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First Record and Molecular Identification of Amantia Manginiana in Jordan

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ABSTRACT

The occurrence of the basidiomycete Amantia manginiana is reported for the first time in Jordan. The large crumbly white wild mushroom was collected from woodland during December 2014 from Dabouq in Jordan. All morphological characteristics including cap, stem, gills, and spores were recorded. The mycelium was isolated by aseptically removing a small part of the fruiting body and transferring it onto the potato dextrose agar plate. After four days of incubation at 25-30 °C in the dark, a pure culture was obtained. Sequence analysis of a partial fragment of 28S nuclear large subunit (nLSU) in the ribosomal RNA gene of the isolated strains included the new strain within taxon Amanita manginiana with 95% similarity to Genebank accession No. KP161281 and 91% to Genebank accession No. AF024463.1 respectively. According to morphological characteristics and molecular sequence analysis, the mushroom was identified as Amanita manginiana and recorded for the first time in Jordan.

Keywords: Amanita, Molecular identification, Mushroom, Fungi.

INTRODUCTION

Amanita mushrooms are important for both human beings and ecosystems. Some members in this genus are valued edible species, whereas some species are deadly poisonous, and most species form ectomycorrhizal (ECM) association with vascular plants and play a significant role in the forest ecosystems by providing plants with essential nutrients. ECM fungi obtain carbon resources from the host plants and simultaneously provide

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water and nutrients to the hosts. It is also generally accepted that ECM fungi are beneficial to the growth and abiotic or biotic resistance of the host plants (Harley 1991; Zhang et al., 2015; Nara, 2006). The genus Amanita is one of the most familiar basidiomycetous genera, comprising about 900-1000 species worldwide. Species of the genus Amanita are important mushrooms, among these described species, about 100 are considered poisonous and about 50 are edible. For the remaining species, their edibility is largely unknown. These species were reported in a broad range of habitats (Tulloss 2005, Kirk et al. 2008). Amanita mushrooms mainly have white, free to sub-free gills with bilateral lamellar trama, white spore print, vulval remnants as warts or patches on the pileal surface and the base of the stipe (Yang and Oberwinkler 1999). In addition, many have an annulus on the stem. Al-Momany and Guecel in 2009 reported two species of Amanita in North Cyprus. In Jordan, no Amanita species was reported until now (Al-Momany, 2018). The Amanita manginiana is also known as "Mangin's False Death Cap is distributed worldwide, especially in East Asia, Central, and South America, South Africa, and Australia (Yang 2005; Cai et al. 2014; Cho et al. 2015; Deng et al; 2014 and Li and Cai 2014). Amanita phalloides are known to have been introduced to Australia, New Zealand, and South Africa together with their host plants (Dunstan et al. 1998). Most of the mushrooms, which are used as food by local communities in Jordan have neither been documented nor studied.

This study aimed to identify the Amanita manginiana found in Jordan based on morphological and morphometrical characteristics. In addition, to confirm the morphological identified characteristics with molecular sequence analysis.

MATERIALS AND METHODS

Study Site, Sample Collection, and Pure Culture Preparation

Mushroom samples were collected during December 2014 from Dabouq hills which are located in Amman Governorate in Jordan renowned for its dense forests which represent a natural habitat for several local and internationally threatened Quercus species. Two hundred hectares of woodland dominated by oak trees and located in the western region of Amman, and lies between 31°59'43.1"N 35°47'49.4" E (Google Maps 31.995306, 35.797056), with mean annual precipitation of around 500 mm.

The habitat and morphological characters of the fruiting body including cap, stem, attachment of gills, spore characters, and spore print were recorded. The keys of Breitenbach and Kränzlin; (1995) Buczacki, (2012); Evans and Kibby, (2010); Haselbach, (2011) and Phillips, (2006) were followed to identify the mushroom morphologically.

The mycelium was isolated from the fruiting body by aseptically removing a small piece of the fruiting body of mushroom samples and transferring it to potato dextrose agar (PDA) medium plates (Stamets, 2000). After four days of incubation, a pure culture was prepared using the same fresh autoclaved media; the plates were incubated at 25-30 °C in the dark for DNA extraction.

DNA Extraction and ITS Sequence Analysis

Genomic DNA was extracted using E.Z.N.A. SP Fungal DNA Mini Kit. The DNA quality was assessed by gel electrophoresis in a 1% agarose gel stained with Red Safe (Intron, Bio-tek, Seoul, Korea). DNA concentration was determined using a spectrophotometer (BIO-RAD, Smart specTm plus spectrophotometer, Hercules, CA, USA). Quantity and Quality of DNA were determined by visualization under UV light. The DNA was loaded into 1% Agarose gel in 1x TAE buffer and electrophoresed on

BIO-RAD, Subcell GT basic, Mini subcell GT. DNA concentrations were estimated by gel electrophoresis. DNA stock solutions were adjusted to 30 ng/µl with sterile distilled water and stored at -20 °C for PCR reaction.

