

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

Mohsen A. Al-Musafir¹, Auid A. Abdulkader², Dhia S. Alwaily³

¹Al-Musayyib Technical Institute, Al Furat Al-Awsat Technical University, Iraq.

E-mail: Mohsen.kareem@atu.edu.iq

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

³Faculty 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

Lepidoptera, 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).



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

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 μ l of buffer CL, 20 μ l of Proteinase K, and 5 μ l 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 μ l 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 μ l of the supernatant solution was carefully transferred to a new (unused) 1.5 ml tube.
- A 200 μ l 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 μ l 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 μ l 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 μ l 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.

3. The COXI Gene Primer Design

The primers for COXI rRNA gen diagnosis *B. amydrula* located on the COXI 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. amydrula*

Sequence (5'→3')	Tm	GC%	Product length
Forward primer	247	60.03	427
Reverse primer	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 / μ l) 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	5 μ l
Forward primer	10 picomols/ μ l (1 μ l)
Reverse primer	10 picomols/ μ l (1 μ l)
DNA	1.5 μ l
Distill water	16.5 μ l
Total volume	25 μ l

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.

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

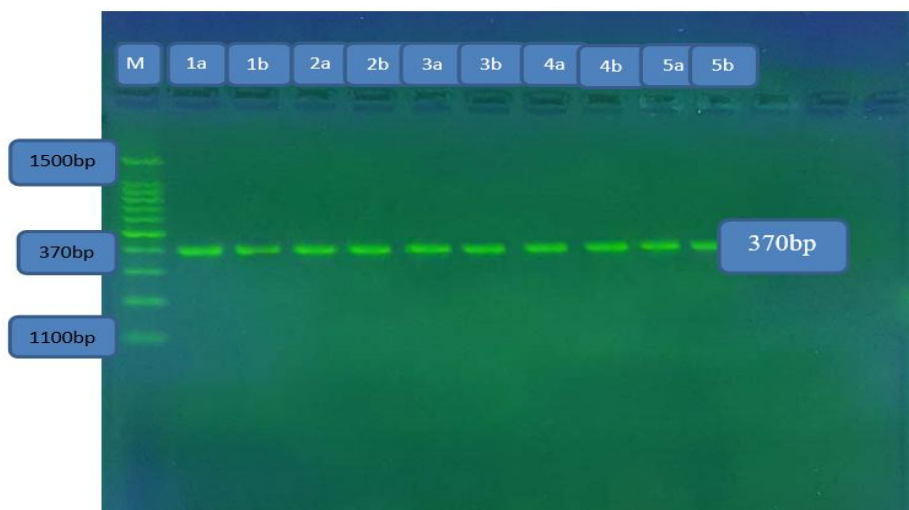


Figure 3. Represents the electrophoresis image of Agarose gel containing the results of the PCR test of the COXI rRNA gene for the diagnosis of the lesser date moth sex

