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Diagnostic Study by PCR Technique for the Date Lesser Moth *Batrachedra Amydrula* Meyrick (Batrachedridae: Lepdoptera) in the Central and Southern Region of Iraq

Mohsen A. Al-Musafir[1], Auid A. Abdulkader[2], Dhia S. Alwaily[3]

[1]Al-Musayyib Technical Institute, Al Furat Al-Awsat Technical University, Iraq.
E-mail: Mohsen.kareem@atu.edu.iq

[2]Faculty of Agriculture, Al-Basrah University, Iraq. E-mail: Ayad.52@hotmail.com

[3]Faculty of Agriculture, Al-Basrah University, Iraq. E-mail: dalwaily@gmail.com

ABSTRACT

A molecular study of this insect was conducted to find out the sex and its genetic fixation. This study was carried out using both techniques of PCR and Nucleotide Sequences to document the classification results and their genetic fingerprinting in Iraq. The results showed that it obtained an identity of 99% with the species registered in a Gene bank of a scientific name *Batrachedra amydruala* (MAD) isolation.

KEYWORDS

Lepdoptera**,** PCR, *Batrachedra Amydraula.*

Introduction

The date palm tree *Phoenix dactylifera* L. belongs to the Palmaceae family, which is an important fruit tree rich in nutrients. Besides, it is believed that Iraq and the Arabian Gulf are the origins of this tree and from them, it spread to the Arab world and the rest of the world. Iraq is an important center of spreading palm trees in the world, as the number of planted palm trees reached more than 30 million date palm trees until 1980, and Iraq was the first in the production of dates in the world (Jarodet, 2003). One of the most dangerous pests that affect palm trees in the areas cultivated in Iraq and the world is the *Batrachedra amydraula* M, which infects immature palm fruits from the beginning of the fruit set until the later stages, causing direct losses in the crop itself. Thus, the losses in the yield can reach from (60 – 100%) when conditions become appropriate in some seasons for the breeding of lesser date moth, as it is found in Iraq in the city of Basra. Many studies were conducted in which it was indicated that there are two species of the insect resulted of a significant decrease in the yield and its quality, as well as its effect on the age and growth of the palm, as it infects palm tree of various parts and dates (Al-Jboory, 2007). The lesser date moth that infects the date palms in Iraq belongs to the genus Batrachedra. On the other hand, the latest studies reported there are only one species because the only species that was collected through a comprehensive survey in the Baghdad location was registered with the species *Batrachedra Sp* (Aziz F.M.2005). Therefore, this study was conducted. due to the absence of a molecular diagnostic study for this insect.

Materials and Methods of Work

- **Study Locations**

Four governorates were chosen from central and southern Iraq, which are Basra (Shatt al-Arab), Maysan (Qalaat Saleh), Dhi Qar (Nasiriyah), and Babylon (Al-Musayyib district and Al-Nakhil Research Station in Al- Mahawil district) as shown in Figure (1). One orchard was chosen from every region; where each orchard contains no less than 100 palm trees, in each orchard placed from 3 to 5 pheromone traps, placed on the first row of palm fronds near the branches Sticky pheromone traps were used to collected adults of lesser date moth, where these traps were placed with nylon bags and transferred to the laboratory. The adults were collected after applying a little diluted alcohol to remove the gums from the trap. After that, the insect adults were placed in clean, sterilized, and labeled glass ampoules. A sample was taken from each governorate containing more than 50 insects to conduct the genetic study, where they were placed in the freezer directly for freezing, (Mohammad et al., 2003).

