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Cytochrome Oxidase Subunit I (COI) Gene Sequencing for Identification of Cephalopina Titillator local Isolates from Camels in Thi-Qar Province/Iraq

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ABSTRACT

This study was conducted for the period from the first of the month of February 2020 to 30 of October 2020 in the **Thi-Qar**Province ,A twenty DNA samples were used to detects the parasite by using specific DNA primer of cytochrome oxidase gene COI which amplify 545 bp PCR products for *Cephalopina titillator*, then the positive products were subjected to DNA sequencing and the result was analyze by **(MEGA X version) and NCBI-BLAST programs**. The results of the PCR showed the positive identity of the *C. titillator* in the samples in agarose gel electrophoresis with in 545bp for the gene specific regions of the *C. titillator*, the DNA sequences appear the presence of a new strain of parasite under study that recorded in NCBI gene bank under IQ 1 (ACCESSION: MW167083). We can conclude that the ***Cephalopeniatitillotar***has a new strain recorded in Iraqi isolates called Cephalopina titillator isolate IQ1 cytochrome c oxidase subunit I version (MW167083.1) and mitochondrial COX I gene is a powerful genetic marker for phylogenetic studies.

Keywords: Cytochrome oxidase gene, *Cephalopina titillator*, Myiasis

INTRODUCTION

Camels in the middle east are a importantconstituent of desert life and have an economic significance as they are an essential source for good quality red meat, milk and leather as well as contributing to the transportation of various goods across regions that are difficult to reach by means of transportation (1), exposed Camel to infection with different types of parasites, including camel nasal bot fly, and its scientific name is *Cephalopina titillator*, which is a compulsory parasite that be located inthe mucous membrane of the nasal cavity and pharynx, and spreads widely in the world at the locations of camels where it affects camels Nasal myiasis (2,3). The adult fly places its larvae around the nostrils of the camel, where the fly is larviparous affection, and the larvae, known as first instar larvae, crawl towards the nasal cavities, causing irritation and damage to the mucous tissues lining the nose and pharynx, and over time turn into the second and third phases and remain inside the nasal and pharyngeal cavities for a period of time. It may reach from (10-11) months and then leave the host during sneezing and fall to the soil where it turns into a virgin Pupa and after a period of (3-9) weeks it comes out as a complete fly (4,5).The clinical signs of the camel that are programmed are characterized by general poor appetite, difficulty breathing, nasal discharge, snoring, involuntary head movement, nosebleeds, and sneezing (6,7). It was indicated to the parasite for the first time in Iraq in 1977 (the infection rate was (47%) (8). The larvae cause damage to the physiological functions of the animal and tissue damage as they cause economic losses through the occurrence of miscarriages, low milk yield, weight loss and low fertility rate for camels. (9), and the larvae may sometimes reach the

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cranial cavity by the penetration of the larvae into the ethmoid bone, where meningitis causes meningitis and nervous signs (10,11).The current study aims to determine the C. titillator strains that associated with the myiasis in Iraq.

MATERIALS AND METHODS

Sample collection:

A total of (20) DNA samples were examined in the Al-Nasiriyah city/Iraq in the September and October /2020 to detects *Cephalopina titillator*strain.

Molecular identification of *Cephalopina titillator*by conventional PCR.

The DNA samples were subjected toPCR-intensified inward interpreted spacer (cox1) district of ribosomal DNA (rDNA) was performed with groundworks cox-1 specific primer forward (5'-GAGGAGGCTTCGCAGTAGAC-3') and cox-1 turn around (5'-GGGGTTTTCTACTGGTCGGG-3') (Bioneer-Korea), the PCR reaction mixture illustrated in table (1). The PCR conditions involved initial denaturation at 94 °C for 5 min. followed by 35 cycles of denaturation 94 °C (1min), annealing 55 °C (2 min) and extension 72 °C (3 min), final extension done at 72 °C for 8 min.

Table 1. PCR contents

No	PCR master mix	Volume µl
1	Master mix (Bioneer-Korea)	5 µl
2	Forward primer 10 mol	1 µl
3	Reverse primer 10 mol	1 µl
4	PR water	5 µl
5	DNA template	8 µl
Total volume		20 µl

DNA sequences

The cytochrome oxidase subunit I (COI) gene was partially sequence to detects the local *Cephalopina titillator* camel strain of IQ isolate after send for the sequencing by using automated DNA sequencer ABI 3730XL (Macrogen Corporation, Korea) in South Korea, The sequence alignment analysis performed by NCBI BLAST. While, the phylogenetic tree analysis was madeby UPGMA tree method in (MEGA X version).

