships in the largest order; Agaricales (Moncalvo et al., 2002; Garnica et al., 2007 and Binder et al., 2010). However, continued assessment of evolutionary relationships within this order is necessary. Also, the species of *Lepista* and some other Tricholomataceae are somewhat difficult to be differentiated by morphological descriptions. So, molecular techniques such as ribosomal RNA gene sequencing had been developed for their identification (Stott, 1998 and Stott et al., 2005).

The aim of the study is to advance the current knowledge of morphology, and molecular analysis of an Egyptian *Lepista* mushroom that grows in El-Sinania Farms at Damietta – Egypt.

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Materials and Methods

Morphology and identification

Descriptions of basidiomes were made according to their external and internal morphology. For external morphology the material was observed for colour, texture, gills morphology, margin, and pileal surface of basidiocarp. For internal morphology, thin hand sections were taken from pileus passing through gills, and mounted in also in Melzer's reagent (Beneke, 1958).

The slides were observed under Optika B-350 compound microscope having a combination of 10x eyepiece and 10x, 45x and oil immersion (100x), objectives. Photographs were taken using Canon digital camera. Measurements of basidiospores were taken using objective micrometer or calibrated ocular.

Isolation into pure culture was carried out directly after collection from the field sites according to the method of El-Gharabawy et al. (2016). Small pieces of either inner layers of the fruiting body tissue were cultured on to plates of potato dextrose agar (PDA), 2% malt extract agar (MA) under sterile conditions. Isolation plates were incubated at 25°C and pure cultures were maintained on PDA slopes at 4°C. Radial growth rate (RGR) was quantified on 9 cm Petridishes of PDA and 2% MA using 1cm discs of actively growing cultures at 25°C.

DNA extraction

Genomic DNA was extracted according to the procedure of Lee & Taylor (1990) with some modifications. Fresh fruiting body was washed with sterile water and frozen with liquid nitrogen followed by grinding with sterilized sonicator. Then 500 µl extraction buffer (Equal volume of 50 mM Tris-HCl (pH 7.5), 50 mM EDTA (pH 8.0) and 1% SDS (Sodium Dodecyl Sulphate) was added to the micro tube sample and incubated at 65°C for 30 min. After incubation the same volume of (25 Phenol: 24 Chloroform: 1 Isoamyl-alcohol) mixture was added, mixed by inverting the tubes and centrifuged at 4°C for 10 min at 12000 rpm. The DNA in the supernatant was precipitated by isopropanol, washed with 75% ethanol, resuspended in water free nuclease and then stored at -20°C until used for PCR amplification.

PCR amplification and sequencing of ITS-5.8S rRNA region

The oligonucleotide primers (ITS4: 5'TCCTC-CGCTTATTGATATGC³ and ITS5: 5'GGAAG-

TAAAAGTCGTAACAAGG³) used for amplification and sequencing of the 5.8S rRNA-ITS regions (White et al., 1990) were made by BI-ONEER, South Korea.

PCR reaction was carried out using a thermal cycler (TECHENE model FTC3102, UK). PCR mixture consisted of 4 µl of each primer (20 pmole ml⁻¹), 1µl of genomic DNA and 25 µl Dream Taq (Thermo scientific- Green PCR Master Mix) to a final volume of 50 µl. PCR was performed with initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, then final extension at 72°C for 10 min. The PCR product was then sequenced using the same ITS4/ITS5 primers by ABI 3730XL DNA Analyzer at Macrogen, South Korea.

Alignment and phylogenetic analyses

Obtained ITS nucleotide sequences were subjected to a BLAST search against the NCBI database (<http://www.ncbi.nlm.nih.gov/>) to match the best similarities with other related ITSs on database (Altschul et al., 1990, 1997). The best DNA sequence similarities with our ITS region were obtained from NCBI GenBank and aligned using CLUSTAL W (Thompson et al., 1994). Unalignable regions were excluded manually and the sequences from the same species and unidentified organisms were discarded. Finally, Phylogenetic tree analysis was done using MEGA version 4 (Tamura et al., 2007). The neighbor-joining was performed using the maximum composite likelihood methods (Tamura & Nei, 1993). The bootstrap values of 50 or above were only considered and represented next to the phylogenetic tree branches with confidence levels estimated by 1000 bootstrap replicates.

