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Original Article

Molecular identification of three novel species of *Ganoderma* from different habitats in Mosul, Iraq

Shimal Y. Abdul-Hadi[1], Mustafa Nadhim Owaid[2, 3*], Raghad Nawaf Gergees[4], and Aswan H. AL-Bayyar[4]

[1] *Department of Biology, College of Education for Pure Science, University of Mosul, Mosul, Ninawa, 00964 Iraq*

[2] *Department of Heet Education, Ministry of Education, Hit, Anbar, 31007 Iraq*

[3] *Department of Environmental Sciences, College of Applied Sciences, University of Anbar, Hit, Anbar, 31007 Iraq*

[4] *College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, 10081 Iraq*

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Abstract

Reishi Mushroom, *Ganoderma*, is considered one of important wood-decaying medicinal mushrooms. This study aimed to identify three samples of this genus in Mosul city in February and April 2019. Three species of *Ganoderma* were collected from three various trees including *Eucalyptus*, *Morus*, and *Olea* (olive) in Mosul City, Northern Iraq. Their identifications and their DNA sequences were genetically identified by using PCR techniques according to detect nuclear ribosomal internal transcribed spacer (ITS) regions. Results exhibited the finding of *Ganoderma resinaceum*, *Ganoderma applanatum*, and *Ganoderma* sp. This study is first attempt to identify Reishi Mushroom by molecular methods in Iraq. Thus, the current study is considered new good data in the field of mushroom in Iraq especially based on the molecular strategies in the identification.

Keywords: biodiversity, ITS, medicinal mushroom, reishi mushroom, rRNA

1. Introduction

Fungi are eukaryotic organisms that have the ability to decompose organic materials and convert them into a living mass. Therefore, fungi convert organic wastes such as cellulose, lignin and others into simple substances by the action of the enzymes that secrete them. Thus, mycoflora are environmentally important in cleaning the environment from pollutants (Martins, 2017). Mycoflora includes microfungi and macrofungi is a great treasure in any country for its important medical and industrial applications. Many kinds of macro fungi (mushrooms) were collected and isolated in different regions of Iraq; some wild fungi were collected from the swestern Iraq such as *Armillaria mellea*, *Coprinus disseminates*, *Pleurotus* spp., *Agaricus* spp., *Calvatia* sp., *Thelephora* sp., *Fomes* sp., *Lepiota* sp. and *Morchella* sp. (Owaid, Muslat, & Tan, 2014). Other researchers found about 34 species belonging to 23 genera of Basidiomycetes from the northern Iraq (Aziz & Toma, 2012). In one another research *Polyporus* spp. was isolated from the ecosystem of Fallujah, Iraq (Muslat & Owaid, 2015), many truffles were also found in the deserts of Anbar (Owaid, 2016), *Ganoderma lucidum* was also reported from Salahadin Governorate of Iraq (Al-

*Corresponding author
Email address: mustafanowaid@uoanbar.edu.iq; mustafanowaid@gmail.com

Khesraji, Shugran, & Augul, 2017). Also, many species of Basidiomycota were isolated from Salahadin and Baghdad Governorates (Al-Khesraji & Suliaman, 2019; Al-Khesraji, Suliaman, Al Hayawi, & Sadiq, 2019).

Iraq is rich with desert truffles (Owaid, Muslim, & Hamad, 2018), also some rid climate in western Iraq showed some edible mushrooms like *Agaricus* sp., *Clitocybe* sp. and *Marasmius* sp. (M. N. Owaid, Seephueak, & Attallah, 2018) and recently, researchers record new species of mushroom at different districts in this country (Owaid, 2021). The genus *Ganoderma* contains species that are associated with dead and declining host trees (Loyd *et al*., 2018). In Iraq, only three species of *Ganoderma* were recorded including *Ganoderma applanatum* in Babylon during 2004-2008 (Imran & Hassan, 2008), *Ganoderma adspersum* in Sulaimaniya during 2015-2016 (Al-Khesraji, Suliaman, & Hassan, 2018), and *Ganoderma lucidum* in Salahadin during 2016-2017 (Al-Khesraji *et al*., 2017).

The aim of this work is to identify three isolates of *Ganoderma* to the species level, collected from Mosul northern Iraq during February to April 2019 using genetic identification according to PCR technique and recording the genetic sequence to know sequence similarity using neighbor-joining phylogenetic trees which showed the relationship between the three isolates and related species based on 23S rRNA sequences.

2. Materials and Methods

2.1 Area of study

Mosul, the second largest city in Iraq, is located in the north on Tigris River sides. Mousl has a moderate climate because of its rise from the sea level that reaches approx. 228 m. The area of the city is 37,323 km^2, and it is 362 km northwest of Baghdad. The city coordinates extend between 36° 20' 6.00" N and 43° 07' 8.00" E. The collected mushroom samples and their habitats were recorded. However, the physical parameters of weather (temperature (°C), precipitation (mm), cloud cover, relative humidity (%)) were recorded for each location during a month were obtained from meteoblue AG database, Basel, Switzerland for one month for each study area in this investigation.

