First recording and molecular identification of two basidiomycetous macrofungi from Mosul/ Iraq

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Received 15 Nov 2022; Accepted 23 Dec 2022; Published 2 Jan 2023

Abstract

In this study, referred to two of macrofungi (Basidiomycota): *Abortiporus biennis* (Polyporales) and *Inocutis Levis* (Hymenochaetales). The first one type of fungus has been collected from Al- Kubaa area located 13 km northwest Mosul. And the fungus *Inocutis Levis* was collected from Al-Nimrud district, located 37 km southwest Mosul, Iraq. These fungi were described based on their morphological and microscopic characteristics. The samples were molecularly identified by DNA sequencing data. These macrofungal species are recorded for the first time from Iraq.

Keywords: basidiomycota, *Poplus euphratica*, mycelia, Mosul/Iraq

1. Introduction

Fungi are considered one of the richest groups of organisms with the highest estimated species diversity, they are including 21679 species that belong to macrofungi. Many of which are edible and have medical purposes. In addition to their importance in the pharmaceutical and environmental fields (Antonelli, *et al*., 2020; Badalyan & Rapior, 2020) [1, 2]. According to the classification of macrofungi, it is distributed into many orders, the largest and most widespread are the Agaricales and Polyporales, they are both within the Basidiomycota (Alli, *et al*., 2017; Teke, *et al*., 2019) [18, 19]. The Polyporales consists of almost 1800 species, distributed on 23 family. It is normally called polypores. It is generally described as having a fertile polypores surface and a woody fruiting body (Hibbett, *et al*., 2007) [3]. This fungal group has multiple purposes in medical, nutritional, economic, and environmental fields.

Polypores are among the most important decomposers of cellulose and lignin. So, this fungus has contributed in recycling the biggest part of the nutrients in forests. In addition, this order includes various genera such as: *Ganodera*, *Fomitopsis*, and *Fomes*. Such fungi cause rotting of living trees roots in forests, which eventually threatens forest cultivation (Bolhassan, *et al*., 2012) [4]. This order is regarded as one of the most studied fungal groups, and received a great attention from the researchers in the fungi and applied sciences fields. This order species constitutes about 1.5 % of the total diagnosed fungal species, and considered of great importance for researchers (Kirk, *et al*., 2008) [5]. It is important to mention that several species of this order have been recorded in Iraq, such as species of genus *Trametes*, and *Lentinus* (Suliaman, *et al*., 2017) [6] *Phaeolus* (Al- Khesraji & Al- Hayawi, 2019) [8] and *Bjerkandera* (Suliaman & Al- Khesraji, 2021) [7].

The research for macrofungi has greatly improved in the last few years, and new recording of macrofungi with describing their types in Iraq, especially in the north took place. The favorable climate and the type of vegetation contributed in a great deal to increase the richness of biodiversity. Therefore, this study aimed at finding new fungal that were not previously recorded in Iraq.

2. Materials and methods

2.1 Sample collection and morphological diagnosis

During the field research in the Nimrud district, located 37 km southeast of Mosul/ Iraq, and Al- Kubba area, located 13 km northwest of Mosul, the fruiting bodies on the tree trunks were collected. Al- Nimrud district is one of the areas through which the Tigris River passes, and there are a lot of fallen leaves of trees *Poplus euphratica.* Whereas, Al- Kubba area, in which it is also located on the Tigris River, is very rich in many agricultural crops. All of these factors made these areas a suitable and rich environment in biodiversity.

The first obtained fruiting body was from Al- Kubba area, in which it was found clustered on the *Pyrus communis* trunk. The second sample was collected from Al- Nimrud area, singly developing on the *Olea europaea* trunk. The fruiting bodies were diagnosed from the shape of cap, its color, petioled or not, the shape and diameter of the holes and number of holes in one millimeter.

Also, the fruiting bodies were microscopically diagnosed as the shape of spores and their dimensions. Photographs of the samples were taken in their natural habitat (tree trunks), and in the laboratory. A compound light microscope was used to examine the samples microscopically, and to be stored in Formalin Acetic Acid (FAA).

2.2 DNA extraction, amplification and nucleotide sequences

DNA was extracted from the purified fungal mycelia, which were taken from pure fungal colonies. Extraction was done with the use of extraction kit provided by Zymoresearch company, catalog No. D6005. By using ITS gene, the Polymerase Chain Reaction (PCR) was conducted and in sequential steps:

Denaturation 1 (temperature 95 °C, for 5 minutes). Denaturation 2 (temperature 94 °C, for 30 minutes). Anealing (temperature 56 °C, for 30 minutes). Extension (temperature 70-72 °C, for 30 minutes). Final Extension (temperature 70-72 °C, for 5 minutes).