Results

Morphological characteristics

The taxonomic position: Fungi – Basidiomycota – Agaricomycetes – Agaricales – Tricholomataceae.

Habitat: The fruit bodies were saprobic, gregarious and form a fairy ring, on soil and in mixed lawn and usually in areas where leaf litter collects (Fig. 1A & B). The *Lepista* isolate had the code of EGDA2 strain (refers to Egypt, Damietta where it found). The samples were collected during field trip to El-Senania lemon fruit farms at Damietta district in 16th of December 2014, at N 31.4403°, E 31.7776°. Temperature was 29.7°C, relative humidity (RH) was 44.6 and the soil moisture was 5.56.

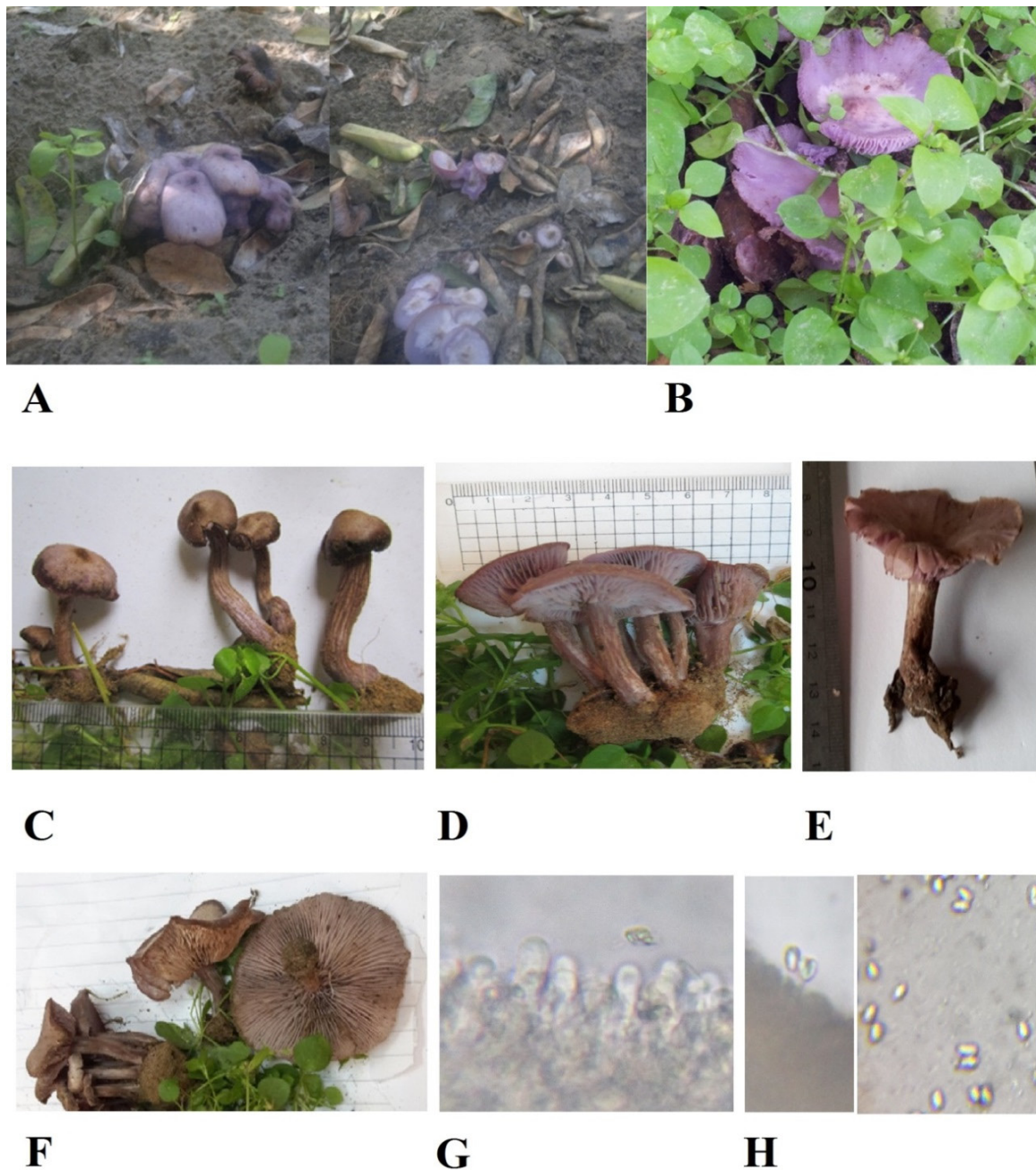


Fig. 1. Fruit body external morphology of *L. sordida* EGDA2 in the field gregarious and form a fairy ring (A & B), Cap is convex with a slight umbo (C) flattening out or developing a central depression with in-rolled margin (D & E). Gills sinuate or emarginate and crowded (F). Basidia (G) and basidiospores (H).

