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MOLECULAR CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF *CULEX QUINQUEFASCIATUS* BY CYTOCHROME C OXIDASE SUBUNIT II

Sahar Abd[1]* and Emad Ahmed[2]

[1]*Lecturer Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq.

[2]Prof. Dr. Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq.

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***Corresponding Author**
Sahar Abd
Lecturer Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq.

ABSTRACT

Molecular Genetic methods were used in this study to find out the genetic diversity of *Culex* collected from Nahrawan region of Baghdad in Iraq, the amplified segments of mitochondrial DNA which consisted of the genes COII by using Polymerase Chain Reaction (PCR) with the existence of pairs from the specific primers. The PCR amplified fragments in these mosquitoes were approximately *685* base pairs and the amino acids were more than 95% similar between *Culex quinquefasciatus* from USA and *C. quinquefosciatus* from Baghdad, and there is a substitution in 31 bases distributed along the nucleotide sequence, the variations ranged between 16 type of transition while the 12 type Transversion. These segments contain the major amino acid residues of cytochrome c oxidase included in electron transport and ligand binding.

KEYWORDS: *Culex* mosquitoes; DNA barcode; cytochrome oxidase II (COII), Phylogenetic Analysi.

INTRODUCTION

Mosquitoes are responsible for the transmission of parasitic and viral infections to both humans and livestock, with substantial morbidity and mortality.[1] There are over 3,500 different species of mosquitoes throughout the world.[2] Some diseases transmitted by mosquitoes include malaria, yellow fever, encephalitis and RVF among others.[3] Prevention of infection of mosquito transmitted diseases mainly rely on vector control as well as

vaccinations to reduce and/or prevent transmission.[2] Mosquito control is carried out by habitat control, use of insecticides, larvicides and breeding control using sterile males.[4] Most mosquito transmitted organisms have an obligatory developmental stage that takes place in the mosquito, and in some cases completely rely on the vectors for transmission. The primary identification of mosquitoes has over time been carried out by morphological characterization.[5] Mosquitoes are observed under a differentiating microscope and only persons with knowledge of the features that differentiate one species from another have the capacity to carry out morphological identification. An unambiguous identification of mosquitoes using morphological characters requires taxonomic experience and specimens as intact as possible.[6] In addition to the morphological tools, molecular methods for sub species classification have been proposed.[7] These include PCRRFLPs which apply differences in the length of DNA fragments after digestion with restriction endonucleases.[8]

Mitochondrial Cytochrome oxidase C subunit I and II (COI and COII), Cytochrome oxidase B, 16S rRNA gene are helpful in species identification, phylogenetic analyses and other related studies.[9] this study used in identification of insect specimens and have proven highly informative for phylogenetic inference.

MATERIALS AND METHODS

Field samples were obtained from different selected locations in Baghdad, Periods of higher mosquito population densities were targeted. DNA was extracted separately for mosquitoes from *Culex quinquefasciatus* by using Genomic DNA Mini Kit Tissue Protocol.

DNA Amplification by (PCR) was used to amplify a segment of the COII gene using the primer pair **table (1)** to find the optimal condition has identified for (Initial denaturation and annealing) after a work several experiments to get for this condition, the temperature has changed through the work of (Gradient PCR) for all samples to select the optimal condition **table (2)**, and also changed the concentration for DNA template between (1.5-2µl) where is considered these two factors from important factors in primer annealing with complement.

The primers used in the interaction

Table (1): The specific and universal primer of gene *COII 685 bp.*

Primer	Sequence	Tm (°C)	GC (%)	Product size
COIIF	AAGTCGCTAAAGCTCCTACTG	62	47.62 %	685
COIIR	CTTCATCAAAGTCGTCTCCA	58	45 %	base pair

Table (2): The optimum condition of detection *COII*.

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	94°C	5 min.	35cycle
2-	Denaturation -2	94°C	40sec.	
3-	Annealing	62°C	35sec.	
4-	Extension-1	72°C	35sec.	
5-	Extension -2	72°C	10 min.	

RESULTS AND DISCUSSIONS

Total genomic DNA was extracted from each mosquito using Genomic DNA Mini Kit Tissue Protocol **Figure 1**. Samples not analyzed immediately were stored at −20°C. The polymerase chain reaction (PCR) was used to amplify a segment of the COII gene using the specific primer *685 bp* pair AAGTCGCTAAAGCTCCTACTG / CTTCATCAAAGTCGTCTCCA and standard assay conditions, find the optimal temperature in ratio of strong fragment, 62°C were chosen for annealing temperature for the PCR experiment **Figure 2**.