In order to determine the sequences of the nitrogenous bases for each fungus, the result of the reaction was sent to Macrogene Korean company. They result of the nucleotide sequences was received from the company, and was compared with the reference sequences in the database of the National Center for Biotechnology Information (NCBI), through Blast program. In addition, in order to accurately diagnose the sample to the species, an alignment of the nucleotide sequences was conducted to know the degree of conformity with the other reference isolate to the same genus previously diagnosed and registered in the Gen Bank. Based on the nucleotide sequences contained with their used initiator, the Phylogenetic tree for each fungus was drawn using Mega 6 program.

3. Results

Phenotypic and microscopic diagnosis

According to the taxonomic keys stated in the scientific references, an appearance and microscopic diagnosis for the obtained fungi was conducted, and as follows:

Abortiporus biennis

Macroscopic features

Cap 12- 15 cm wide, semi-circular, fan-like, the outer surface is reddish-hazel color, the fruiting bodies are clustered and overlapped. The bottom surface (fertile surface) is light hazel color, the holes are elongated, the number of holes 1- 2/ mm.

Microscopic features

Spores: 7.5- 10 x 5 – 7.5 5 µm, oval, spherical with an oily drop. Nature and habitant: clustered on the *Pyrus communis* trunk, no information when this fungus is edible or poisonous. Fruiting time: March- May. Figure 1 showed appearance and Microscopic characteristic for the fungus.

The appearance and microscopic characteristics are similar with the ones mentioned by O'Reilly (2016) [9], and Kou (2019) [10]. *Abortiporus biennis* fungus was recorded in Italy by (Sicoli, *et al*., 2004) [11]. This fungus is widespread in North America, where it is mostly common in the temperate forests of the northern hemisphere (Zhou, *et al*., 2016) [12]. The fungus has unique ability to decompose all wood components for living trees, the dead and living roots of trees, and the woody debris buried in soil. This shows that the fungus ability to compete under various condition This fungus can use the wood at different stages of decomposition. It also has the ability to dissolve toxic metal oxides but the fungus is sensitive to different stress conditions (Martinez, *et al*., 2005; Graz, *et al*., 2009) [13, 14].

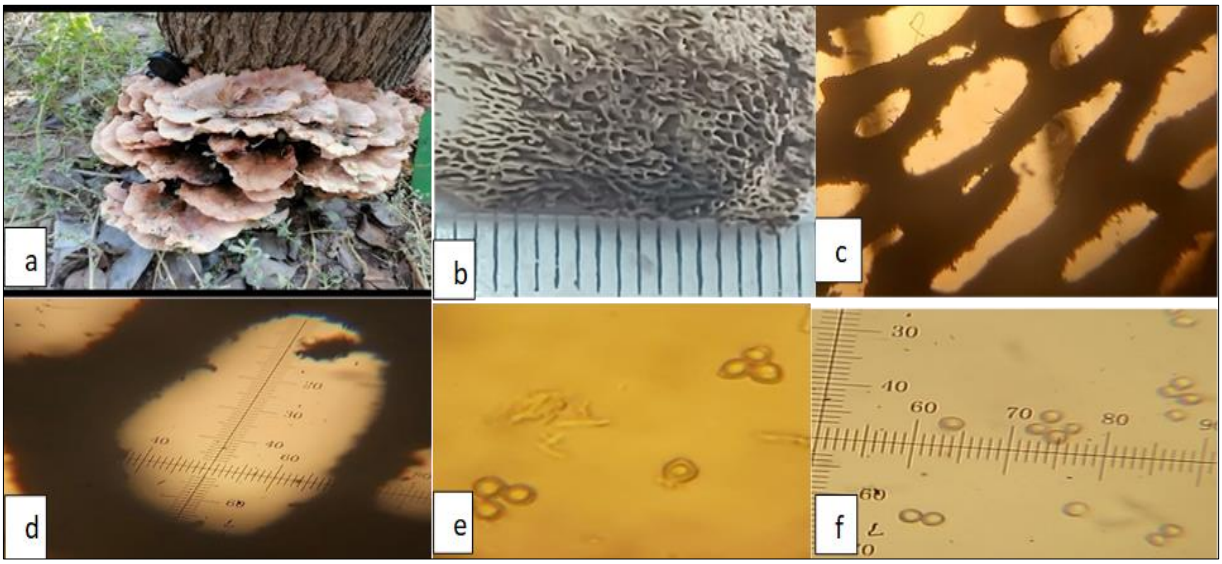

Fig 1: *A. biennis*: a fruiting body in natural habitat, b. lower surface magnified, c elongated pores, d. pores in section, and e, f. spores

Inocutis Levis

Macroscopic features

Cap 14- 20 cm wide, short neck, the outer surface is light brown, gray. The fertile layer is dark brown, the holes are circular to polygon shape, 3 holes/mm.