Description: The basidiocarp is deep violet colour with a thin cap margin. At maturity it is not distinguishable from *Lepista nuda*. Cap is 2 to 6 cm across; initially convex sometimes with a slight umbo (Fig. 1C), flattening out or developing a central depression at maturity (Fig. 1D, E), usually with a slightly a wavy margin and in-rolled; deep lilac, turning brown from the centre in dry weather. Cap surface is smooth not sticky even in moist weather. Gills sinuate or

emarginated and crowded (Fig. 1E & F), the gills are initially greyish lilac fading to buff with age. Stem is 2 to 4 cm long and 4 to 10 mm in diameter; fibrillose; lilac; downy and white at base with no ring. Basidia are narrow, clavate (Fig. 1G) with parallel trama, 2 or 4 spored. Spores are ellipsoidal, 6-9 by 4-5 μm (Fig. 1H) nonamyloid, colourless, hyaline, thin walled, roughened and ornamented with tiny spines. Spore print is creamy-white.

The pure culture of this species is white with faster radial growth rate on PDA (8.5 mm/ day) than on MA (7.8 mm/ day) (Fig. 2). Mycelium colonized all the Petridish within 8 days of incubation. Culture showed more aggressive

appearance on PDA as oyster mushrooms with thick growing tenacious mycelium and fluffy cottony surface. It also pins readily and easily on agar with pale violet reverse.