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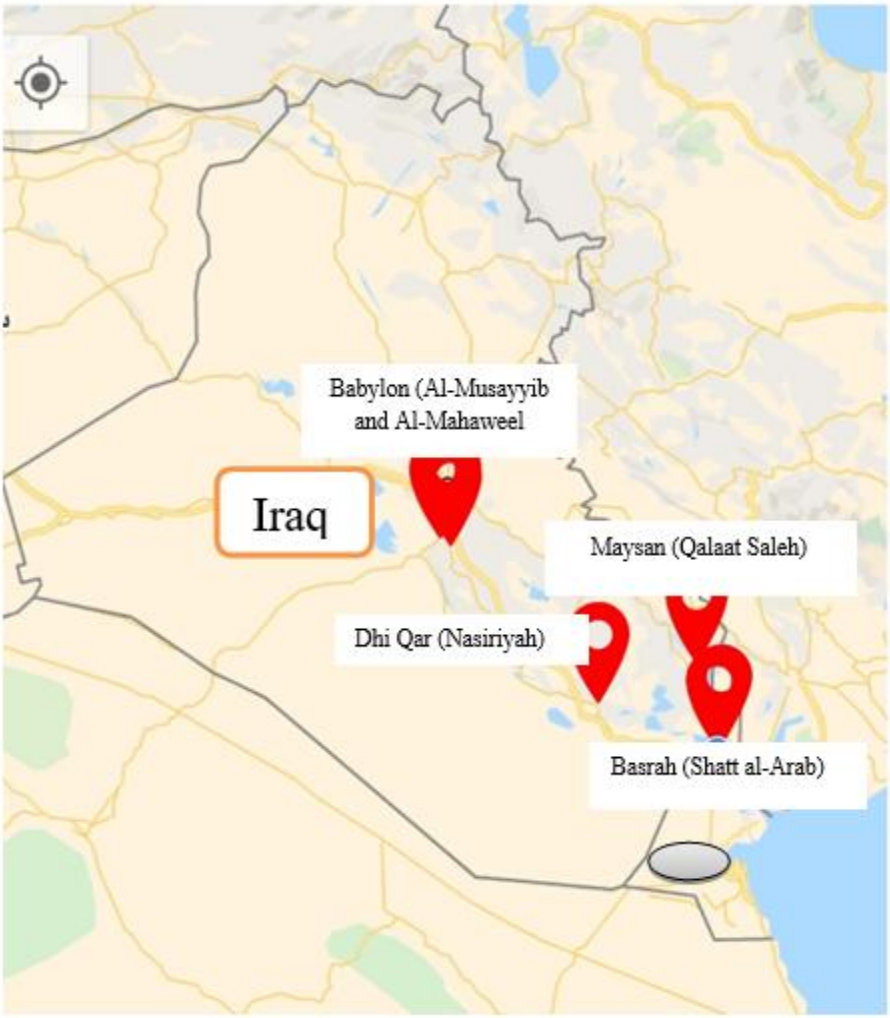

Figure 1. Iraqi map, showing the study locations

- **Insect Diagnostics Using Polymerase Chain Reaction (PCR) Technology**

Samples of lesser date moth adults were collected from each of the study locations (Basra, Maysan, Dhi Qar, and Babylon. Then, these samples were sent to the laboratories of the Wahj Al-DNA Company (Baghdad / Karada Kharj) to carry out the diagnosis with PCR technology and the Sequencing tests as follows:

1. Materials and Devices Used

Seq.	Material name	Kit type and name	The manufacturing company
1	Agarose	8100.11	Conda/USA
2	Red safe staining solution	21141	Intron/Korea
3	Loading dye	21161	Intron/Korea
4	Ladder 100bp	24073	Intron/Korea
5	Pre mix pcr	25025	Intron/Korea
6	TBE- Buffer (10X	IBS.BT004	Conda/USA
7	Primer	-------	Integrated DNA technologies /USA

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8	G-spinDNA extraction Kit	17045	Intron biotechnology/ Korea

Contents of the kit for extracting DNA from the insect

Contents of 50 columns	Label
25 ml	Buffer CL
25 ml	Buffer BL
40 ml	Buffer WA
10 ml	Buffer WB
20 ml	Buffer CE
50 ea	Spin Column / Collection Tube
3 mg x 1 vial	RNase A (Lyophilized powder)
22 g x 1 vial •	Proteinase K (Lyophilized powder)

2. Extraction of Insect DNA

G-spin DNA Extraction Kit Tissue Protocol provided from Intron Biotechnology / Korea were used for DNA extraction and followed method (5) as follows:

- One adult insect was taken from the samples for the genetic study for each location, after removing the wings and legs, and placed in a 1.5 ml Eppendorf tube. Then, it was crushed with the addition of liquid nitrogen for 5 minutes and by using a clean and sterile glass rod.
- A 25 mg of the insect tissue sample was taken, then it was transferred to a 1.5 ml tube using a clean and sterile spoon.
- A 200 µl of buffer CL, 20 µl of Proteinase K, and 5 µl of RNase A solution were added to the sample tube and mixed it by magnetic stirrer vigorously (vortexing).
- The solution was incubated at a temperature of 56 ° C (using an electric heater or a water bath) for 10-30 minutes.
- After completely dissolving, 200 µl of BL solution was added into the upper sample tube and mixed well. Then the mixture was incubated at 70° C for 5 minutes.
- The sample tube was placed in a centrifuge at 13000 rpm for 5 minutes to remove the insect tissue particles that are not dissolved. Then 350 - 400 µl of the supernatant solution was carefully transferred to a new (unused) 1.5 ml tube.
- A 200 µl of absolute ethanol to was added the solution and mixed well by gently stirring 5 - 6 times or by pipette. (without using a magnetic shaker), after mixing, the tube 1.5 ml was then cooled to remove the droplets from inside the cap.
- The mixture was then carefully transferred from Step 6 to the binding column (in a 2 mL collection tube) without wetting the tip; the cap was closed and placed in the centrifuge at 13,000 rpm for one minute. The filtrate was then discarded and the 2 mm tube placed in the binding column (reuse).
- A 700 µl of WA solution was added to the binding column without wetting the tip, and placed in a centrifuge for 1 min at 13,000 rpm. The flow was then discarded and re-use the collection tube.
- A 700 µl of the WB solution was added to the binding column without wetting the tip, and placed in a centrifuge for 10 min at 13,000 rpm. The supernatant liquid was then discarded and placed the separation column in a 2.0 mL collection tube (reuse), then place it again in the centrifuge for an additional minute to dry the membrane. Supernatant liquid was then discarded by collecting it in another tube.
- The binding column was placed into a new 1.5 mL tube (not used), and 100 µl of CE solution was added directly to the membrane. The solution was incubated for 10 minutes at room temperature, and then it was placed in a centrifuge for 1 minute at 13,000 rpm.

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3. The COXI Gene Primer Design

The primers for COXI rRNA gen diagnosis B. amydrula located on the COX1 GenBank were designed using Primer 3 plus software provided by Integrated DNA Technologies / Canada, as shown in Table (1).

Table 1. Primer design for the COXI gene of B. amydraula

Sequence (5'->3')		Tm	GC%	Product length
Forward primer	GGAGCCCCAGATATAGCTTTCC	247	60.03	427
Reverse primer	AAATTGGATCTCCCCCTCCTG	632	59.14	

4. Measuring the Concentration and Testing of the Extracted DNA

Detection of the extracted DNA was performed by a Nanodrop Spectrophotometer, by determining the concentration of DNA (ng / µl) and measuring the purity of the DNA through absorbance at a wavelength of 260/280.

5. Preparation of the PCR Master Mix

The PCR mixture was prepared using a PreMixKit (i-Taq) PCR kit provided from the German company Bio San, according to the ingredients in the following Table:

Ingredients	**Volume**
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl (1 µl)
Reverse primer	10 picomols/µl (1 µl)
DNA	1.5µl
Distill water	16.5 µl
Total volume	25µl

6. Gene Detection

The polymerase chain reaction test was carried out using the PCR Thermocycler under ideal conditions as in the following Table:

No.	**Phase**	**Tm (°C)**	**Time**	**No. of cycle**
1-	Initial Denaturation	95°C	5 min.	1 cycle
2-	Denaturation -2	95°C	45sec	35 cycle
3-	Annealing	58°C	45sec	
4-	Extension-1	72°C	45sec	
5-	Extension -2	72°C	7 min.	1 cycle

7. Gel Electrophoresis

Electrophoresis was performed with Agarose Gel at a concentration of 1.5% to read the PCR result, according to the supplier instructions.

8. Sequencing Method of DNA Sequencer

The DNA sequencing method for determining the sex of B. amydrualia was performed by a morphological method and by PCR method by carrying out the phylogenetic tree analysis for the COI rRNA gene. Then, the reaction product was sent to the South Korean company Macrogen for DNA sequencing using the AB DNA Sequencing System.

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Results and Discussion

The results of the molecular classification using PCR and Sequences showed their conformity with the morphological description of lesser date moth on the date palm in Figure 2. Along with the different regions and environmental conditions for each location, and their conformity with the information recorded in the GEN BANK.