RESULTS

The results of the PCR showed the positive identity of the *C. titillator* in the samples in agarose gel electrophoresis with in 545bp PCR products of **PCR product COX1 primer of *C. titillator*.**

DNA Sequence

The DNA sequencing analysis of *C. titillator*COI gene was show clear genetic variation between *C. titillator* isolates from different hosts according to phylogenetic tree analysis that analyzed

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local *C. titillator*camel isolates with Standard NCBI-BLAST *C. titillator* isolates. As show in (figure 1).

The Nucleotide variations Substitution analysis (Table 2) between local *C. titillator*camel isolates gene and NCBI BLAST *C. titillator*isolates were not found transitional substitutions between nucleotide at from total gene nucleotides.

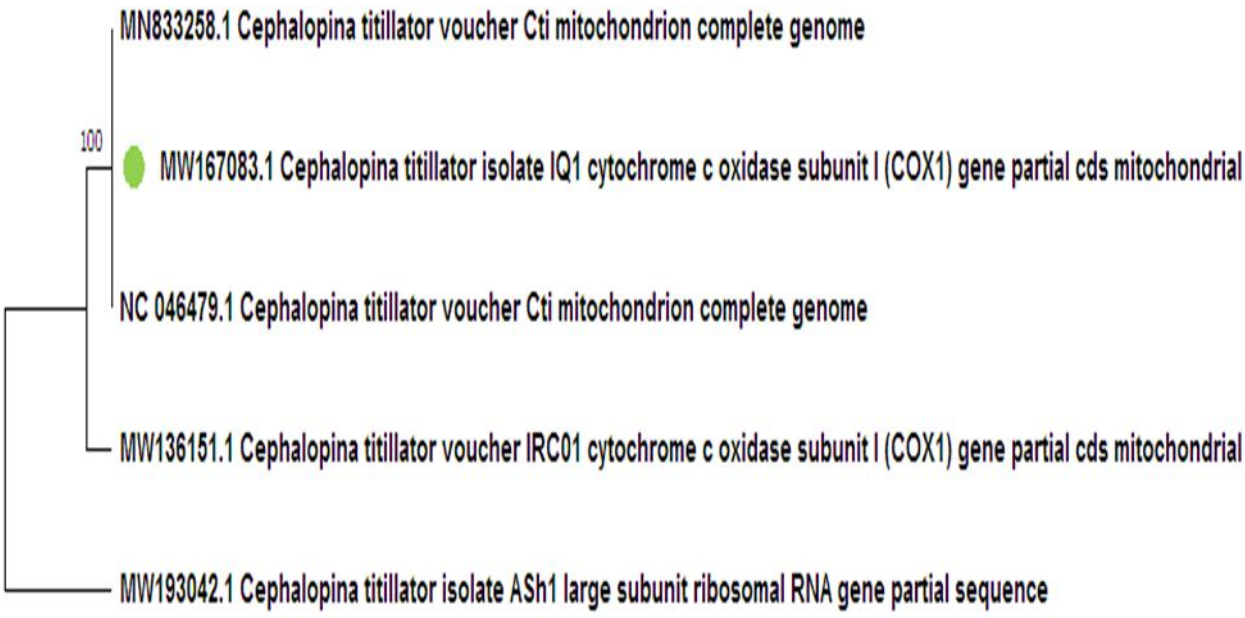

Figure 1. Phylogenetic tree analysis based on cytochrome oxidase subunit I (COI) gene partial sequence in local *Cephalopina titillator* Camel bot fly IQ isolate that used for genetic relationship analysis . The phylogenetic tree was constructed using UPGMA tree method in (MEGA X version). The local *Cephalopina titillator* Camel bot fly IQ isolate were showed genetic closed related to NCBI-BLAST *Cephalopina titillator* (MN833258.1) and (NC_046479.1). Whereas other NCBI-BLAST *Cephalopina titillator* were showed genetic variants to local *Cephalopina titillator* Camel bot fly IQ isolate.

Table 2. the NCBI-BLAST Homology Sequence identity (%) between local *Cephalopina titillator* isolates and NCBI-BLAST submitted *C.titillator*isolates:

NCBI Cephalopina titillator	**Genbank Accession number**	**NCBI-BLAST Homology Sequence identity (%)**
Cephalopina titillator isolate No.1	MN833258.1	100%
Cephalopina titillator isolate No.2	NC_046479.1	100%
Cephalopina titillator isolate No.3	MW136151.1	87%
Cephalopina titillator isolate No.4	MW193042.1	80%

Multiple sequence alignment ofcytochrome oxidase subunit I (COI) gene in *C. titillator*isolates and NCBI-Gene bank *C. titillator* isolate.The sequence alignment analysis using NCBI BLAST.

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That showed the nucleotide alignment identity at 100%.