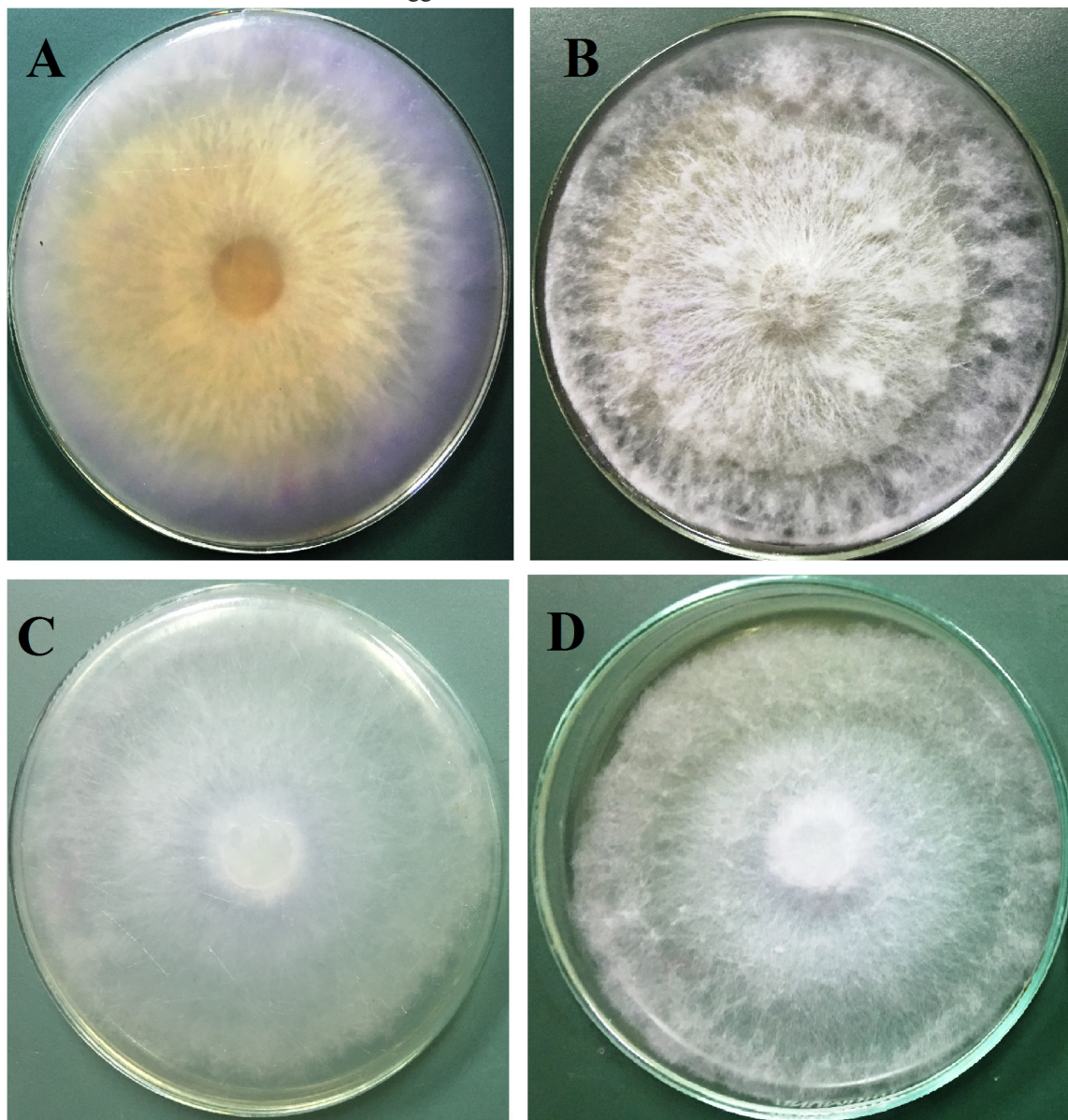


Fig.2. Culture of *Lepista sordida* EGDA2 growing on PDA (A & B) and MA(C & D).

ITS based identification

The PCR product sequencing of the ITS1-5.8S-ITSII rDNA region for *L. sordida* EGDA2 revealed 663 bp, which were submitted in the GenBank with accession number LN827702. The DNA sequence alignment of the studied ITS region for *L. sordida* EGDA2 showed the highest identity (100%) with *L. sordida* (KF874612) and (99%) with *L. sordida* (KJ137272). Some other different *L. sordida* and

L. tarda strains showed less similarities reached 95%-98%. Also, it exhibited 94%-95% identity with *L. nuda*, *L. saeva* and *L. personata* strains. *Tricholoma mongolicum* isoletes aligned with our strain at 96% identity, while different species of *Clitocybe*, *Collybia* and *Lyophyllum* showed 94%-95% similarities. All the previously mentioned alignments were significant as they possessed E value 0.0 using Blast program.

The phylogenetic tree based on ITS DNA sequence (Fig.3) clustered *L. sordida* EGDA2 in one clade with some other isolates of the

same *L. sordida* species possessing approximate dissimilarity distance reached 0.015 with *Tricholoma mongolicum* clade.