Figure 2. An image of the *Batrachedra amydraula* M. adults

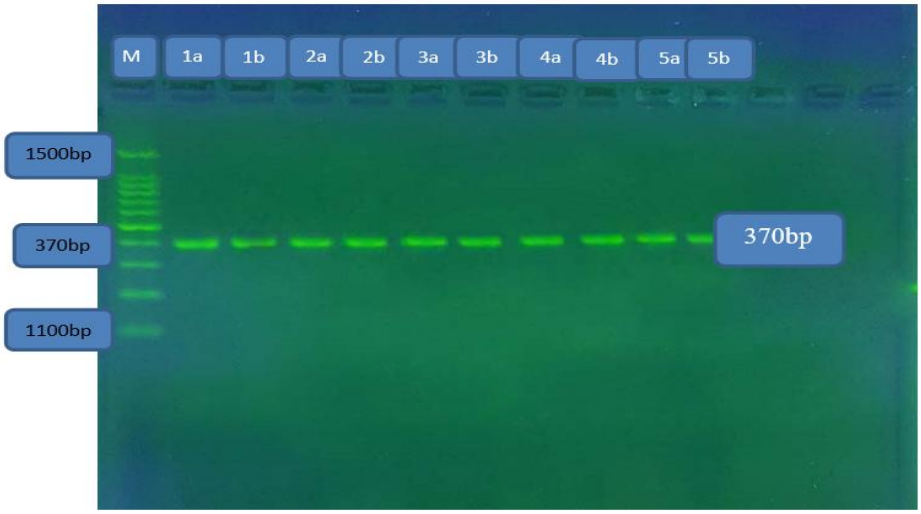

Figure 3. Represents the electrophoresis image of Agarose gel containing the results of the PCR test of the COXI rRNA gene for the diagnosis of the lesser date moth sex

*the symbol M (Marker ladder 1500-100bp) and pits (1a and 1b) represent the grouped species from Basra Governorate. (2a and 2) 2b) represent the grouped species from Maysan governorate and (3a and 3b) represent the grouped species from Dhi-Qar governorate. (4a and 4b) represent the grouped species from Babylon Governorate, Al-Musayyib district. (5a and 5b) represent the grouped species from Babylon Governorate, Al-Nakhil Research Station in Al Mahawil district.

Genetic Identity

The genetic sequences in the nitrogenous bases chain in the studied samples showed a high degree of identity, reached (99%). Therefore, these samples can be highly dependent on the study location, as well as the COXI gene is

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an accurate indication for the sex diagnosis of B. amydraula, as in Table (2).

Table 2. Genetic identity between the local insect (species and sex) with the species registered globally on the Global Genbank

No. of sample	Type of substitution	Location	Nucleotide	Sequence ID with compare	Sequence ID registry	Identities	Source
1	Transvertion	272	G\C	KT827248.1	MT890535.1	99%	Batrachedra amydraula (COXI) gene
	Transvertion	329	C\G				
	Transvertion	341	G\C				
	Transition	522	A\G				
2	Transvertion	272	G\C	KT827248.1	MT890536.1	99%	Batrachedra amydraula (COXI) gene
	Transvertion	341	G\C				
	Transition	522	A\G				
	Transition	589	T\C				
3	Transvertion	272	G\C	KT827248.1	MT890537.1	99%	Batrachedra amydraula (COXI) gene
	Transvertion	341	G\C				
	Transition	522	A\G				
4	Transvertion	272	G\C	KT827248.1	MT890538.1	99%	Batrachedra amydraula (COXI) gene
	Transvertion	341	G\C				
	Transition	466	T\C				
	Transition	522	A\G				
5	Transvertion	272	G\C	KT827248.1	MT890539.1	99%	Batrachedra amydraula (COXI) gene
	Transvertion	341	G\C				
	Transition	522	A\G				
	Transition	553	T>C				
	Transvertion	554	T>G				
6	Transvertion	272	G\C	KT827248.1	MT890551.1	99%	Batrachedra amydraula (COXI) gene
	Transvertion	329	C\G				
	Transvertion	341	G\C				
	Transition	522	A\G				
7	Transvertion	272	G\C	KT827248.1	MT890552.1	99%	Batrachedra amydraula (COXI) gene
	Transvertion	341	G\C				
	Transition	522	A\G				
	Transition	589	T\C				
8	Transvertion	272	G\C	KT827248.1	MT890553.1	99%	Batrachedra amydraula (COXI) gene
	Transvertion	341	G\C				
	Transition	522	A\G				
9	Transvertion	272	G\C	KT827248.1	MT890554.1	99%	Batrachedra amydraula (COXI) gene
	Transvertion	341	G\C				
	Transition	466	T\C				
	Transition	522	A\G				
10	Transvertion	272	G\C	KT827248.1	MT890555.1	99%	Batrachedra amydraula (COXI) gene
	Transvertion	341	G\C				
	Transition	522	A\G				
	Transition	553	T\C				
	Transvertion	554	T\G				