Score	Expe	Identities	Gaps	Strand
1115 bits(1236)	**0.0**	**618/618(100%)**	**0/618(0%)**	**Plus/Plus**

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Query  1     TTAAGAATATTAATTCGAATAGAGCTAGGACACCCCGGAACGCTCATTGGAAATGATCAA  60
Sbjct  1715  ............................................................  1774

Query  61    ATTTATAATGTAATTGTCACCGCACATGCTTTCATCATAATTTTCTTTATAGTTATACCA  120
Sbjct  1775  ............................................................  1834

Query  121   ATTATAATTGGAGGATTTGGAAATTGACTAGTCCCACTAATACTAGGGGCCCCAGATATA  180
Sbjct  1835  ............................................................  1894

Query  181   GCATTCCCTCGAATAAATAATATAAGATTCTGACTTTTACCTCCCGCTCTTACACTCCTT  240
Sbjct  1895  ............................................................  1954

Query  241   CTAACAAGAAGAATAGTAGAAAGCGGCGCTGGCACTGGATGAACTGTTTATCCTCCTCTT  300
Sbjct  1955  ............................................................  2014

Query  301   TCATCAAATATTGCCCACAGAGGAGCCTCCGTAGATTTAGCAATTTTCTCACTTCATTTA  360
Sbjct  2015  ............................................................  2074

Query  361   GCTGGAATTTCATCCATTTTAGGAGCAGTTAATTTTATTACAACAATCATCAATATACGA  420
Sbjct  2075  ............................................................  2134

Query  421   TCTATTGGAATAACTTTAGATCGAACACCCTTATTTGTATGATCTGTAATAATTACAGCA  480
Sbjct  2135  ............................................................  2194

Query  481   ATTCTTTTACTTCTATCTCTACCAGTTTTAGCAGGAGCCATTACAATACTATTAACCGAC  540
Sbjct  2195  ............................................................  2254

Query  541   CGGAATTTAAACACATCATTTTTTGACCCTGCAGGAGGGGGAGACCCCATTCTTTATCAA  600
Sbjct  2255  ............................................................  2314

Query  601   CACTTATTTTGATTTTTT  618
Sbjct  2315  ..................  2332
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DISCUSSION

Camels infestations due to the tropical botfly (*Cephalopina titillator*) as a causative agent nasal myiasis were reported in a number of countries including Iraq C. titillator is an obligate parasite infecting both sexes of camels in all stages of life leading to health risks and greatest economic losses in camels (12,13). One- to three-year-old animals are most severely affected. The plague seasonality of the fly larva has been studied in different countries of Asia and Africa involving Iraq. The adult fly lives liberally around the head of the camels and It places larvae round of the nostrils, which migrates to the nasopharyngeal area and they stays for months then develops to adults (14).

Mitochondrial DNA (mtDNA) has been used recently as a tool in the investigations and studying of the evolution and taxonomy of animal populations. protein-coding genes in mitochondria are mostly occur underneath purifying selection. The changes in DNA sequences reflected on the amino acid sequences in the proteins, even though the amino acid exchanges are rare in some mitochondrial DNA genes particularly in the cytochrome oxidase genes(15,16). The cytochrome oxidase subunit I (COI) gene is an important gene in mtDNA genes cassette, this gene is responsible for the production of cytochrome oxidase is a last enzyme in the mitochondrial electron transport chain which drives oxidative phosphorylation(17).

The cytochrome oxidase subunit I (COI) gene considered as an important goal for taxonomic and evolutionary studies of the eukaryotes because of its existence in most members of eukaryotes and its chief function in the cell metabolism and energy production and the patterns of COI evolution may vary between taxa (18).. Furthermore, COI gene is the highly conserved mitochondrial genes in animalia. In addition, the size and structure of COI gene have been suitable especially for evolutionary studies and its represents a good phylogeny signal, and thus displays. COI has been known to be as DNA barcoding that been used very successfully in many animal. For examples, it has been differentiated species in birds (19,20).

The results of COX gene sequence in the current study showed aslightly difference in nucleotides genes composition of local isolates when compared with other isolates of *C.titillator* that recording in NCBI gene bank by using NCBI blast. The sequence alignment showed identity at 100% among *C. titillator* local isolates. The phylogenetic pedigree showed the gene sequence variation between the local isolates (MW167083.1) *C. titillator* IQI (COX) gene of mitochondria and other previously recorded *C. titillator*strains . But the Iraqi isolate IOI has been nearly phylo-genetically from China isolates (MN833258.1) and (NC046479.1) isolates than those that recorded in Middle east (21). Also, camel bot fly IQ isolate showed genetic variants according to DNA partial cds mitochondrial COX I gene sequence (22,23).

CONCLUSION

The current study indicates the recording of a new isolates of the camel botfly *C. titillator* that has a different genetic variants, and the cytochrome oxidase subunit I represents a powerful genetic marker for taxonomic and phylogenetic studies of the parasites.

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