Macroscopic features

Spores 7.5- 10 x 5 – 6.25 µm, elliptical or oval.

Nature and habitant

Is found on the *Olea europaea* tree trunk, inedible.

Fruiting time

October-November. Figure 2 show the appearance and microscopic characteristics for the fungus.

Inocutis Levis usually grows in Asia, this fungus can be considered as a potential treatment in which it has medical

properties. It is effective in regulating blood glucose, regulating insulin secretion, and reduce the risk in diabetes as the fungus's extracts contain anti- diabetic and anti- cholesterol effect (Ehsanifard, *et al*., 2017; Chaharmiri, *et al*., 2020; Dokhaharani, *et al*., 2020) [15-17].

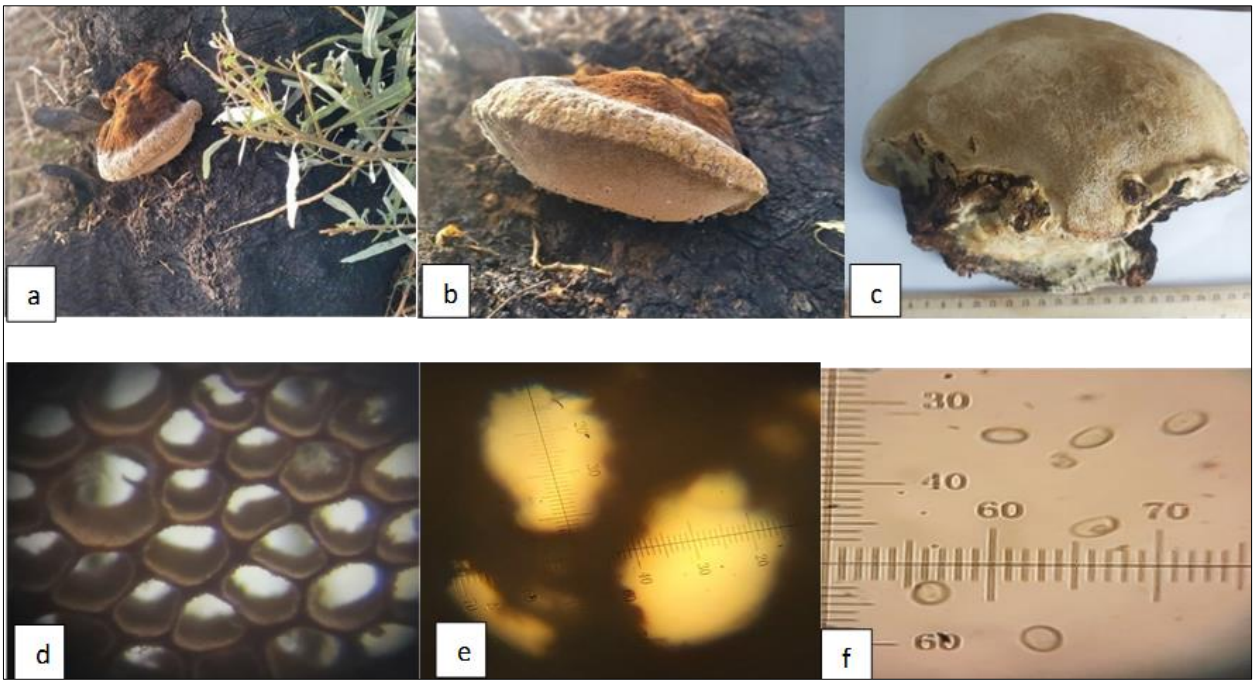

Fig 2: *I. Levis*: (a, b) - fruiting body in natural habitat, c - porous upper surface, (d, e) - pores in section, f- spores

Molecular diagnosis of the obtained local fungi

In order to ensure the appearance diagnosis for the obtained isolate fungal, DNA extraction was purely conducted using extraction kit provided by Zymoresearch Company, Catalog No. D6005.