- **Dendrogram**

Figure 4 represents the Phylogenetic tree analysis of B.amydraula species for the current study samples. However, the use of MEGA6 program and the UPGMA tree analysis showed a clear identity was found for the insect species from the samples taken from the study areas with the species recorded in the NCBI Genbank. Accordingly, the registration symbols (Cod) for the insect species registered in the study was obtained from the NCBI Genbank with the official registration document for genus B.amydraula, which was diagnosed in this study at the aforementioned locations.

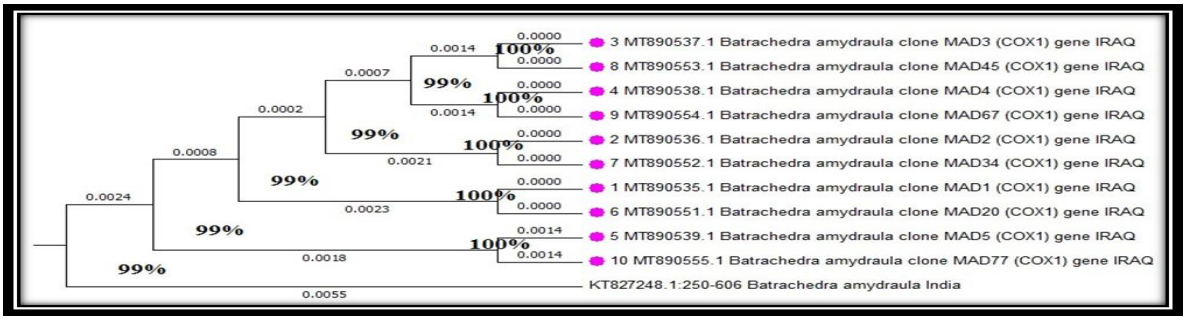

Figure 4. Dendrogram of the identities and difference of B. amyaraula species by using the PCR technique with the species registered in the NCBI.

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- **Analysis of DNA Sequencer Results**

The data for analyzing the DNA sequences results indicated that there is a great similarity for the alignments of COXI gene bases in the local B. amydraula insect with the species registered in the NCBI GenBank as shown in Figure 5.