2.2 Collection of mushroom samples

The fruiting bodies of mushrooms were collected from trunks of some fruitful trees in different locations in Mosul city during February to April 2019. Some photographs of samples were captured. All samples were preserved in polyethylene bags and brought to the Lab. The mushroom samples were washed by tape water for several times to remove all soils, cut to small pieces, sterilized using ethyl 70% for 2 min, then washed by DW and dried using filter paper Whatman No.1. Some mushroom pieces were transferred to fresh PDA plates and incubated at 28±2 °C for 10 days.

All these fungal isolates were cultured on PDA (potato dextrose agar) and GSM (Ganoderma selective media) as mentioned by Ariffin and Idris (Darus & Abu Seman, 1992) (Ariffin and Idris, 1992). The DNA was extracted from the mycelium according to Chong *et al*. (2011). The DNA concentration was determined by Spectrophotometer Biodrop at 260 nm. The DNA purity for the extracted genomic DNA (gDNA) was also determined by the same Spectrophotometer at 260 nm and 280 nm.

2.3 Genetic identification of the fungal isolates

2.3.1 The DNA extraction

The DNA of fungal isolates was extracted from fresh mycelia 10-days old by the extraction kit of Bioneer, Korea according to the company instructions.

2.3.2 Electrophoresis

To achieve electrophoresis**,** 1.4% Agarose gel was prepared in TBE (1X) (40 mM Tris, 20 mM boric acid and 1M of EDTA) by using microwave till boiling. Then, it was left to cool at 50-60 °C. The agarose gel was poured in the instrument tray after putting the comb to make wells without bubbles. It was stand for solidification and the comb was lift, then the gel was put in the tank and TBE solution was poured to cover the gel. Only 5 µl of samples were prepared by mixing with 5-7 µl of the loading buffer in the wells with the ladder 100bp DNA (Bioneer, Korea). It took 2.5-3.0 hrs to finish by using 70 V/cm. The gel picked up and soaked in Ethidium bromide dye (50 µg/ml) for 30 min. The gel was transformed to destain by distilled water, then it was tested under UV light.

2.3.3 Polymerase chain reaction of DNA

Polymerase Chain Reaction (PCR) of DNA was conducted after the DNA extraction and purification using forward primer ITS1 (TCCGTAGGTGAACCTGCGG) and reverse primer ITS4 (TCCTCCGCTTATTGATATGC), Nucleotide sequence (5'-3'), Specificity ITS rRNA. The amplification was done according to Chong et al (2011). DNA bands were extracted using GEL/PCR Purification Kit of Favor Gen Korean Company.

2.3.4 The DNA sequence

Samples were prepared for determination DNA sequence of nitrogen bases. DNA was amplified using the reaction mixture with a final volume reached 20µl which composed from 5µl DNA template, 1.5µl Forword primer, and 1.5µl Reverse primer then its volume was completed to 12µl by Free nuclease water. The thermal amplification condition of PCR was as the following: 95 °C for 5 min for initial denaturation, 95 °C for 1 min for denutaration, 52 °C for 1 min for annulaing, 72 °C for 2 min for elongation and 72 °C for 10 min for final elongation.

The prepared samples were sent to Macrogen Company to determine DNA sequence. When the result reached, the sequence was used in the international website of biotechnology information by the link http://blast.ncbi.nlm.nih.gov\blast.cgi to classify the isolates according to DNA sequence in Gene Bank.

2.3.5 The phylogenetic tree

The determined sequences were compared with those retrieved from the NCBI GenBank nucleotide sequence databases (Tables 1 to 3). A distance matrix tree was constructed by the neighbor-joining method (Saitou & Nei, 1987), and the topology of the phylogenetic tree was constructed by bootstrap (500X) analysis using the MEGA-6 software (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013).

3. Results and Discussion

The first mushroom sample was collected from dead *Eucalyptus* trees at Mosul Forest (36.38° N, 43.12° E, 294 masl) in February 2019. The second sample was collected from *Morus* tree at Bashiqa distract (36.45° N, 43.35° E, 380 masl) in February 2019. The third sample was collected from *Olea* (olive) tree at the campus of University of Mosul (36.38° N, 43.14° E, 273 masl) in April 2019.