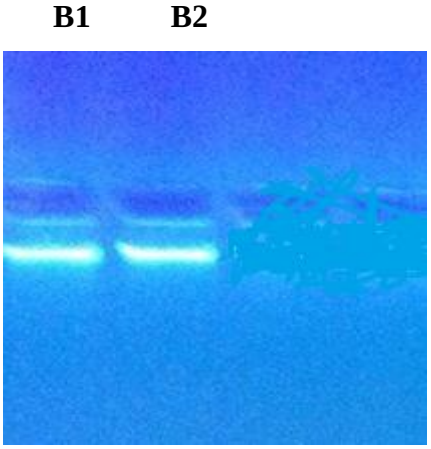

Figure (1): Gel electrophoresis of DNA extraction from *Culex quinquefasciatus*, 2% agarose gel at 5 vol /cm for 1:15 houre.

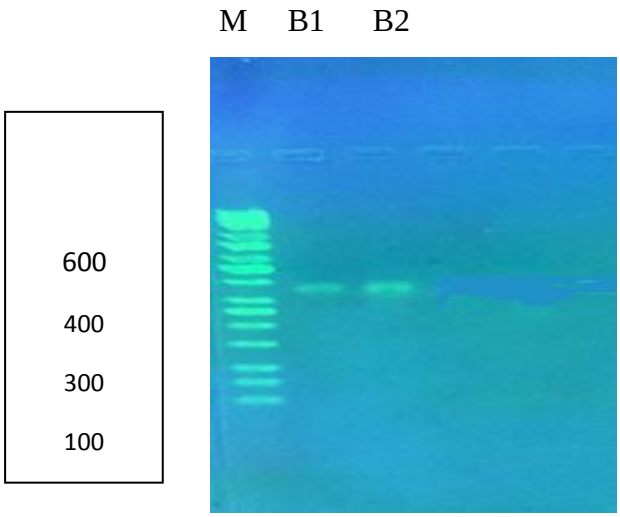

Figure 2: Electrophoresis of PCR products of the COII gene using the specific primer *685 bp* pair collected from of Iraq. PCR product from Iraq sample, M: Marker; B1 female Baghdad; B2 male Baghdad.

Fig 3 shows the locations of nucleotide substitutions and the sequences showed very low variation between species on Alignment and phylogenetic analysis, Table (3) shows the substitutions classified according to the type of base change between these2 species. The COII gene is 95% similar between *Culex quinquefasciatus* from USA and *C. quinquefosciatus* from Baghdad. The number of transversion was less than that of transitions In all transversions, the frequency of change between A and T. For a comparison of COII, amino acid sequences were deduced by using the insect mitochondrial code.685 amino acid residues are present and similarity is shown in Figure 3 This is similar from comparisons of mtDNA between closely related mammalian species in which transitions occur more frequently than transversions.[10] There was also variation between species on phylogenetic analysis Figure 4 the evolutionary history was inferred using the Neigh bour-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to estimate the phylogenetic tree. The rate variation among sites was modelled with genetic distances = 0.030. DNA barcode approach based on DNA sequences of mitochondrial cytochrome oxidase gene sequences could identify 62 species among these, in confirmation with the conventional taxonomy.[11] Jakarta, Bangladesh, Turkey and Tunisia were located together in one linage, in this phylogenetic tree, Iraq was considered as an out group but was genetically closer to the USA and Thailand.

Table (3): Type substitutions classified according sequence of partial mitochondrial COII sequences of *Culex quinquefasciatus* from *Baghdad* compared with *Cx. quinquefasciatus* from USA.