The results of the polymerase chain reaction (PCR) showed a clear, bright fluorescent bands with molecular weight of 650 base pairs, that are corresponding to the result of the initiator used for the ITS gene in both of its front and back types, as illustrated in Fig (3).

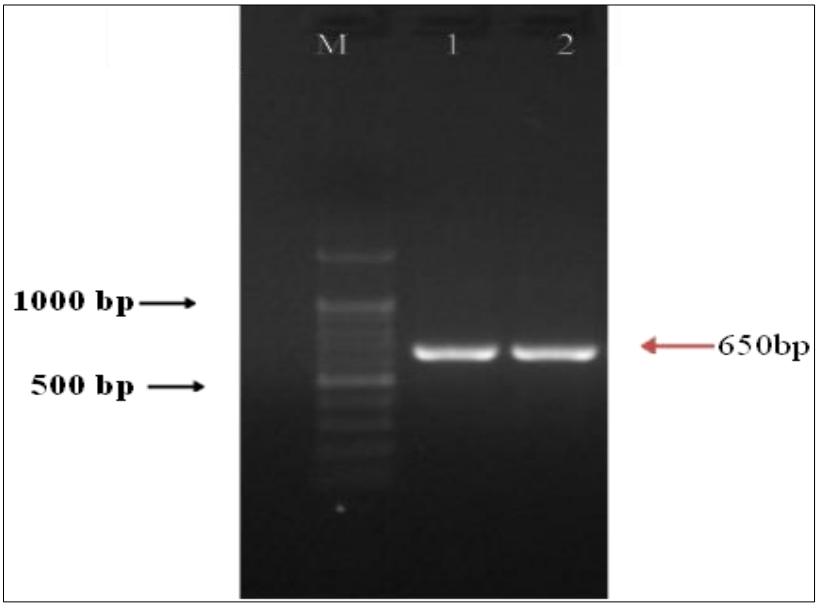

Fig 3: The results of DNA amplification for the *Abortiporus biennis, Inocutis Levis* on agarose gel. Path M represents: size marker, pathways 1- 2 are results of DNA amplification

The use of polymerase chain reaction (PCR) is considered the best method in diagnosing the fungal species with high accuracy. In which the Internal Transcribed Spacer (ITS) for the rDNA is the main diagnosis target. Therefore, ITS represents one of the most used genomic areas in identifying and diagnosing the biodiversity for the large fungi in different parts of the world.

The nucleotide sequence of the fungal *Abortiporus biennis*

After completing the ITS gene amplification, and to determine

the nucleotide sequence of fungal isolate, the amplification result was sent to Microgen company. The results were received after 30 days. Figure 4 explains the company results of appearance diagnosis.

>H220504-023_A23_A5_AF.ab1 954
TAATGCCTTGGTTGTTGCTGGCCTTTTATTAGGCATGTGCACGCCTCGGTTATTTCCAAATTCTTTACACCT
CTGTGCACTTTACATGGATTTTTATATTTTCTTATTGATGGACGTTCGAGCTTGCCACCGCGAGTTTGACG
AAAGTCAATTGAAGATTTAAAGTCTGTGGTTTACACATTTATACGCTTCAGTTTAAAGAATGTAATACTCC
GTTTAACGCAATTAAATAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAG
CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTC
CTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGTATTCTCAATTCTCTCAACTTTTGTTGTCAGA
ATTGGACTTGGAGGTATGCCGGTGTTTTAATCAACATCAGCTCCTCTGAAATGTATTAGTGTGAATGTGT
TGCACATTTTTCAGTGTGATAATTATCTGCGCTGTAGATGTTGTAACAAAATTTATAGTTCATGCTTCTAA
CCGTCTGTTTACTCAGACAAACTTATATACTTTGAAATCTGACCTCAGATCAGGTAGGACTACCCGCTGA
ACTTAAGCATTAATATAAGGAGAGAGAAAAAAAATGGAATTTTTAAGGCCTGGTGGTGGGGGCCTTTTA
TTAGGCATGTCACGCCTCGGTTATTTCCAAATTCTTTACACCTCTGTCCCTTTACATGGATTTTTTATATTTT
TCTTTATTGAGGGGGCGAGCTTCCACCCGAGTTTACGAAAAGTAATTGAAGAAATTTAAAGGTTGGGTT
TATAAATTTTTCCCCTCCTTTTAAAAAAAGAAAAAAACCCTTTAGGCCTTTAATTAAAAACACTTTAGAAA
GAAGTTTTTGGTTTCCCTCCAGATAAAAAAACCGC

Fig 4: Nucleotide sequences of local fungus *Abortiporus biennis*

It has become evident from the process of alignment for the nucleotide sequence of the local fungal isolate, and after coping and pasting it in the site of NCBI http://www.ncbi.nlm.nih.gov/blast, that the nucleotide sequence of the local fungal isolate belongs to the type *Abortiporus biennis*, which was registered in the US Gen Bank with ID: ON667915.1 Accession number for the first time in Iraq, by gen bank team after completing and verifying nucleotide and related private information.