The weather of these districts varied that exhibited the daily number of sunny, partly cloudy, overcast and precipitation days. The temperature of Mosul Forest in February 2019 reached 21-7 ℃ (max/min), 5.9 days overcast, 12 days partly cloudy, and 13 days sunny whereas the precipitation days was 7.9 days at average approx. 30 mm. The second location is Bashiqa distract which exhibited weather close from Mosul Forest in February 2019, but the precipitation rate increased to 48.5 mm. The third location is University of Mosul campus in April 2019 showed the temperature 28-12 ℃ (max/min), 3.4 days overcast, 12.4 days partly cloudy and 14.2 days sunny, while the precipitation days was 7.4 days at average approx. 56 mm. Thus February and April 2019 in Mosul were considered sunny with different degrees of cloudiness because less than 20% cloud cover. Generally, days with less than 20% cloud cover are considered as sunny, with 20-80% cloud cover as partly cloudy and with more than 80% as overcast. Results agree with the finding of (Owaid *et al*., 2014), who recorded the monthly distribution of the wild mushrooms in Heet city (weastern Iraq) to February, November and December (2009-2013). The distribution of the mushroom in these disctricts dependens on the weather and precipitation days and rates (Owaid *et al*., 2018). Generally, the Iraqi climate is classified as the dry and semi-dry region in summer, and cold and rainy in winter. The variance of rainfall amount is very high from year to another. The amount of annual rainfall mainly depends on the type of the low pressure system (cyclone), location of the region and its intensity and speed and period of continuity and the amount of moisture loaded (Mohammed & Hadi, 2012). The annual rainfull was necessary for the fungal mycelium to fruit and the spring rainfall was the main infulence on fruiting stage and positive correlation between carpophores and rainfall rate in oak forests in Italy (Salerni, LaganÀ, Perini, Loppi, & Dominicis, 2002). Climate change affects ecological systems across various spatiotemporal scales and disrupts the life cycles of res- ident organisms. Precipitation amounts and temperature means determined fungal activity. Enhanced growth conditions and extended growing seasons appear beneficial to fungi from both a socioeconomic and an ecological perspective, because most vascular plants interact with mycorrhizal fungi to generate biomass (Büntgen, Kauserud, & Egli, 2012), thus Mushrooms are considered as rainmakers in the ecosystem (Hassett, Fischer, & Money, 2015).

However, areas of highest precipitation amounts in Iraq are concentrated on its northern districts/parts like Mousl (Mohammed & Hadi, 2012), thus thius area is rich with many species of mushrooms. Generally, drought and high temperature in Iraq at summer discourage growth mushrooms except some species like *Polyporus* spp. in orchards/gardens of Fallujah City near the rivers (Muslat & Owaid, 2015) and that agrees with the results of Mousl City.

There are three samples of fruiting bodies were collected from various trees in Mousl, including sample 1 (*Ganoderma resinaceum*), sample 2 (*Ganoderma applanatum*), and sample 3 (*Ganoderma* sp.) as shown in Figures 1a-c, respectively. *Ganoderma resinaceum* isolate CH160999.3 grown on dead *Eucalyptus* (Eucalypteae) trees in the Mosul Forest. *Ganoderma applanatum* isolate FC20141001.25 grown on a mulberries *Morus* (Moraceae) tree in Bashiqa distract and *Ganoderma* sp. strain CMW45101 grown on olive *Olea* (Oleaceae) trees in the gardens of

Figure 1. Fruiting bodies and mycelial cultures of *Ganoderma* sp. on PDA. Isolate 1: *Ganoderma resinaceum* (a,d), isolate 2: *Ganoderma applanatum* (b,e), and isolate 3: *Ganoderma* sp. (c,f), respectively

University of Mosul. The fruiting bodies of these isolated mushroom samples were cultured on PDA to obtain their mycelia as shown in Figures 1d-f, respectively. The results of identification of three samples using PCR show in Tables 1-3, respectively. The results in these tables agree with results of Rajesh et al (Rajesh, Dhanasekaran, & Panneerselvam, 2014) who found two species of *Ganoderma* in India. The genus *Ganoderma* contains species that are associated with dead and declining host trees (Loyd *et al*., 2018). In USA, some species of *Ganoderma* like *G. curtisii*, *G. meredithiae*, *G. sessile*, and *G. zonatum* exhibited pathogenicity on young, healthy landscape trees including Unknown hardwood, *Pinus elliottii*, Unknown hardwood, and *Serenoa repens*, respectively (Loyd *et al*., 2018). Also, species of *G. boninense* grow on and kill oil palm trees in South East Asia (Hushiarian, Yusof, & Dutse, 2013; Ramzi, Me, Ruslan, Baharum, & Muhammad, 2019).

The DNA sequences of the fungal isolates after conducting PCR shows as below: The result of sequencing isolate No. 1 was recorded in Figure 2. This sequence was submitted into NCBI-Blast and found to have e-value of 0 and percent identity of 99.85% in respect to *Ganoderma resinaceum* isolate CH 160999.3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence (Table 1). This table showed most related isolates with their accession numbers that show homology with isolate No. 1 (*Ganoderma resinaceum*) retrieved from NCBI database. This sample was identified as *Ganoderma resinaceum* which isolated from dead *Eucalyptus* trees in Mosul Forest in February 2019, see its fruiting bodies and mycelium as in Figures 1a and 1d, respectively. Besides, the alignment of the first obtained sequence in Blast tool is shown in Figure 3. However, *Ganoderma* sp. isolate 1 exhibited 99.84%, 99.84%, 99.85%, 99.84%, and 99.68% sequence similarity to *G. resinaceum* CCBAS (MG706242.1), *G. pfeifferi* CBS 747.84 (JQ520198.1), *G. resinaceum* CH 160999.3 (EF060007.1), *G. resinaceum* GLS/1 (JQ627588.1) and *G. resinaceum* IUM 3651 (JQ520204.1), respectively (Table 1).