*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

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	Transversion	272	G\C	KT827248.1	MT890535.1	99%	Batrachedra amydraula (COXI) gene
	Transversion	329	C\G				
	Transversion	341	G\C				
	Transition	522	A\G				
2	Transversion	272	G\C	KT827248.1	MT890536.1	99%	Batrachedra amydraula (COXI) gene
	Transversion	341	G\C				
	Transition	522	A\G				
	Transition	589	T\C				
3	Transversion	272	G\C	KT827248.1	MT890537.1	99%	Batrachedra amydraula (COXI) gene
	Transversion	341	G\C				
	Transition	522	A\G				
4	Transversion	272	G\C	KT827248.1	MT890538.1	99%	Batrachedra amydraula (COXI) gene
	Transversion	341	G\C				
	Transition	466	T\C				
	Transition	522	A\G				
5	Transversion	272	G\C	KT827248.1	MT890539.1	99%	Batrachedra amydraula (COXI) gene
	Transversion	341	G\C				
	Transition	522	A\G				
	Transition	553	T>C				
	Transversion	554	T>G				
6	Transversion	272	G\C	KT827248.1	MT890551.1	99%	Batrachedra amydraula (COXI) gene
	Transversion	329	C\G				
	Transversion	341	G\C				
	Transition	522	A\G				
7	Transversion	272	G\C	KT827248.1	MT890552.1	99%	Batrachedra amydraula (COXI) gene
	Transversion	341	G\C				
	Transition	522	A\G				
	Transition	589	T\C				
8	Transversion	272	G\C	KT827248.1	MT890553.1	99%	Batrachedra amydraula (COXI) gene
	Transversion	341	G\C				
	Transition	522	A\G				
9	Transversion	272	G\C	KT827248.1	MT890554.1	99%	Batrachedra amydraula (COXI) gene
	Transversion	341	G\C				
	Transition	466	T\C				
	Transition	522	A\G				
10	Transversion	272	G\C	KT827248.1	MT890555.1	99%	Batrachedra amydraula (COXI) gene
	Transversion	341	G\C				
	Transition	522	A\G				
	Transition	553	T\C				
	Transversion	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.

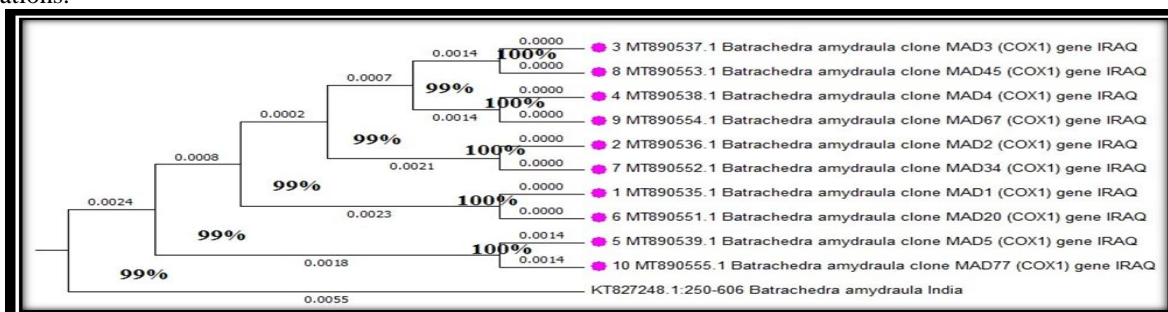


Figure 4. Dendrogram of the identities and difference of *B. amyraula* species by using the PCR technique with the species registered in the NCBI.

• **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.