Multiple sequence alignment

The nucleotide sequence analysis technique was carried out for the local fungus *Abortiporus biennis* and comparing it with the rest of the strains obtained from the rest of the world. In which the results were already deposited as reference isolates at the NCBI site. The results showed the presence of point mutations in some isolates or as it is called Single Nucleotide Polymorphism (SNP) distributed among various isolates.

When comparing the local isolate with the isolate registered in America, and has the serial No. KP135300.1, the genetic match rate was 100%. Also, the matching rate was 100% was the isolate from Czech Republic that has the serial No. LN714515.1

In addition, it was noted that there was not point mutation, as in the Polish, and Chinese isolates. Whereas, it appeared that there is 99% matching rate with the Swedish isolate that has the serial No. JN649325.1, and it was noticed there one point mutation available in the site (218). It is from Transition type that converted cytosine into thymine (C to T).

The matching rate was 97% with Nepalese isolate, that has serial No. LC149599.1, and it was noticed there approximately (9) point mutations distributed in several sites. The first one is in site (94) and it is from Transversion type that converted guanine into thymine (G to T). The second is in site (101), and it also from Transition type that converted cytosine into thymine (C to T). While the third one is in site (105), and it is from type Transition as well. It changed thymine into cytosine (T to C). The fourth is available on site (155), and it is also from type Transition. It led to convert guanine to adenine (G to A). The fifth is in site (165), and it is from type Transition as well. It changed adenine to guanine (A to G). The sixth one is in site (174), and it is also from type Transition that led to convert cytosine to thymine (C to T). The seventh one is available on site (453), and it is from type Transition. It converted cytosine to thymine (C to T). The eighth, the last one, is from type Transition as well. It converted cytosine into thymine (C to T), as illustrated in tab (1).

Table 1: Genetic similarity ratios for the local fungus *A. biennis* compared to the reference sequences global loaded in the NCBI

Sr. No.	Accession	Country	Source	Isolation_source	Compatibility
1.	ID: KP135300.1	USA	*Abortiporus biennis*	Root of hardwood	99%
2.	ID: LN714515.1	Czech Republic	*Abortiporus biennis*	----------	99%
3.	ID: KC862285.1	Poland	*Abortiporus biennis*	----------	99%
4.	ID: KJ094473.1	China	*Abortiporus biennis*	----------	99%
5.	ID: MZ159547.1	United Kingdom	*Abortiporus biennis*	----------	99%
6.	ID: JN649325.1	Sweden	*Abortiporus biennis*	----------	99%
7.	ID: MN294799.1	South Korea	*Abortiporus biennis*	----------	99%
8.	ID: EU232187.1	Taiwan	*Abortiporus biennis*	----------	99%
9.	ID: LC149599.1	Nepal	*Abortiporus biennis*	Wood	97%

Phylogenetic tree

Based on the nucleotide sequence obtained data for the local fungal isolate, the obtained sequence from the sequence process via Mega program, version 6 freely available on NCBI website, was entered. The ideal evolutionary tree was constructed with a total length of branches 545.42441502. The tree was drawn within fixed scales with the length of branches in the same units, and evolutionary distances used to build the phylogenetic tree with the local fungus. The evolutionary distances were measured by the use of Maximum Composite Likelihood Method. This is based on the process of changing bases at each site on the gene to be examined. The analysis included (9) isolates in addition to the local isolate, and to know the genetic affinity rate.

Based on the results of evolutionary tree analysis which was branched into two main clusters, in which the first cluster branched into two secondary branches, showed that the first branch from first cluster is the nearest homogenous type to the local isolate, and to a large extent, it the group that carries the serial numbers registered in the Gen bank (KP135300.1, LN714515.1, KC862285.1, KJ094473, MN294799.1 and EU232187.1) with 99% genetic matching rate.