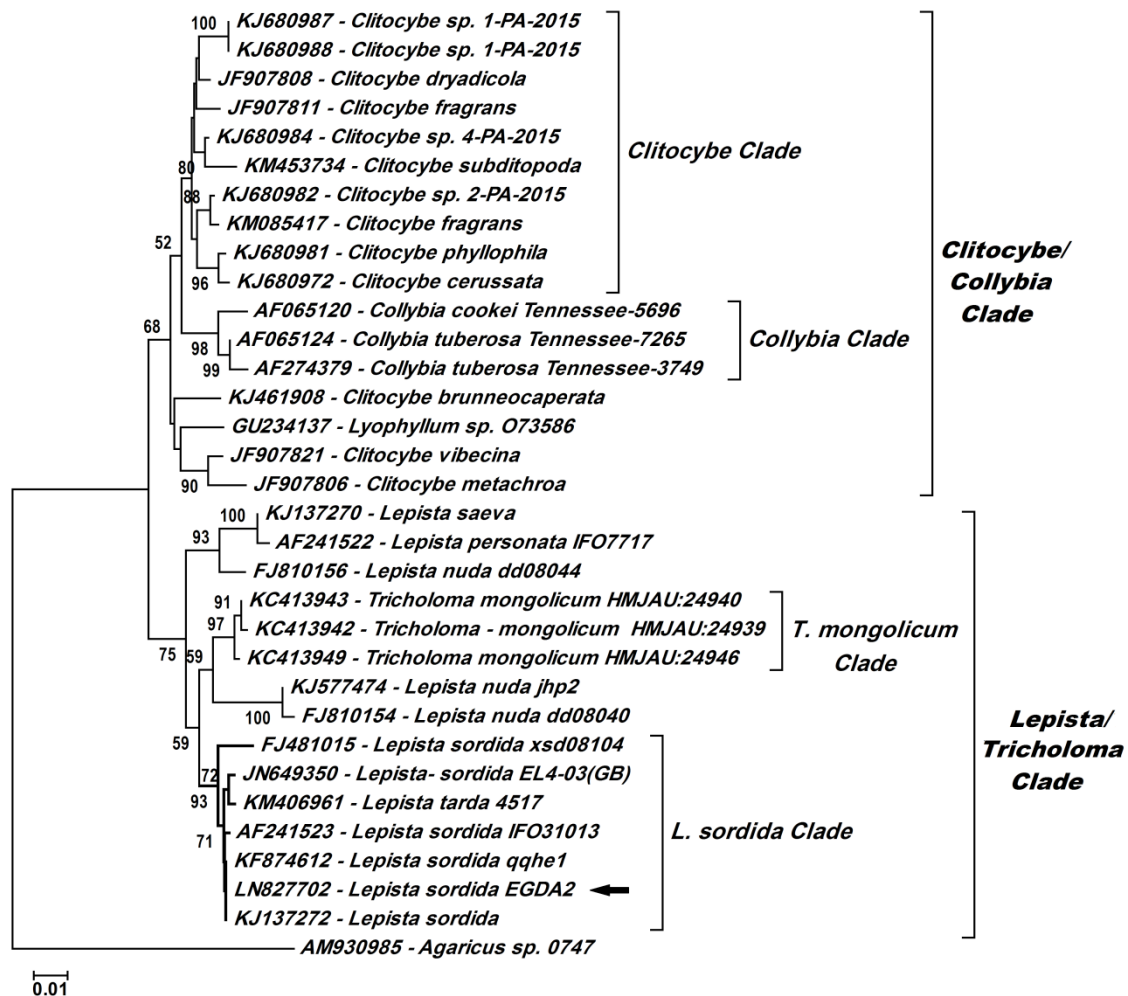


Fig. 3. Phylogenetic tree analysis based on the ITS1-5.8S-ITS2rDNA sequence alignment for *L. sordida* EGDA2 (AC: LN827702) with some other related genera and *Lepista* species which possessed the best similarity. The ITS sequence of *Agaricus* sp. (AC: AM930985) was used as outgroup to root the tree. The bootstrap values 50 or above were represented next to the phylogenetic tree branches.

Discussion

Lepista sordida (Schumach) Singer is a basidiomycete fungus that produces an excellent tasting light purple mushroom. *L. sordida* usually occurs so late in the season than other mushroom, it was observed growing in December and late winter in current study. Tricholomataceae was only represented by *Lyophyllum buxum* in the North East Nile delta (El-Fallal, 2003, 2013), until we recorded *L. sordida* EGDA2 isolate in 2014. However, *Lyophyllum* is now belonging to Lyophyllaceae (Species Fungorum, 2013). The identification of the genus *Lepista* looks almost, but

not quite like either a *Tricholoma* or a *Clitocybe*. Its cap is soft fleshy and gills are attached to a fleshy stem. The stem is central and fibrous (when broken, fibers leave a ragged edge) with no ring. Although *Tricholoma* species typically have gills notched at the stem, as do several *Lepista* species, the spores of *Tricholoma* are white, never colored, whereas *L. sordida* produces roughened pale buff or pinkish-buff basidiospores. *Tricholoma* caps never change color on drying; in several *Lepista* species color does so change. Young *Tricholoma* cap margins typically incurve, young *Lepista* cap margins typically inroll. *Tricholoma* species do not have purple colors; whitish *Tricholomas*

are grayish while whitish *Lepistas* are typically brownish or buff tinged.

L. sordida is an attractive mushroom; its flesh has striking lilac to violet colour when young while the caps may take on a brown colour and begin to fade from the centre toward the margin with age. Because of its purple colours, late appearance and lack of any association with trees *L. sordida* is fairly easy to identify. It is probably most similar in morphological characters to the closely related *L. nuda*, however, *L. sordida* is more slender than *L. nuda* and has more of a tendency to grow in clusters, as is seen in Fig. 1. Hence, more criteria are required as ITS analysis for accurate differentiation.