While the result of sequencing isolate No. 2 was recorded in Figure 4. This sequence was submitted into NCBI-Blast and found to have e-value of 0 and percent identity of 100% in respect to *Ganoderma applanatum* voucher Mushroom Observer 363942 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence (Table 2). This table showed most related isolates with their accession numbers that show homology with isolate No. 2 (*Ganoderma applanatum*) retrieved from NCBI database. This sample was identified as *Ganoderma applanatum* which isolated from *Morus* tree in Bashiqa distract in Mosul in February 2019, see its fruiting bodies and mycelium as in Figures 1b and 1e, respectively. Besides, the alignment of the first obtained sequence in Blast tool is shown in Figure 5. However, *Ganoderma* sp. isolate 2 exhibited 100% sequence similarity to *G. applanatum* K(M)120830 (AY884178.1), *G. Applanatum* SFC20141001-25 (KY36425 6.1) and *G. applanatum* strain 407, (Table 2).

The result of sequencing isolate No. 3 was recorded in Figure 6. This sequence was submitted into NCBI-Blast and found to have e-value of 0 and percent identity of 100% in respect to *Ganoderma* sp. strain CMW45101 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence Sequence (Table 3). This table showed most related isolates with their accession numbers that show homology with isolate No. 3 (*Ganoderma* sp.) retrieved from NCBI database. This sample was identified as *Ganoderma* sp. which isolated from *Olea* (olive) tree in gardens of the campus of University of Mosul in April 2019, see its fruiting bodies and mycelium as in Figures 1c and 1f, respectively. Besides, the

ATCGAGTTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTACTGTGGG
TTCCAGACGTTGTGAAGCGGGCTCTTTACGGGGCTTGTAAAGCGGCGTGCCTGTGCCTGCGTTTATCACAAACTCTATAAAGTATT
AGAATGTGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGA
AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGC
ATGCCTGTTTGAGTGTCATGAAATCTTCAACTTACAGACCTTTGCGGGTTTGTAGGCTTGGACTTTGGAGGCTTGTCGGCCGTGTTT
CGGTCGGCTCCTCTTAAATGTATTAGCTTGATTCCTTGCGGATCGGCTCTCGGTGTGATAATGTCTACGCCGTGACCCGTGAAGCG
TTTTGGCGAGCTTCTAACCGTCTCTGTTTGTGAGACAGCTTTATGACCTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTA
AGCATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCCTAGTAACTGCGAGTGA

Figure 2. Result of sequencing isolate of *Ganoderma resinaceum*

Table 1. Most related isolates with their accession numbers that show homology with isolate No. 1 (*Ganoderma resinaceum*) retrieved from NCBI database.

Description	Query coverage	E-value	Identity	Accession No.
Ganoderma resinaceum isolate CH 160999.3	100%	0.0	99.85%	EF060007.1
Ganoderma pfeifferi strain CBS 747.84	94%	0.0	99.84%	JQ520198.1
Ganoderma resinaceum strain GLS/1	93%	0.0	99.84%	JQ627588.1
Ganoderma resinaceum strain IUM 3651	94%	0.0	99.68%	JQ520204.1
Ganoderma sessile strain KRT_Iso_10	100%	0.0	97.58%	MN430930.1
Ganoderma resinaceum voucher CCBAS	92%	0.0	99.84%	MG706242.1
Ganoderma sessile voucher MS188x	100%	0.0	97.58%	MG654320.1
Ganoderma sessile voucher 165MO	100%	0.0	97.58%	MG654312.1
Ganoderma sessile voucher 118SC	100%	0.0	97.58%	MG654310.1
Ganoderma sessile voucher 103SC	100%	0.0	97.58%	MG654304.1

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Query 1   ATCGAGTTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCA 60
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1   ATCGAGTTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCA 60
Query 61  CTCTACACCTGTGCACTTACTGTGGGTTCCAGACGTTGTGAAGCGGGCTCTTTACGGGGC 120
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 61  CTCTACACCTGTGCACTTACTGTGGGTTCCAGACGTTGTGAAGCGGGCTCTTTACGGGGC 120
Query 121 TTGTAAAGCGGCGTGCCTGTGCCTGCGTTTATCACAAACTCTATAAAGTATTAGAATGTG 180
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 121 TTGTAAAGCGGCGTGCCTGTGCCTGCGTTTATCACAAACTCTATAAAGTATTAGAATGTG 180
Query 181 TATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATC 240
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 181 TATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATC 240
Query 241 GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG 300
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 241 GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG 300
Query 301 AATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCA 360
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 301 AATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCA 360
Query 361 TGAAATCTTCAACTTACAGACCTTTGCGGGTTTGTAGGCTTGGACTTTGGAGGCTTGTCG 420
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 361 TGAAATCTTCAACTTACAGACCTTTGCGGGTTTGTAGGCTTGGACTTTGGAGGCTTGTCG 420
Query 421 GCCGTGTTTCGGTCGGCTCCTCTTAAATGTATTAGCTTGATTCCTTGCGGATCGGCTCTC 480
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 421 GCCGTGTTTCGGTCGGCTCCTCTTAAATGTATTAGCTTGATTCCTTGCGGATCGGCTCTC 480
Query 481 GGTGTGATAATGTCTACGCCGTGACCCGTGAAGCGTTTTGGCGAGCTTCTAACCGTCTCT 540
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 481 GGTGTGATAATGTCTACGCCGTGACCCGTGAAGCGTTTTGGCGAGCTTCTAACCGTCTC- 539
Query 541 GTTTGTGAGACAGCTTTATGACCTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTT 600
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 540 GTTTGTGAGACAGCTTTATGACCTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTT 599
Query 601 AAGCATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCCTAGTAACTGCGAGTG 660
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 600 AAGCATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCCTAGTAACTGCGAGTG 659
Query 661 A 661
          |
Sbjct 660 A 660
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Figure 3. Alignment of the first obtained sequence in Blast tool