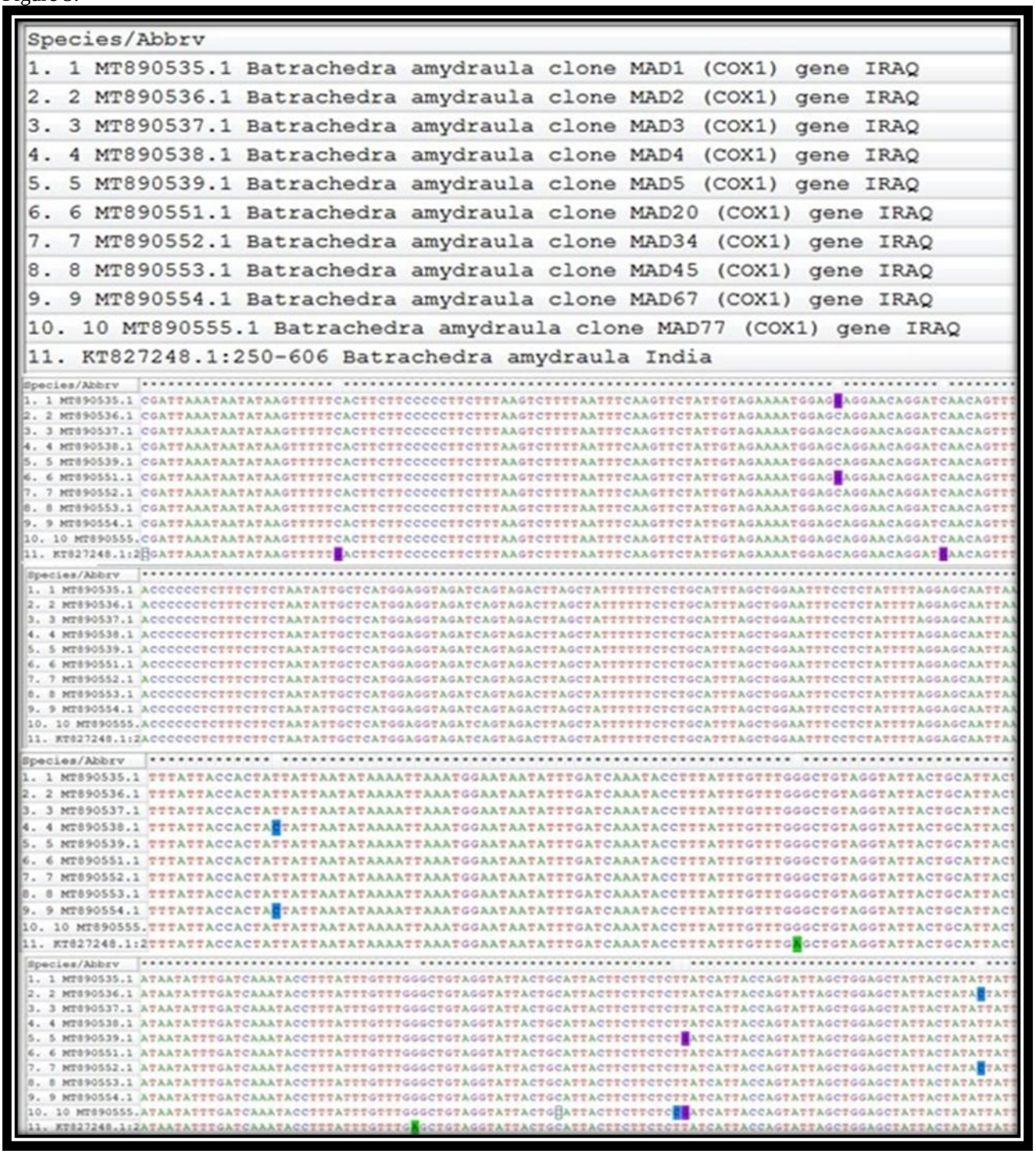

Figure 5. Represents the multiple sequence alignment analysis by using the MEGA6 program of the PCR test results for COXI gene of the studied B.amydraula

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The Subsequences of Nitrogenous Bases

The results indicated the sites of identities between the nucleotide sequences of the COI gene with the nucleotide sequences taken from the Genbank for each of the study areas (Basra, Maysan, Dhi Qar and Babylon (Al-Musayyib and Al-Muhawil).

1: Basrah- 1a

Batrachedra amydraula cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KT827248.1Length: 676Number of Matches: 1
Range 1: 250 to 606

Score	Expect	Identities	Gaps	Strand
627 bits(694)	0.0	353/357(99%)	0/357(0%)	Plus/Plus

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Query  61   TCTATTGTAGAAAATGGAGGAGGAACAGGATCAACAGTTTACCCCCCTCTTTCTTCTAAT  120
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Sbjct  310  TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCTCTTTCTTCTAAT  369

Query  121  ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT  180
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Sbjct  550  CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT  606
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2: Basrah -1b

Batrachedra amydraula cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KT827248.1Length: 676Number of Matches: 1
Range 1: 250 to 606

Score	Expect	Identities	Gaps	Strand
627 bits(694)	0.0	353/357(99%)	0/357(0%)	Plus/Plus

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Sbjct  550  CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT  606
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3: Maysan-2a

Batrachedra amydraula cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KT827248.1Length: 676Number of Matches: 1
Range 1: 250 to 606

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4: Maysan-2b

Batrachedra amydraula cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KT827248.1Length: 676Number of Matches: 1
Range 1: 250 to 606