The partial sequences of the large subunit nLSU gene, containing rRNA coding regions were amplified using LR0R-F (5`-ACCCGCTGAACTTAAGC-3`) and LR16-R(5`-TTCCACCCAAACACTCG-3`) primers (Junior et al., 2010). The PCR amplification reaction was carried out using a thermal cycler (BIO-RAD C10000Tm Thermal Cycler, USA) in a 25 µL total volume reaction consisting of 12.5 µL PCR master mix (Intron, Bio-tek, Seoul, Korea), 1.25 µL of 10 pmol of both primers, 2.5 µL of template DNA from the stock (30 ng/µL), and 7.5 µL nuclease-free water.

PCR was performed to amplify above nLSU region in a 25 µL total volume reaction under the following program conditions: 94 °C for 5 min for initial genomic DNA denaturation, 40 cycles at 94 °C for 40 s, 55 °C for 30 s (annealing), and 72 °C for 1 min (DNA amplification), and a final elongation (extension) at 72 °C for 5 min. The amplified products were purified using E.Z.N. A Gel Extraction Kit-Spin Protocol and analyzed to test the integrity and concentration on 1.5 % Agarose gel using electrophoresis. The PCR products were directly sequenced in both directions in Macrogene Inc. (Seoul-Korea) and were sequenced using an ABI 3730XL capillary electrophoresis sequencing station (Applied Biosystem, USA).

Sequencing reactions were performed, and the sequences were automatically determined in the genetic analyzer using PCR primers mentioned above. Sequences were used to a query in a blastn (http://blast.ncbi.nlm.nih.gov) search against the GenBank nr DNA sequences database (http://blast.ddbj.nig.ac.jp/top-e.html) and a multiple sequence alignment was carried out using Clustal X.

RESULTS:

Morphological and Molecular Identification

The characteristics of mushroom samples were described as follows: The sporocarp of this mushroom was found singly on the ground of mixed woodlands. Cap diameter was 14 - 19 cm, 4 cm thickness with concave shape (Umbo), off-white to cottony in color, with the margin more pallid and smoother, fibrous, and bearing fine hairs (Figure1).

Figure 1: the lower surface of Amanita manginiana showing long gills.

The gills are adnate as blades and creamy white. Long gills are present around 4 cm in length and are centrally attached to the stem. The stem is crumbly with 23 cm in length, 4.5-5.5 cm in diameter, cylindrical, stuffed, and white The flesh was white and very thin with a crumbly texture. with a basal bulb. The bulb is fleshy, globose to ovoid (Figure 2). The ring is membranous, white, superior, and skirt- like, apical, membranous, fragile, soon falling to pieces. Volva measured 5-6 × 3-4 cm, limbate, membranous, and white. The spore print and spore color are white. White warty spores measure 5.9-11.5 × 5.1-7.7 µm and are ovoid or broadly ellipsoid and truncate from one end. Basidia probably lacks clamps because of its assigned section. Clamp connections were

present. Based on macro-and microscopic features, this mushroom was identified as *Amanita manginiana* in accordance with previous referenced descriptions.

Figure 2: Stipe or stem of Amanita manginiana showing basal bulb and brittle gills.

Results revealed that the length of the sequence for large subunit *nLSU* was 600 bp. Blast results identified the strain to be close to taxon *Amanita manginiana* with ID 95% similarity to Genebank accession No. KP161281 and 91% to Genebank accession No. AF024463.1 respectively. Phylogenetic analysis (Figure 3) showed that the *Amanita* sequences were distributed across two monophyletic clades supported by high clade credibility values. Two large monophyletic clades were identified which revealed a close relationship between *Amanita* species in the first clade and among species of the second clade separate. The closest species in this tree was a sequence of *A. cyanochlorisoma* in the first clade and *A. manginiana* in the second one. The new *Amanita sp.* from Jordan showed a distant relationship with other samples with clear grouping with *Amanita manginiana* sequences.

According to morphological characteristics and molecular analysis, the mushroom was identified as *Amanita manginiana* and recorded for the first time in Jordan. This mushroom belongs to Kingdom Fungi, Division Basidiomycota, Subdivision Agaricomycotina, Class Agaricomycetes, Order Agaricales, Family Amanitaceae. This mushroom was found growing naturally in the Jordan forest on soil under oak trees.