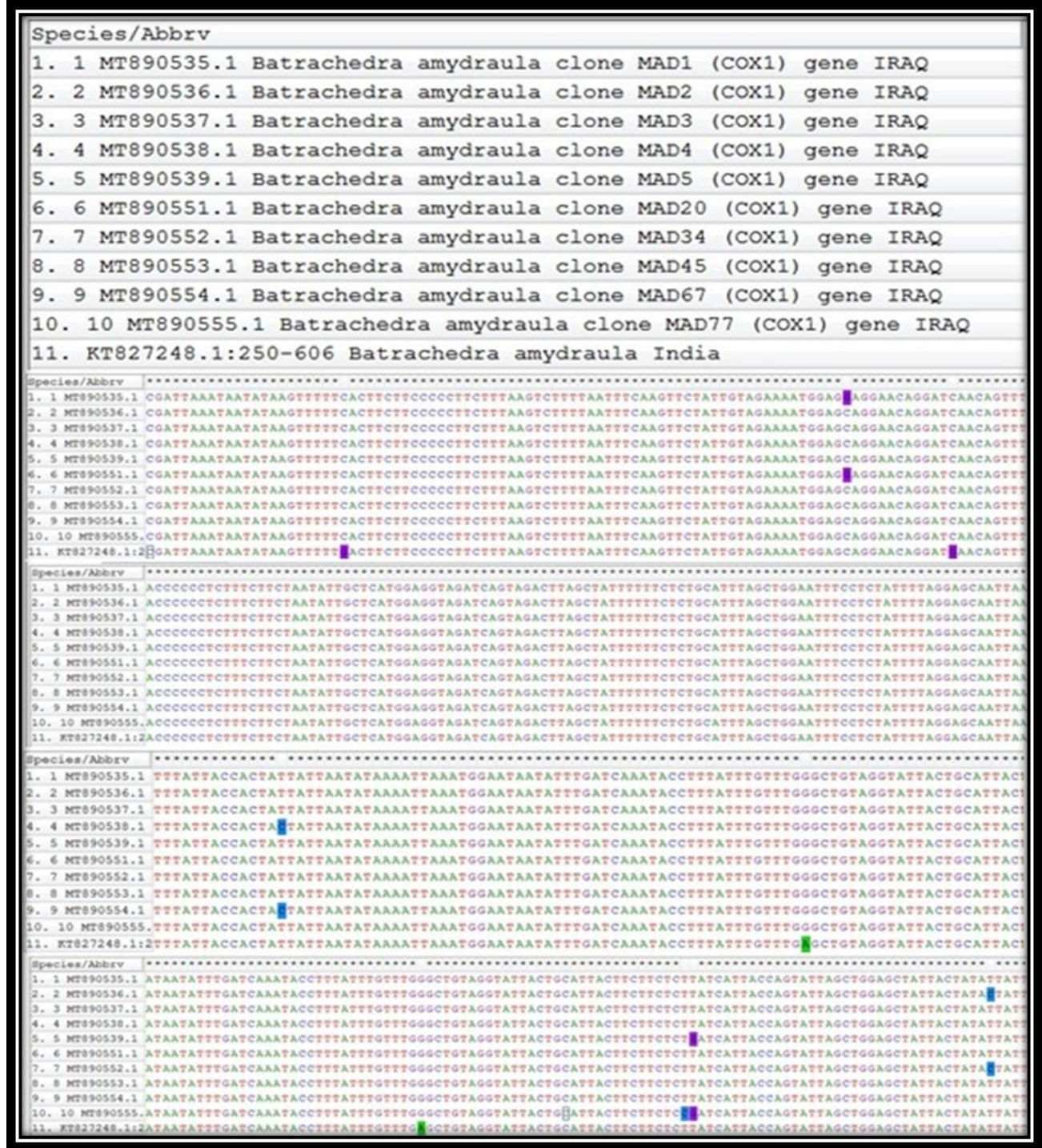


Figure 5. Represents the multiple sequence alignment analysis by using the MEGA6 program of the PCR test results for COXI gene of the studied *B. amydraula*

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
Query	1	CGATTAAATAATATAAGTTTTTCACTTCTTCCCCTTCTTTAAGTCTTTTAATTTCAAGT	60	
Sbjct	250	CGATTAAATAATATAAGTTTTTGACTTCTTCCCCTTCTTTAAGTCTTTTAATTTCAAGT	309	
Query	61	TCTATTGTAGAAAATGGAGGAGGAACAGGATCAACAGTTTACCCCCTCTTTCTTCTAAT	120	
Sbjct	310	TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCTCTTTCTTCTAAT	369	
Query	121	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	180	
Sbjct	370	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	429	
Query	181	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAAATGGA	240	
Sbjct	430	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAAATGGA	489	
Query	241	ATAATATTTTGATCAAATACCTTTATTTGTTTGGGCTGTAGGTATTACTGCATTACTTCTT	300	
Sbjct	490	ATAATATTTTGATCAAATACCTTTATTTGTTTGGGCTGTAGGTATTACTGCATTACTTCTT	549	
Query	301	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	357	
Sbjct	550	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	606	