While the second branch of the first cluster has included the isolate with the serial number registered in the Gen bank (MZ159547.1 and JN649325.1), which showed genetic match rate of 99%. The second branch of the isolate evolutionary tree with serial number registered in the Gen bank (LC149599.1) and showed a genetic match rate of 99%, as shown in table (1).

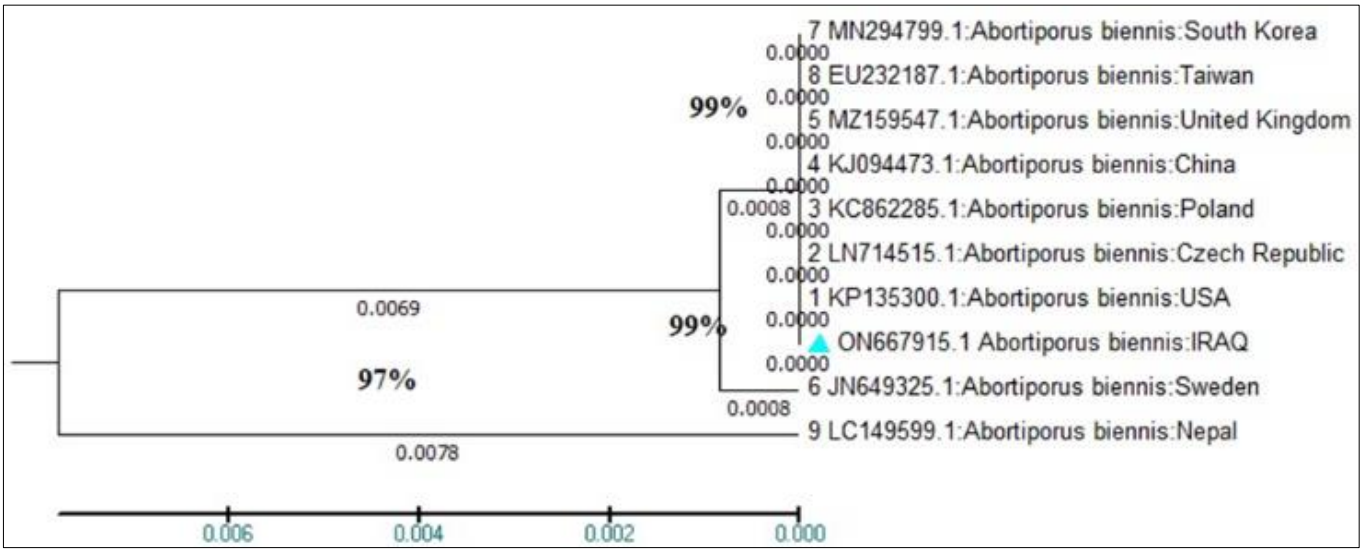

Fig 5: Genetic affinity tree for the local fungus *A. biennis*, and the global isolate reference

>H220504-023_C23_A6_AF.ab1 744

CATAACGAGGCGTTTGTATCGAGGGTCTGTGCTGGTCGGAAACGTACATGTGCACGACCTTTGTATTCA
AATCCATTTAAACCCCTGGTCACTTTGGCGGGTGAAGCTTGGAGGTAGGAAAACCTTTTTCGACTGGCAT
AACTTTAGACTTGGGAGGATACAAGGAGGCCGGACTTGGGATGGGGCCCTTTGGACGAGCCGAAGGA
AGGAAACCGGTGCAACGAAGAAAGGGGTCGGGAGGCCAACGGCTTGGCTTAGGTGGTTTAAAAAACG
TTCCAATGGTATTGGGATGTAAAGGTCCCTGGGGGGGAAAATTGGAGGGCAACCTTCAACGATGGAGC
TCCTGGCTCTCGGCTCGGTGAAGAACGCAGCGGAAAGTCAAAAGTAATGTGGAATTTCGGATTCAGTGA
ATCATCGGAACTTTGGACGCGCTTGGTGCCCGTGGTATTCCTGGGTATTCCCCCTTGGGGGCCTGCTTTC
CCTAATAATGGACGTTTACTGCGACTAGGCCTAGCGCTTATTGCGCCCGAGTCTGCCTTTGAGGTGTCCT
TGGTTAACCTCACCCGCCAAAGTACTAGGCGGACTTCTGTCGAGTTGTAGTACTAAGTCTATCGTCAGAA
TAAGCGTACGACTGCTAACGCGACGCTAAGAGGCCCGGTTAATGATTTGATACAAGGCAGGACCCTTAC
GTCTGGACATGCAGACTGACTCATACGGAGACTATCCGTCGATACACTACGAGCG

Fig 6: The nucleotide sequences for the local fungus *I. Levis*

The nucleotide sequences of the amplified ITS gene for the second local fungus obtained from Al- Nimrud district/ Nineveh, confirmed its affinity to the fungus *Inocutis Levis*, which was later registered in the Gen bank with serial No. (ON667917.1).