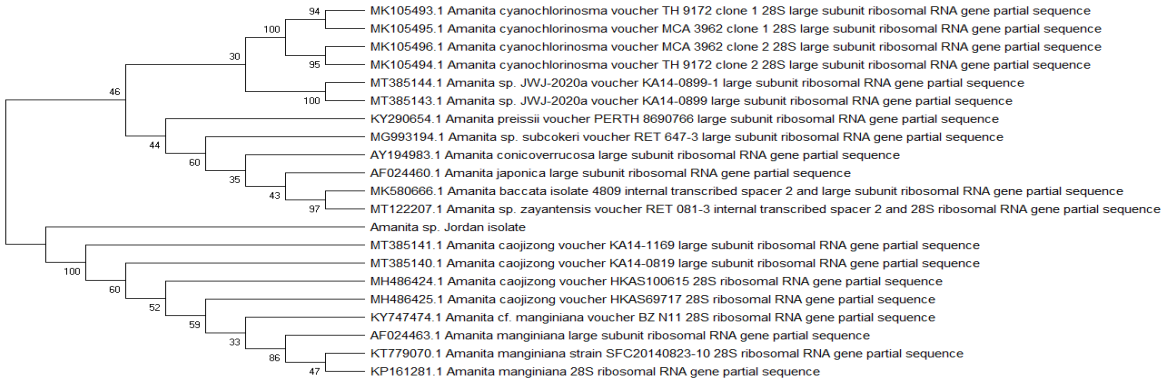

Figure 3. Phylogenetic tree for *Amanita* species based on maximum likelihood analysis of large subunit of nuclear ribosomal RNA (*nLSU*) sequences

Discussion:

Wild mushrooms played an important role in the ecosystem as organic matter decomposers as well as food for human beings or for medicinal purposes. Some mushrooms could be highly toxic or deadly. Many genera may contain several edibles as well as poisonous species. So, the identification of wild mushrooms is very important for mushroom collectors to avoid any toxicity. *Amanita* is known to contain several deadly species and some species are edible and formed a volva like a cup at the lower part of the stipe. Sanmee et al (2008) measured the spore drawings show length varying from 7 - 9 µm, the width is 4 - 6 µm originally were described from Thailand. This species is very poorly known. Yang reports a species similar to *A. manginiana* from China under the name *A. manginiana sensu* W.F. Chiu. *A. manginiana* appears to belong with a group of edible species that now are classed in section *Phalloideae*.

Taxonomic studies based on DNA sequence analysis have increased confidence in identification and showed that Asian fungal species are different from North American and European fungal species with identical morphological characteristics (Park *et al*., 2013).

Sequence comparison of the *nLSU* region is widely used in taxonomy and molecular phylogeny because it is easy to amplify even from small quantities of DNA due to the high copy number of *rRNA* genes with a high degree of variation even between closely related species (Abdulmalk, 2013). Molecular identification using both the ITS and *nLSU* sequences complemented the morphology-based identification. Sequence similarity and phylogenetic analysis confirmed the identity of six *Amanita* species, in which there were high ITS (over 99.0%) and *nLSU* (over 99.0%) sequence similarities (Cho *et al*. 2015). In this study, the amplification of the *LSU* region from the Jordanian isolate gave band sizes of around 600 bp. Molecular identification of the Jordanian isolate using *nLSU* sequence analysis demonstrated that it shares high sequence similarity with *A. manginiana.*

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أول تسجيل والتعريف الجزيئي لفطر Amantia manginiana في الاردن

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ملخص

تم الإبلاغ عن ظهور الفطر basidiomycete Amantia manginiana لأول مرة في الأردن. تم جمع الفطر البري الأبيض المتفتت الكبير من غابة دابوق خلال شهر كانون الاول 2014 في الأردن. تم تسجيل جميع الخصائص المورفولوجية بما في ذلك القبعة والساق والخياشيم والجراثيم. تم عزل العزل الفطري عن طريق إزالة جزء صغير من الجسم المثمر معقمًا ونقله إلى طبق أجار دكستروز البطاطس. بعد أربعة أيام من الحضانة عند 25−30 درجة مئوية في الظلام، تم الحصول على مزرعة نقية. تضمّن التحليل التسلسلي الجزئي لجزء من28S من الوحدة الفرعية النووية الكبيرة (nLSU)في جين ال DNA الريبوسومي للسلالات المعزولة، السلالة الجديدة ضمن تصنيف Amanita manginiana مع تشابه 95٪ من رقم انضمام Genebank KP161281و91٪ من رقم انضمام1. Genebank AF024463 ، على التوالي. وفقًا للخصائص المورفولوجية والتحليل التسلسلي الجزيئي، تم تحديد الفطر على أنهAmanita manginiana ، وتم تسجيله لأول مرة في الأردن.

الكلمات الدالة: أمانيتا، التعريف الجزيئي، الفطر، الفطريات.