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
Query 1	CGATTAAATAATATAAGTTTTTCACTTCTTCCCCCTTCTTAAAGTCTTTAATTTCAAGT	60		
Sbjct 250	CGATTAAATAATATAAGTTTTTGACTTCTTCCCCCTTCTTAAAGTCTTTAATTTCAAGT	309		
Query 61	TCTATTGTAGAAAATGGAGCAGGAACAGGATCAACAGTTTACCCCCCTTCTTCTTCTAAT	120		
Sbjct 310	TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCTTCTTCTTCTAAT	369		
Query 121	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	180		
Sbjct 370	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	429		
Query 181	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAATGGA	240		
Sbjct 430	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAATGGA	489		
Query 241	ATAATATTTGATCAAATACCTTTATTTGTTGGGCTGTAGGTATTACTGCATTACTTCTT	300		
Sbjct 490	ATAATATTTGATCAAATACCTTTATTTGTTGAGCTGTAGGTATTACTGCATTACTTCTT	549		
Query 301	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATACTATTAACAGATCGAAAT	357		
Sbjct 550	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATACTATTAACAGATCGAAAT	606		

3: Maysan-2a**Batrachedra amydraula cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial****Sequence ID: KT827248.1 Length: 676 Number of Matches: 1**

Range 1: 250 to 606

Score	Expect	Identities	Gaps	Strand
631 bits(699)	0.0	354/357(99%)	0/357(0%)	Plus/Plus
Query 1	CGATTAAATAATATAAGTTTTTCACTTCTTCCCCTTCTTTAAGTCTTTTAATTTCAAGT	60		
Sbjct 250	CGATTAAATAATATAAGTTTTTGACTTCTTCCCCTTCTTTAAGTCTTTTAATTTCAAGT	309		
Query 61	TCTATTGTAGAAAATGGAGCAGGAACAGGATCAACAGTTTACCCCCTCTTCTTCTAAT	120		
Sbjct 310	TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCTCTTCTTCTAAT	369		
Query 121	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	180		
Sbjct 370	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	429		
Query 181	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAATGGA	240		
Sbjct 430	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAATGGA	489		
Query 241	ATAATATTTGATCAAATACCTTTATTTGTTGGGCTGTAGGTATTACTGCATTACTTCTT	300		
Sbjct 490	ATAATATTTGATCAAATACCTTTATTTGTTGAGCTGTAGGTATTACTGCATTACTTCTT	549		
Query 301	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	357		
Sbjct 550	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	606		

4: Maysan-2b**Batrachedra amydraula cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial****Sequence ID: KT827248.1 Length: 676 Number 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	TCTATTGTAGAAAATGGAGCAGGAACAGGATCAACAGTTTACCCCCTCTTCTTCTAAT	120
Sbjct	310	TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCTCTTCTTCTAAT	369
Query	121	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	180
Sbjct	370	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	429
Query	181	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTACTATTAATATAAAAATTAATGGA	240
Sbjct	430	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTACTATTAATATAAAAATTAATGGA	489
Query	241	ATAATATTTGATCAAATACCTTTATTTGTTTGGGCTGTAGGTATTACTGCATTACTTCTT	300
Sbjct	490	ATAATATTTGATCAAATACCTTTATTTGTTTGGGCTGTAGGTATTACTGCATTACTTCTT	549
Query	301	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	357
Sbjct	550	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	606

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	TCTATTGTAGAAAATGGAGCAGGAACAGGATCAACAGTTTACCCCCCTCTTCTTCTAAT	120		
Sbjct 310	TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCTCTTCTTCTAAT	369		
Query 121	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	180		
Sbjct 370	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	429		
Query 181	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAATGGA	240		
Sbjct 430	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAATGGA	489		
Query 241	ATAATATTTGATCAAATACCTTTATTTGTTGGGCTGTAGGTATTACTGCATTACTTCTT	300		
Sbjct 490	ATAATATTTGATCAAATACCTTTATTTGTTGAGCTGTAGGTATTACTGCATTACTTCTT	549		
Query 301	CTCCGATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	357		
Sbjct 550	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	606		