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AACCTGCGGAAGGATCATTATCGAGTTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACA
CCTGTGCACTTACTGTGGGTATCAGATCGTGAAGCGTGCTCTTTTACCGGAGCTTGTGAAGCGTGTCTGTGCCTGCGTTTATCACA
AACACTATAAAGTATCAGAATGTGTATTACGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGA
TGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTT
GGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTATAAGCTTTTGTGGTTTGTAGGCTTGGACTTGGAGGCT
TGTCGGCCTTGATCGGTCGGCTCCTCTTAAATGCATTAGCTTGATTCCTTGCGGATCGGCTCTCGGTGTGATAATATCTACGCCGCG
ACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTCACTTGAGAGACAACTTTATGACCTCTGACCTCAAATCAGGTAGGACTACCCG
CTGAACTTAAGCATATCAATAAGCGGAGGA
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Figure 4. Second obtained sequence of *Ganoderma applanatum*

Table 2. Most related isolates with their accession numbers that show homology with isolate No. 2 (*Ganoderma applanatum*) retrieved from NCBI database.

Description	Query coverage	E-value	Identity	Accession No.
Ganoderma applanatum voucher Mushroom Observer 363942	100%	0.0	100.00%	MN173820.1
Ganoderma applanatum strain 407	100%	0.0	100.00%	MH320562.1
Ganoderma applanatum isolate SFC20141001-25	100%	0.0	100.00%	KY364256.1
Ganoderma applanatum strain BL26	100%	0.0	100.00%	JX501311.1
Ganoderma applanatum strain IUM 3985	100%	0.0	100.00%	JQ520162.1
Ganoderma applanatum voucher K(M)120830	100%	0.0	100.00%	AY884178.1
Ganoderma sp. JM97/3	99%	0.0	100.00%	AF255094.1
Ganoderma sp. CBS187.31	99%	0.0	100.00%	AF255093.1
Ganoderma applanatum voucher LE 287671	99%	0.0	99.84%	MN435140.1
Ganoderma applanatum voucher K(M)120829	99%	0.0	99.84%	AY884179.1

alignment of the first obtained sequence in Blast tool is shown in Figure 7. However, *Ganoderma* sp. isolate 3 exhibited 100%, 99.09% and 99.09% sequence similarity to *Ganoderma* sp. CMW45101 (MG020265.1), *G. gibbosum* XSD-B33 (EU273555.1) and *G. gibbosum* XSD-B35 (EU273557.1), respectively (Table 3).