When comparing the nucleotide sequences for the local isolate with the nucleotide sequences for the deposited isolate registered in the Gen bank after the alignment process, it showed there are some points mutations with the Iranian isolate that carries the serial number (KU058398.1).

It was noticed that there are (3) point mutations distributed in several sites, in which the first one is at site (170), and it is from Transversion type. It converted cytosine into guanine (C to G). The second one is available on site (178), and it is from Transition type, that led to conversion of guanine into adenine (G to A). Whereas the third one is in site (260), and it is from type Transition as well. It changed thymine into cytosine (T to C), and genetic match rate was 99%.

The alignment results with nucleotide sequences for the isolate with serial No. (MN990351.1) with match rate of 99%. It included (4) point mutations distributed in several sites. The first one was in site (157) and it was from Transition type that led to convert thymine into cytosine (T to C). The second one was in site (170), and it is from type Tranversion, that led to convert cytosine to guanine (C to G). Whereas the third one was in site (178) and it is from Transition type that changed guanine to adenine (G to A). The fourth one was in site (260), and it is from Transition type, that led to convert thymine to cytosine (T to C), as shown in table (2).

Table 2: Genetic similarity ratios for the local fungus *I. Levis* compared to the reference sequences global loaded in the NCBI

Sr. No.	Accession	Country	Source	Isolation_source	Compatibility
1.	ID: KU058398.1	Iran	*Inocutis Levis*	elm tree	99%
2.	ID: MN990351.1	Italy	*Inocutis Levis*	----------	99%
3.	ID: MK422156.1	Tunisia	*Inocutis Levis*	Mushrooms	99%

Phylogenetic tree

After depositing the obtained nucleotide sequences during the sequences process via MEGA6 program, an evolutionary tree was drawn. It was constructed with a total length of branches estimated by (0.01411105). The tree was drawn with fixed scales with the length of branches in the same units, and the evolutionary distances used to construct the phylogenetic tree with the local fungus. The evolutionary distances were measured based on the process of changing bases in each site of the target gene. The analysis included (3) isolates in addition to the local isolate, and to know the genetic affinity rate. The genetic tree was divided into two main clusters. The first one branched into two secondary clusters, included the homogenous species, and most related to the local isolate to a great extent, and they are the reference isolates with serial numbers (KU058398.1, MN990351.1 and MK422156.1). It showed a genetic match rate of 99%, as shown in table (2).

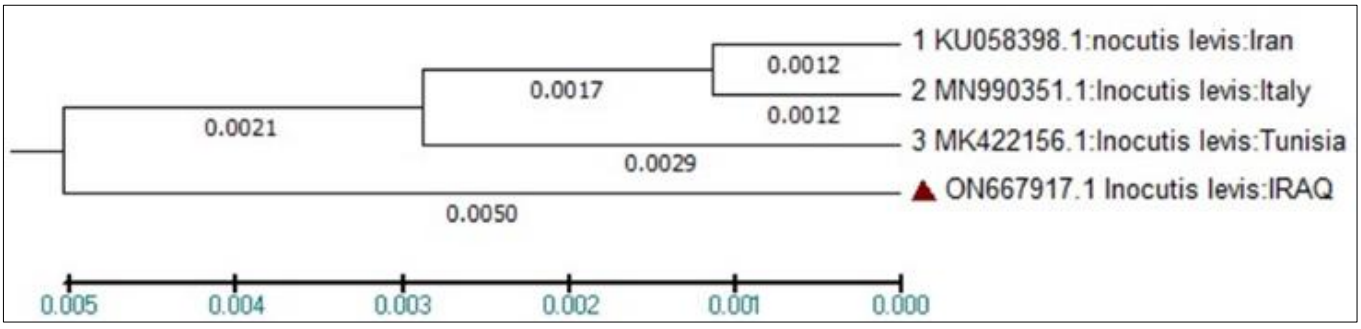

Fig 7: Genetic affinity tree for local fungus *I. Levis*, and global reference isolates

Many thanks and sincere appreciation to the Department of Biology, College of Education for Pure Sciences, University of Mosul, to facilitate work in research laboratories.