Score	Expect	Identities	Gaps	Strand
627 bits(694)	0.0	353/357(99%)	0/357(0%)	Plus/Plus

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Sbjct  550  CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT  606
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5: Dhi Qar-3a

Batrachedra amydraula cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KT827248.1Length: 676Number of Matches: 1
Range 1: 250 to 606

Score	Expect	Identities	Gaps	Strand
622 bits(689)	0.0	352/357(99%)	0/357(0%)	Plus/Plus

```
Query  1    CGATTAAATAATATAAGTTTTTCACTTCTTCCCCCTTCTTTAAGTCTTTTAATTTCAAGT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  250  CGATTAAATAATATAAGTTTTTGACTTCTTCCCCCTTCTTTAAGTCTTTTAATTTCAAGT  309

Query  61   TCTATTGTAGAAAATGGAGCAGGAACAGGATCAACAGTTTACCCCCCTCTTTCTTCTAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  310  TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCTCTTTCTTCTAAT  369

Query  121  ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  370  ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT  429

Query  181  TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAATTAAATGGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  430  TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAATTAAATGGA  489
Query  241  ATAATATTTGATCAAATACCTTTATTTGTTTGGGCTGTAGGTATTACTGCATTACTTCTT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  490  ATAATATTTGATCAAATACCTTTATTTGTTTGAGCTGTAGGTATTACTGCATTACTTCTT  549

Query  301  CTCCGATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT  357
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  550  CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT  606
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Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 8117 - 8134
Received 16 February 2021; Accepted 08 March 2021.

6: Dhi Qar-3b

Batrachedra amydraula cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KT827248.1Length: 676Number of Matches: 1
Range 1: 250 to 606GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
627 bits(694)	0.0	353/357(99%)	0/357(0%)	Plus/Plus

```
Query  1    CGATTAAATAATATAAGTTTTTCACTTCTTCCCCCTTCTTTAAGTCTTTTAATTTCAAGT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  250  CGATTAAATAATATAAGTTTTTGACTTCTTCCCCCTTCTTTAAGTCTTTTAATTTCAAGT  309

Query  61   TCTATTGTAGAAAATGGAGGAGGAACAGGATCAACAGTTTACCCCCCTCTTTCTTCTAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  310  TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCTCTTTCTTCTAAT  369

Query  121  ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  370  ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT  429

Query  181  TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAATTAAATGGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  430  TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAATTAAATGGA  489

Query  241  ATAATATTTGATCAAATACCTTTATTTGTTTGGGCTGTAGGTATTACTGCATTACTTCTT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  490  ATAATATTTGATCAAATACCTTTATTTGTTTGAGCTGTAGGTATTACTGCATTACTTCTT  549

Query  301  CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT  357
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  550  CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT  606
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Received 16 February 2021; Accepted 08 March 2021.

7: Babylon, AL- Musayyib -4a

Batrachedra amydraula cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KT827248.1Length: 676Number of Matches: 1
Range 1: 250 to 606