6: Dhi Qar-3b**Batrachedra amydraula cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial****Sequence ID: KT827248.1 Length: 676 Number of Matches: 1**

Range 1: 250 to 606 GenBank Graphics Next Match Previous Match

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

Query	1	CGATTAAATAATATAAGTTTTTCACTTCTTCCCCTTCTTTAAGTCTTTTAATTTCAAGT	60
Sbjct	250	CGATTAAATAATATAAGTTTTTGACTTCTTCCCCTTCTTTAAGTCTTTTAATTTCAAGT	309
Query	61	TCTATTGTAGAAAATGGAGGAGGAACAGGATCAACAGTTTACCCCCTCTTCTTCTAAT	120
Sbjct	310	TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCTCTTCTTCTAAT	369
Query	121	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	180
Sbjct	370	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	429
Query	181	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAATGGA	240
Sbjct	430	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAATGGA	489
Query	241	ATAATATTTGATCAAATACCTTTATTTGTTGGGCTGTAGGTATTACTGCATTACTTCTT	300
Sbjct	490	ATAATATTTGATCAAATACCTTTATTTGTTGAGCTGTAGGTATTACTGCATTACTTCTT	549
Query	301	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	357
Sbjct	550	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	606

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	CGATTAAATAATATAAGTTTTTCACTTCTTCCCCTTCTTTAAGTCTTTTAATTTCAAGT	60
Sbjct	250	CGATTAAATAATATAAGTTTTTGACTTCTTCCCCTTCTTTAAGTCTTTTAATTTCAAGT	309
Query	61	TCTATTGTAGAAAATGGAGCAGGAACAGGATCAACAGTTTACCCCCTCTTCTTCTAAT	120
Sbjct	310	TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCTCTTCTTCTAAT	369
Query	121	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	180
Sbjct	370	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	429
Query	181	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAATGGA	240
Sbjct	430	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAATGGA	489
Query	241	ATAATATTTGATCAAATACCTTTATTTGTTGGGCTGTAGGTATTACTGCATTACTTCTT	300
Sbjct	490	ATAATATTTGATCAAATACCTTTATTTGTTGAGCTGTAGGTATTACTGCATTACTTCTT	549
Query	301	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATACTATTAACAGATCGAAAT	357
Sbjct	550	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATACTATTAACAGATCGAAAT	606

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
Query 1	CGATTAAATAATATAAGTTTTTCACTTCTTCCCCCTTCTTTAAGTCTTTTAATTTCAAGT	60		
Sbjct 250	CGATTAAATAATATAAGTTTTTGACTTCTTCCCCCTTCTTTAAGTCTTTTAATTTCAAGT	309		
Query 61	TCTATTGTAGAAAATGGAGCAGGAACAGGATCAACAGTTTACCCCCCTCTTCTTCTAAT	120		
Sbjct 310	TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCCTCTTCTTCTAAT	369		
Query 121	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	180		
Sbjct 370	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	429		
Query 181	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAATGGA	240		
Sbjct 430	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAATGGA	489		
Query 241	ATAATATTTGATCAAATACCTTTATTTGTTGGGCTGTAGGTATTACTGCATTACTTCTT	300		
Sbjct 490	ATAATATTTGATCAAATACCTTTATTTGTTGAGCTGTAGGTATTACTGCATTACTTCTT	549		
Query 301	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	357		
Sbjct 550	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	606		