Interestingly, the phylogenetic tree based on the ITS sequencing clustered *L. sordida* EGDA2 with *Tricholoma mongolicum*, which is morphologically different, in the same *Lepista* clade. The same observation was recorded by Yu et al. (2011) who renamed *T. mongolicum* to *Leucocalocybe mongolicum* which clustered with *Lepista irina*.

The ITS phylogram analysis for some agaric fungi showed also that *L. sordida* and *L. nuda* were in the same clade with *L. mongolicum* and closely clustered with some species of *Clitocybe* and *Collybia* (Cooper, 2014). However, *Clitocybe* species typically have gills running down the stem and in-rolled cap margins. *Lepista* species with such characteristics may not be readily distinguished, except that if gills run down stem, they are nearly always short decurrent. White, buff and pinkish tan colors are common to both genera. Furthermore, *Clitocybe* species have no purple colors. Most *Lepista* species have a growth habit that is clustered, sub-clustered or at least gregarious. Only *C. dilatata* in genus *Clitocybe* appears to have a clustered growth habit. In the Pacific Northwest species, *Clitocybe* has white spores while *Lepista* has pale pinkish buff or pinkish buff spores (Bigelow, 1982, 1985). It was not surprise to find that *L. tarda* and *L. sordida* EGDA2 were located in the same clade as they are morphologically similar except that *L. tarda* stem is tapering (Butler, 2004).

Lepista. sordida as a valuable edible and medicinal mushroom is widespread in northern temperate zones throughout the world (Terashima & Fujiie, 2005). *L. sordida* was successfully cultivated for the first time in Thailand from a wild strain (Thongbai et al., 2017). This strain was identified by morphological description and ITS

molecular technique and a temperature of 25 - 30 °C was the best for mycelial growth. *L. sordida*, as a fairy-ring-forming fungus, was examined for its effect on the growth of turfgrass in Japan (Terashima & Fujiie, 2007). A plant growth-stimulating compound, 2-azahypoxanthine (AHX) was purified from its culture found to promote the growth of plant roots (Choi et al., 2010). Furthermore, *L. sordida* proved active role in lignin degradation, dye removal and other industrial applications. Laccases produced by *L. sordida* was characterized by Cavallazzi et al. (2004).

In conclusion, the combination of the ITS sequence analysis and morphological characters confirmed that the *Lepista* sp. isolated from Damietta District at North East Nile delta is belonging to *L. sordida* species. It is also more related to *L. tarda* and *T. mongolicum* (*L. mongolicum*) than *Clitocybe* and *Collybia* species.

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التسجيل الأول لفطر *Lepista sordida* (Schumach) Singer في شمال شرق أفريقيا

أميرة على الفلال ، أحمد قاسم عبد الصمد السيد و هدى محمد الغراباوى
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تم جمع عذلة جديدة لفطر *Lepista sordida* من مزرعة فاكهة في السنانية في منطقة دمياط في شمال دلتا مصر في ديسمبر 2014. وقد تم تعريفه باستخدام التقنيات المورفولوجية (الكبيرة والمجهرية) و الجزيئية. تم وصف الأجسام الثمرية التي تم جمعها والمزارع النقية المعزولة كاملا. وقدر معدل النمو القطري على أوساط الأجار من دكستروز مستخلص البطاطس ومستخلص الشعير (8.5 و 7.8 مم/ يوم ، على التوالي). تم تأكيد التعريف من خلال تحليل تسلسل الحمض النووي من الجين الريبوسومي 5.8S rRNA بما في ذلك الفواصل الداخلية (ITS). ثم تم مناقشة موقعها التصنيفي بين بعض الأجناس من عائلة Tricholomataceae وعلاقتها ببعض أنواع *Lepista* الأخرى. كما لوحظت البيئة المحيطة بالفطر وتم تسجيل الظروف البيئية مثل درجة الحرارة (29.7 م) والرطوبة النسبية (44.6) ورطوبة التربة (5.56). وقد سجل فطر *Lepista sordida* سابقا في جنوب أفريقيا ونيجيريا (في الجنوب) والجزائر وتونس في الشمال ، وهنا هو أول تسجيل له في شمال شرق أفريقيا.