Score	Expect	Identities	Gaps	Strand
627 bits(694)	0.0	353/357(99%)	0/357(0%)	Plus/Plus

```
Query  1    CGATTAAATAATATAAGTTTTTCACTTCTTCCCCCTTCTTTAAGTCTTTTAATTTCAAGT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  250  CGATTAAATAATATAAGTTTTTGACTTCTTCCCCCTTCTTTAAGTCTTTTAATTTCAAGT  309

Query  61   TCTATTGTAGAAAATGGAGCAGGAACAGGATCAACAGTTTACCCCCCTCTTTCTTCTAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  310  TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCTCTTTCTTCTAAT  369

Query  121  ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  370  ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT  429

Query  181  TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAATTAAATGGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  430  TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAATTAAATGGA  489

Query  241  ATAATATTTGATCAAATACCTTTATTTGTTTGGGCTGTAGGTATTACTGCATTACTTCTT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  490  ATAATATTTGATCAAATACCTTTATTTGTTTGAGCTGTAGGTATTACTGCATTACTTCTT  549

Query  301  CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATACTATTAACAGATCGAAAT  357
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  550  CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT  606
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8: Babylon, AL- Musayyib -4b

Batrachedra amydraula cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KT827248.1Length: 676Number of Matches: 1
Range 1: 250 to 606GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
631 bits(699)	0.0	354/357(99%)	0/357(0%)	Plus/Plus

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Query  1    CGATTAAATAATATAAGTTTTTCACTTCTTCCCCCTTCTTTAAGTCTTTTAATTTCAAGT  60
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Sbjct  250  CGATTAAATAATATAAGTTTTTGACTTCTTCCCCCTTCTTTAAGTCTTTTAATTTCAAGT  309

Query  61   TCTATTGTAGAAAATGGAGCAGGAACAGGATCAACAGTTTACCCCCCTCTTTCTTCTAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  310  TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCTCTTTCTTCTAAT  369

Query  121  ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  370  ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT  429

Query  181  TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAATTAAATGGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  430  TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAATTAAATGGA  489

Query  241  ATAATATTTGATCAAATACCTTTATTTGTTTGGGCTGTAGGTATTACTGCATTACTTCTT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  490  ATAATATTTGATCAAATACCTTTATTTGTTTGAGCTGTAGGTATTACTGCATTACTTCTT  549

Query  301  CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT  357
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  550  CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT  606
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9: Babylon, Mahawil – 5a

Batrachedra amydraula cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: KT827248.1Length: 676Number of Matches: 1
Range 1: 250 to 606

Score	Expect	Identities	Gaps	Strand
627 bits(694)	0.0	353/357(99%)	0/357(0%)	Plus/Plus

```
Query  1    CGATTAAATAATATAAGTTTTTCACTTCTTCCCCCTTCTTTAAGTCTTTTAATTTCAAGT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  250  CGATTAAATAATATAAGTTTTTGACTTCTTCCCCCTTCTTTAAGTCTTTTAATTTCAAGT  309

Query  61   TCTATTGTAGAAAATGGAGCAGGAACAGGATCAACAGTTTACCCCCCTCTTTCTTCTAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  310  TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCTCTTTCTTCTAAT  369

Query  121  ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  370  ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT  429

Query  181  TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTACTATTAATATAAAATTAAATGGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  430  TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAATTAAATGGA  489

Query  241  ATAATATTTGATCAAATACCTTTATTTGTTTGGGCTGTAGGTATTACTGCATTACTTCTT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  490  ATAATATTTGATCAAATACCTTTATTTGTTTGAGCTGTAGGTATTACTGCATTACTTCTT  549

Query  301  CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT  357
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  550  CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT  606
```

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Received 16 February 2021; Accepted 08 March 2021.

10: Babylon, Mahawil-5b

Batrachedra amydraula cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial
Sequence ID: KT827248.1Length: 676Number of Matches: 1
Range 1: 250 to 606

Score	Expect	Identities	Gaps	Strand
622 bits(689)	0.0	352/357(99%)	0/357(0%)	Plus/Plus

```
Query  1    CGATTAAATAATATAAGTTTTTCACTTCTTCCCCCTTCTTTAAGTCTTTTAATTTCAAGT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  250  CGATTAAATAATATAAGTTTTTGACTTCTTCCCCCTTCTTTAAGTCTTTTAATTTCAAGT  309

Query  61   TCTATTGTAGAAAATGGAGCAGGAACAGGATCAACAGTTTACCCCCCTCTTTCTTCTAAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  310  TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCTCTTTCTTCTAAT  369

Query  121  ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  370  ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT  429

Query  181  TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAATTAAATGGA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  430  TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAATTAAATGGA  489

Query  241  ATAATATTTGATCAAATACCTTTATTTGTTTGGGCTGTAGGTATTACTGCATTACTTCTT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  490  ATAATATTTGATCAAATACCTTTATTTGTTTGAGCTGTAGGTATTACTGCATTACTTCTT  549

Query  301  CTCCGATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT  357
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  550  CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT  606
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Figure 6. Sites of match between the nucleotide sequences of the COXI gene for the study locations (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10) with the gene sequences sites in the GEN BANK.

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Scientific Classification of Lesser Date Moth

Kingdom : Animalia
Phylum : Arthopoda
Class : Insecta
Order : Lepdoptera
Suborder : Ditrysia
Inferaorder: Heteroneura
Superfamily: Gelechoidea
Family : Batrachedridae
Subfamily : Batrachedrinae
Genus : Batrachedra
Species : *amydraula* (MAD)* Iraq. (6).

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