9: Babylon, Mahawil – 5a**Batrachedra amydraula cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial****Sequence ID: KT827248.1 Length: 676 Number 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	CGATTAAATAATATAAGTTTTTCACTTCTTCCCCTTCTTTAAGTCTTTTAATTTCAAGT	60		
Sbjct 250	CGATTAAATAATATAAGTTTTTGACTTCTTCCCCTTCTTTAAGTCTTTTAATTTCAAGT	309		
Query 61	TCTATTGTAGAAAATGGAGCAGGAACAGGATCAACAGTTTACCCCCTCTTCTTCTAAT	120		
Sbjct 310	TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCTCTTCTTCTAAT	369		
Query 121	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	180		
Sbjct 370	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	429		
Query 181	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTACTATTAATATAAAAATTAATGGA	240		
Sbjct 430	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTACTATTAATATAAAAATTAATGGA	489		
Query 241	ATAATATTTGATCAAATACCTTTATTTGTTGGGCTGTAGGTATTACTGCATTACTTCTT	300		
Sbjct 490	ATAATATTTGATCAAATACCTTTATTTGTTGAGCTGTAGGTATTACTGCATTACTTCTT	549		
Query 301	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	357		
Sbjct 550	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	606		

10: Babylon, Mahawil-5b

Batrachedra amydraula cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial
Sequence ID: KT827248.1 Length: 676 Number 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	CGATTAAATAATATAAGTTTTTCACTTCTTCCCCTTCTTTAAGTCTTTTAATTTCAAGT	60		
Sbjct 250	CGATTAAATAATATAAGTTTTTGACTTCTTCCCCTTCTTTAAGTCTTTTAATTTCAAGT	309		
Query 61	TCTATTGTAGAAAATGGAGCAGGAACAGGATCAACAGTTTACCCCCTCTTTCTTCTAAT	120		
Sbjct 310	TCTATTGTAGAAAATGGAGCAGGAACAGGATGAACAGTTTACCCCCTCTTTCTTCTAAT	369		
Query 121	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	180		
Sbjct 370	ATTGCTCATGGAGGTAGATCAGTAGACTTAGCTATTTTTTCTCTGCATTTAGCTGGAATT	429		
Query 181	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAATGGA	240		
Sbjct 430	TCCTCTATTTTAGGAGCAATTAATTTTATTACCACTATTATTAATATAAAAATTAATGGA	489		
Query 241	ATAATATTTGATCAAATACCTTTATTTGTTGGGCTGTAGGTATTACTGCATTACTTCTT	300		
Sbjct 490	ATAATATTTGATCAAATACCTTTATTTGTTGAGCTGTAGGTATTACTGCATTACTTCTT	549		
Query 301	CTCCGATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	357		
Sbjct 550	CTCTTATCATTACCAGTATTAGCTGGAGCTATTACTATATTATTAACAGATCGAAAT	606		

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.

Scientific Classification of Lesser Date Moth

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

References

- [1] Jarodet, A.A. (2003). Agriculture in Iraq: Resources potential, constraints, and research need and priorities. *Food Agriculture and Environment, 1*(2), 160-166.
- [2] Al-Jboory, A.G. (2007). Inventory and diagnosis of biological factors in the date palm environment and adopted to develop an integrated management program for palm pests in Iraq. *Aden University Journal of Applied Sciences, 11*(3), 446-451.
- [3] Aziz, F.M. (2005). *Biological and ecological studies on the lesser date moth Batrachedra spp. (Lepidoptera: Cosmopterygidae) and prediction of its appearance infesting the date palm early in the Spring* (Doctoral dissertation, Ph.D. Dissertation. College of Science University, Baghdad).
- [4] Mohammad, A., & AL-Saeedy, A.A. (2003). *Basics of Insect Ecology. Egypt, Cairo*. Library House Arab Book.
- [5] Rattan, R.S., Reineke, A., Hadapad, A., Gupta, P.R., & Zebitz, C.P.W. (2006). Molecular phylogeny of *Cotesia* species (Hymenoptera: Braconidae) inferred from a 16S gene. *Current Science, 91*(11), 1460-1461.
- [6] Hodges, R.W. (1978). *The Moths of America North of Mexico (R.B.) Dominick et al., eds.) fasc.6.1 (Cosmopterygidae)*. London.