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Query 1   AACCTGCGGAAGGATCATTATCGAGTTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATG 60
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 42  AACCTGCGGAAGGATCATTATCGAGTTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATG 101
Query 61  TGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTACTGTGGGTATCAGATCGTGAAG 120
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 102 TGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTACTGTGGGTATCAGATCGTGAAG 161
Query 121 CGTGCTCTTTTACCGGAGCTTGTGAAGCGTGTCTGTGCCTGCGTTTATCACAAACACTAT 180
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 162 CGTGCTCTTTTACCGGAGCTTGTGAAGCGTGTCTGTGCCTGCGTTTATCACAAACACTAT 221
Query 181 AAAGTATCAGAATGTGTATTACGATGTAACGCATCTATATACAACTTTCAGCAACGGATC 240
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 222 AAAGTATCAGAATGTGTATTACGATGTAACGCATCTATATACAACTTTCAGCAACGGATC 281
Query 241 TCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA 300
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 282 TCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA 341
Query 301 ATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCAT 360
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 342 ATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCAT 401
Query 361 GCCTGTTTGAGTGTCATGAAATCTTCAACCTATAAGCTTTTGTGGTTTGTAGGCTTGGAC 420
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 402 GCCTGTTTGAGTGTCATGAAATCTTCAACCTATAAGCTTTTGTGGTTTGTAGGCTTGGAC 461
Query 421 TTGGAGGCTTGTCGGCCTTGATCGGTCGGCTCCTCTTAAATGCATTAGCTTGATTCCTTG 480
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 462 TTGGAGGCTTGTCGGCCTTGATCGGTCGGCTCCTCTTAAATGCATTAGCTTGATTCCTTG 521
Query 481 CGGATCGGCTCTCGGTGTGATAATATCTACGCCGCGACCGTGAAGCGTTTGGCGAGCTTC 540
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 522 CGGATCGGCTCTCGGTGTGATAATATCTACGCCGCGACCGTGAAGCGTTTGGCGAGCTTC 581
Query 541 TAACCGTCTCACTTGAGAGACAACTTTATGACCTCTGACCTCAAATCAGGTAGGACTACC 600
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 582 TAACCGTCTCACTTGAGAGACAACTTTATGACCTCTGACCTCAAATCAGGTAGGACTACC 641
Query 601 CGCTGAACTTAAGCATATCAATAAGCGGAGGA 632
          ||||||||||||||||||||||||||||||||
Sbjct 642 CGCTGAACTTAAGCATATCAATAAGCGGAGGA 673
```

Figure 5. Alignment of the second obtained sequence in Blast tool

GGGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATCGAGTTCTGACTGGGTTGTAGCTGGCCT
TCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTACTGTGGGTTTACGGGTCGTGAAACGGGCTCGTTTATT
TGGGCTTGTTGAGCGCACTTGTTGCCTGCGTTTATCACAAACTCTATAAAGTATCAGAATGTGTATTGCGATGTAACGCATCTATA
TACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC
AGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAATC
TACAAACTTCTTATGGGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGTCCTTTTACAGGTCGGCTCCTCTTAAATGCATTAGCTTG
GTTCCTTGCGGATCGGCTTGTCGGTGTGATAATGTCTACGCCGCGACCGTGAAGCGTGTTTGGGCGAGCTTCTAACCGTCTCGTTA
CAGAGACAGCTTTTATGACCTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAA

Figure 6. Third obtained sequence belonging to *Ganoderma* sp.

Table 3. Most related isolates with their accession numbers that show homology with isolate No. 3 (*Ganoderma* sp.) retrieved from NCBI database.

Description	Query coverage	E-value	Identity	Accession No.
Ganoderma sp. strain CMW45101	100%	0.0	100.00%	MG020265.1
Ganoderma gibbosum isolate XSD-35	100%	0.0	99.55%	EU273514.1
Ganoderma gibbosum isolate Pvc62	99%	0.0	99.39%	MK280717.1
Ganoderma gibbosum isolate XSD-34	99%	0.0	99.39%	EU273513.1
Ganoderma gibbosum isolate XSD-62	99%	0.0	99.24%	EU326218.1
Ganoderma gibbosum AS5.624	99%	0.0	99.24%	AY593854.1
Ganoderma sp. 4 YD-2015	98%	0.0	99.23%	MK605939.1
Ganoderma gibbosum isolate XSD-B35	99%	0.0	99.09%	EU273557.1
Ganoderma gibbosum isolate XSD-B33	99%	0.0	99.09%	EU273555.1
Ganoderma sp. 4 YD-2015	99%	0.0	99.24%	KM229671.1

3.1 Phylogenetic tree

Analysis of 23S rRNA from the three isolated fungal strains indicated that G. sp. isolate 1, G. sp. isolate 2 and G. sp. isolate 3 are different fungal strains belong to the *Ganoderma* genus. Neighbor-Joining phylogenetic trees (Figure 8) showing the relationship between the three isolates (G. sp. isolate 1,G. sp. isolate 2 and G. sp. isolate 3) and related species based on 23S rRNA sequences using MEGA-6 software. *Ganoderma* sp. isolate 1 exhibited 99.84%, 99.84%,

```
Query  1    GGGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATCGA  60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GGGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATCGA  60
Query  61   GTTCTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTA  120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GTTCTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTA  120
Query  121  CACCTGTGCACTTACTGTGGGTTTACGGGTCGTGAAACGGGCTCGTTTATTTGGGCTTGT  180
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CACCTGTGCACTTACTGTGGGTTTACGGGTCGTGAAACGGGCTCGTTTATTTGGGCTTGT  180
Query  181  TGAGCGCACTTGTTGCCTGCGTTTATCACAAACTCTATAAAGTATCAGAATGTGTATTGC  240
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TGAGCGCACTTGTTGCCTGCGTTTATCACAAACTCTATAAAGTATCAGAATGTGTATTGC  240