References

1. Antonelli A, Fry C, Smith RJ, Simmonds MSJ, Kersey PJ, Pritchard HW, *et al*. State of the World's Plants and Fungi. Royal Botanic Gardens, Kew, 2020. DOI: 10.34885/172. https://doi.org/10.34885/172.
2. Badalyan SM, Rapior S. Perspectives of Biomedical Application of Macrofungi. Current Trends Biomedical Engineering & Biosciences. 2020;19(5):556024.
3. Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, *et al*. A higher-level phylogenetic classification of the Fungi. Mycological Research. 2007;111:509-547.
4. Bolhassan MH, Abdullah N, Sabaratnam V, Tsutomu H, Abdullah S, Rashid NMN *et al*. Diversity and distribution of Polyporales in peninsular Malaysia. Sains Malaysiana. 2012;41(2):155-161.
5. Kirk P, Cannon P, Minter D, Stalpers J. Dictionary of the Fungi (10thedn). Wallingford, UK, 2008.
6. Suliaman SQ, AL-Khesraji TO, Hassan AA. New records of basidiomycetous macrofugi from Kurdistan Region-Northern Iraq. African Journal of Plant Science. 2017;11(6):209-219.
7. Suliaman SQ, Al-Khesraji TO. Morphological and

molecular characterization of *Bjerkandera adusta* (Meruliaceae), a new addition to maceomycota of Iraq. Annals of R. S. C. B. 2021;25(4):11968-11975.
8. Al-Khesraji TO, Al-Hayawi AY. Five new taxa records for the macromycota of Iraq from suliamaniya and tikrit provinces (Iraq). Plant Archives. 2019;19(2):4067-4072.
9. O'Reilly P. Fascinated by Fungi: Exploring the History. Mystery. Facts. and Fiction of the Underworld Kingdom of Mushrooms. First Nature, 2016.
10. Kuo M. *Abortiporus biennis*. Retrieved from the MushroomExpert.Com, 2019, Oct. Available from: http//www.mushroomexpert.com/abortiporus_biennis.html
11. Sicoli G, Colella C, Luisi N, Cirulli M. First reports of *Abortiporus biennis* (Bull.: Fr.) Sing and Oxyporus latemarginatus (Bur. & Mont. Ex Mont.) Donk on cherry trees in Apulia (southern Italy). Micrologia Italiana, 2004, 3.
12. Zhou LW, Nakasone KK, Burdsall HH, Ginns J, Vlasak J, Miettinen O, *et al*. Polypore diversity in North America with an annotated checklist. Mycol Progress, 2016. DOI: 10.1007/s11557-016-1207-7.
13. Martinez AT, Speranza M, Ruiz-Duenas FJ, Ferreira P, Camarero S, Guillen F, *et al*. Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. Int Microbiol. 2005;8:195-204.
14. Graz M, Wilkolazka AJ, Pawlega BP. *Abortiporus biennis* tolerance to insoluble metal oxides: oxalate secretion oxalate oxidase activity, and mycelial morphology. Biometals. 2009;22:401-410.
15. Ehsanifard Z, Mir-Mohammadrezaei F, Safarzadeh A, Ghobad-Nejhad M. Aqueous extract of *Inocutis Levis* improves insulin resistance and glucose tolerance in high sucrose- fed Wistar rats. J Herbmed Pharmacol. 2017;6(4):160-164.
16. Chaharmiri S, Neijhad M, Moghimi H, Farazmand A, Rahmani H. Investigating Antibacterial and Antioxidant Activity of *Inocutis Levis* Extracts and Evaluating its Phenolic Compounds. Biological Journal of Microorganism, 2020. DOI: 10.22108/BJM.120139.1240.
17. Dokhaharani S, Ghobad-Nejhad M, Moghimi H, Farazmand A, Rahmani H. Investigating antibacterial and Antioxidant Activity of *Inocutis Levis* Extracts and Evaluating its Phenolic Compounds. Biological Journal of Microorganism, 2020, 35.
18. Alli H, Col B, Sen I. Macrofungi biodiversity of Kutahya (Turkey) province. Biological Diversity and Conservation. 2017;10(1):133-143.
19. Teke AN, Kinge TR, Bechem EET, Ndam LM, Mih AM. Mushroom species richness, distribution and substrate specificity in the Kilum-Ijim forest reserve of Cameroon. Journal of Applied Biosciences. 2019;133:13592-13617.