Query  241  GATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA  300
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  GATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAA  300
Query  301  GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT  360
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT  360
Query  361  TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAAT  420
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  TGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAAT  420
Query  421  CTTCAATCTACAAACTTCTTATGGGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGTCCT  480
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  CTTCAATCTACAAACTTCTTATGGGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGTCCT  480
Query  481  TTTACAGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTTGTCGGT  540
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  TTTACAGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCTTGCGGATCGGCTTGTCGGT  540
Query  541  GTGATAATGTCTACGCCGCGACCGTGAAGCGTGTTTGGGCGAGCTTCTAACCGTCTCGTT  600
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541  GTGATAATGTCTACGCCGCGACCGTGAAGCGTGTTTGGGCGAGCTTCTAACCGTCTCGTT  600
Query  601  ACAGAGACAGCTTTTATGACCTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAA  660
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601  ACAGAGACAGCTTTTATGACCTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAA  660
```

Figure 7. Alignment result of third obtained sequence

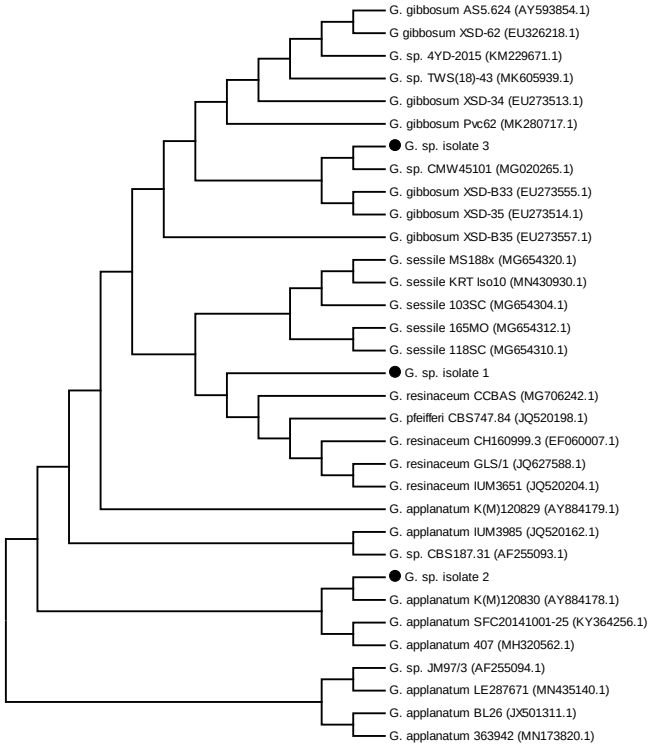

Figure 8. Neighbor-joining phylogenetic trees showing the relationship between the isolates (G. sp. isolate 1,G. sp. isolate 2 and G. sp. isolate 3) and related species based on 23S rRNA sequences using MEGA-6 software.

99.85%, 99.84%, and 99.68% sequence similarity to *G. resinaceum* CCBAS (MG706242.1), *G. pfeifferi* CBS 747.84 (JQ520198.1), *G. resinaceum* CH 160999.3 (EF060007.1), *G. resinaceum* GLS/1 (JQ627588.1) and *G. resinaceum* IUM 3651 (JQ520204.1), respectively. *Ganoderma* sp. isolate 2 exhibited 100% sequence similarity to *G. Applanatum* K(M)120830 (AY884178.1), *G. applanatum* SFC20141001-25 (KY364256.1) and *G. applanatum* strain 407. Finally, *Ganoderma* sp. isolate 3 exhibited 100%, 99.09% and 99.09% sequence similarity to *Ganoderma* sp. CMW45101 (MG020265.1), *G. gibbosum* XSD-B33 (EU273555.1) and *G. gibbosum* XSD-B35 (EU273557.1), respectively.

Many species of edible mushrooms were collected and identified from districts of northen Iraq (in forests of Suliamaniya which are rich in trees of *Quercus* spp. and *Juglans* sp.) which considered a suitable habitat to grow macrofungi naturally (Alkhesraji, 2016). In this country, only three species of *Ganoderma* were recorded including *G. applanatum* in Babylon during 2004-2008 (Imran & Hassan, 2008), *G. adspersum* in Sulaimaniya during 2015-2016 (Al-Khesraji *et al*., 2018), and *G. lucidum* in Salahadin during 2016-2017 (Al-Khesraji *et al*., 2017). Thus, the current study is considered new good data in the field of mushroom in Iraq especially based on the molecular strategies in the identification. This work encourages researchers to seek about new species for improving the mushroom list in Iraq.

4. Conclusions

Results of DNA sequences showed the finding three species of *Ganoderma* (*G. resinaceum*, *G. applanatum* and *Ganoderma* sp.) during February to April 2019, which collected from three various trees (*Eucalyptus*, *Morus* and *Olea* (olive)) in Mosul city northern Iraq. Hence, the current study is considered new data in the field of mushroom in Iraq especially based on the molecular strategies in the identification.

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References

Al-Khesraji, T. O., Shugran, A. H. M., & Augul, R. S. (2017). Some basidiomycota macrofungal species from Salahadin Governorate (North Central Iraq), with the addition of four new species to Iraq. *International Journal of Current Research in Biosciences and Plant Biology*, *4*(10), 74–84. doi:10.20546/ijcrbp.2017.410.008

Al-Khesraji, T. O., & Suliaman, S. Q. (2019). New taxa records for macromycota of Iraq from Salahadin Governorate. *Journal of Research on the Lepidoptera*, *50*(3), 125–135. doi:10.36872/lepi/v50i3/201032

Al-Khesraji, T. O., Suliaman, S. Q., Al Hayawi, A. Y., & Sadiq, S. T. (2019). First report and molecular identification of Iraqi macrofungi. *Proceeding of the international Agriculture and Forest Congress* (pp. 400–410). Izmir, Turky: Ege University.

Al-Khesraji, Talib O., Suliaman, S. Q., & Hassan, A. A. (2018). First Record of fourteen basidiomycetous macrofungi (Agaricomycetes) from Iraq. *International Journal of Current Research in Biosciences and Plant Biology*, *5*(6), 25–44. doi:10.20546/ijcrbp.2018.506.003

Alkhesraji, T. O. (2016). Seven new records of ascomycetous macrofungi from Suliamaniya province (Northeast of Iraq). *Journal of Biology, Agriculture and Healthcare*, *6*(16), 94–107.

Aziz, F. H., & Toma, F. M. (2012). First observations on the mushroom in mountain area of Iraqi Kurdistan Region. *Journal of Advanced Laboratory Research in Biology*, *3*(4), 302–312.

Büntgen, U., Kauserud, H., & Egli, S. (2012). Linking climate variability to mushroom productivity and phenology. *Frontiers in Ecology and the Environment*, *10*(1), 14–19. doi:10.1890/110064

Chong, K. P., Lum, M. S., Foong, C. P., Wong, C. M. V. L., Atong, M., & Rossall, S. (2011). First identification of ganoderma boninense isolated from sabah based on PCR and sequence homology. *African Journal of Biotechnology*, *10*(66), 14718–14723. doi:10.5897/AJB11.1096

Darus, A., & Abu Seman, I. (1992). The Ganoderma selective medium (GSM). *PORIM Information Series*.

Hassett, M. O., Fischer, M. W. F., & Money, N. P. (2015). Mushrooms as rainmakers: How spores act as nuclei for raindrops. *PLoS ONE*, *10*(10), 1–10. doi:10.1371/journal.pone.0140407

Hushiarian, R., Yusof, N. A., & Dutse, S. W. (2013). Detection and control of Ganoderma boninense: Strategies and perspectives. *SpringerPlus*, *2*(1), 1–12. doi:10.1186/2193-1801-2-555

Imran, Z. K., & Hassan, K. M. A. (2008). New record for three mushrooms associated with the trunk of trees for the first time in Iraq. *Journal of University of Babylon*, *16*(1), 400–413.

Loyd, A. L., Linder, E. R., Anger, N. A., Richter, B. S., Blanchette, R. A., & Smith, J. A. (2018). Pathogenicity of ganoderma species on landscape trees in the Southeastern United States. *Plant Disease*, *102*(10), 1944–1949. doi:10.1094/PDIS-02-18-0338-RE

Martins, A. (2017). The numbers behind mushroom biodiversity. In I. C. F. R. Ferreira, P. Morales, & L. Barros (Eds.), *Wild Plants, Mushrooms and Nuts*: *Functional Food Properties and Applications* (1st ed., pp. 15–64). New York, NY: John Wiley and Sons.

Mohammed, T. R. S., & Hadi, A. S. (2012). Annual deviations in the amounts of rainfall falling in Iraq from the general rates for the period 1970-2000. *Journal of Research Diyala Humanity*, *54*, 456–485.

Muslat, M. M., & Owaid, M. N. (2015). Polyporus spp. (Polyporaceae, Basidiomycota): Rare record from ecosystem of Fallujah, Iraq. *International Journal of Environment*, *4*(3), 185–189. doi:10.3126/ije.v4i3.13245

Owaid, M. N., Seephueak, P., & Attallah, R. R. (2018). Recording novel mushrooms in Heet district, Iraq. *Songklanakarin Journal of Science and Technology*, *40*(2), 367–369. doi:10.14456/sjst-psu.2018.58

Owaid, M. N., Muslat, M. M., & Tan, W. C. (2014). First collection and identification of wild mushrooms in western Iraq. *Journal of Advanced Laboratory Research in Biology*, *5*(2), 29–34.

Owaid, M. N., Muslim, R. F., & Hamad, H. A. (2018). Mycosynthesis of silver nanoparticles using terminia sp. desert truffle, pezizaceae, and their antibacterial activity. *Jordan Journal of Biological Sciences*, *11*(4), 401–405.

Owaid, M. N. (2016). Biodiversity and bioecology of Iraqi desert truffles (Pezizaceae) during season 2014. *Journal of Aridland Agriculture*, *2*, 22–25. doi:10.19071/jaa.2016.v2.3046

Owaid, M. N. (2021). Isolation of pisolithus sp., (Sclerodermataceae), the first recording in western Iraq. *Songklanakarin Journal of Science and Technology*, *43*(2), 520-523.

Rajesh, K., Dhanasekaran, D., & Panneerselvam, A. (2014). Isolation and taxonomic characterization of medicinal mushroom Ganoderma spp. *Academia Journal of Microbiology Research*, *2*(2), 61–70. doi:10.15413/ajmr.2014.0109

Ramzi, A. B., Me, M. L. C., Ruslan, U. S., Baharum, S. N., & Muhammad, N. A. N. (2019). Insight into plant cell wall degradation and pathogenesis of Ganoderma boninense via comparative genome analysis. *PeerJ*, *7*, e8065. doi:10.7717/peerj.8065

Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*(4), 406–425. doi:10.1093/oxfordjournals.molbev.a040454

Salerni, E., Lagana, A., Perini, C., Loppi, S., & Dominicis, V.D. (2002). Effects of temperature and rainfall on fruiting of macrofungi in oak forests of the mediterranean area. *Israel Journal of Plant Sciences*, *50*(3), 189–198. doi:10.1560/GV8J-VPKL-UV98-WVU1

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725–2729. doi:10.1093/molbev/mst197