sample	Type of substitution	Location	Nucleotide	Range of nucleotide	Sequence ID
Sample from Baghdad	Transition	3139	C>T	3124 **to** 3735	gb|HQ724617.1|
	Transversion	3159	T>A		
	Insertion	3162	▼T		
	Transversion	3171	A>T		
	Insertion	3180	▼T		
	Transition	3187	C>T		
	Transition	3195	T>C		
	Insertion	3195	▼T		
	Transition	3201	G>A		
	Transition	3243	T>C		
	Transition	3256	T>C		
	Transversion	3258	A>T		
	Transversion	3264	T>A		
	Transversion	3295	T>A		
	Transversion	3330	A>T		
	Transversion	3426	T>A		
	Transversion	3429	T>A		
	Transition	3447	C>T		
	Transition	3457	C>T		
	Transition	3471	C>T		
	Transition	3474	C>T		
	Transversion	3489	T>A		
	Transition	3510	C>T		
	Transition	3537	T>C		
	Transition	3556	C>T		
	Transversion	3579	A>T		
	Transition	3607	T>C		
	Transversion	3624	A>T		
	Transversion	3627	A>T		
	Transition	3630	C>T		
	Transition	3651	T>C		

Score	Expect	Identities	Gaps	Strand
955 bits(1058)	0.0	583/615(95%)	3/615(0%)	Plus/Plus

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Query  3     CATGATCATACAGTTCTAATTTTAATTATAATTACTGTATATAATTACATATGTAATATG  62
             ||||||||||||||| |||||||||||||||||| ||| |||||||| ||||||||| |
Sbjct  3124  CATGATCATACAGTTTTAATTTTAATTATAATTACAGTA-ATAATTACTTATGTAATA-G  3181

Query  63    GTATACTATTTTTTTAATAAGTTTACAAATCGATATTTATTACATGGACAAACTATTGAA  122
             |||||  |||||||  |||||  ||||||||||||||||||||||||||||||||||||
Sbjct  3182  GTATATTATTTTTC-AATAAATTTACAAATCGATATTTATTACATGGACAAACTATTGAA  3240

Query  123   ATTATTTGAACAATTTTACCTGCTATTATTTTAATATTTATTGCTTTTCCATCTCTTCGA  182
             ||  |||||||||||| | |||||  ||||||||||||||||||||||||||  ||||||
Sbjct  3241  ATCATTTGAACAATTCTTCCTGCAATTATTTTAATATTTATTGCTTTTCCATCACTTCGG  3300

Query  183   TTATTATATTTATTAGATGAAATTAATTCACCTTTAATTACTTTAAAGGCTATTGGACAT  242
             ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  3301  TTATTATATTTATTAGATGAAATTAATTCTCCTTTAATTACTTTAAAGGCTATTGGACAT  3360

Query  243   CAATGATACTGAAGTTATGAATATTCTAATTTTATAAATTTAGAATTTGATTCATATATA  302
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3361  CAATGATACTGAAGTTATGAATATTCTAATTTTATAAATTTAGAATTTGATTCATATATA  3420

Query  303   ATTCCTACTAATGAATTAGATTTAAACGGATTCCGACTATTAGATGTTGACAACCGAATT  362
             |||||  ||  ||||||||||||||||  |||||||||  ||||||||||||  ||  ||||||
Sbjct  3421  ATTCCAACAAATGAATTAGATTTAAATGGATTCCGATTATTAGATGTTGATAATCGAATT  3480

Query  363   ATTTTACCTTTAAATAATCAAATTCGAATCTTAGTAACTGCTACTGATGTTCTTCATTCA  422
             ||||||||  ||||||||||||||||||||  ||||||||||||||||||||||||  |||
Sbjct  3481  ATTTTACCATTAAATAATCAAATTCGAATTTTAGTAACTGCTACTGATGTTCTTCACTCA  3540

Query  423   TGAACAGTTCCTTCTCTAGGAGTAAAAATTGATGCTACACCAGGACGATTAAATCAAACT  482
             |||||||||||||  ||||||||||||||||||||  ||||||||||||||||||||||
Sbjct  3541  TGAACAGTTCCTTCTTTAGGAGTAAAAATTGATGCTACTCCAGGACGATTAAATCAAACT  3600

Query  483   AATTTTTTAATTAATCAATCTGGACTATTCTTTGGACAATGTTCTGAAATTTGTGGAGCT  542
             ||||||  ||||||||||||||||  ||  ||  ||||||||||||||||||  |||||||||
Sbjct  3601  AATTTTCTAATTAATCAATCTGGTCTTTTTTTTGGACAATGTTCTGAAATCTGTGGAGCT  3660

Query  543   AATCATAGTTTTATACCTATTGTTATTGAAAGAATTCCAATAAATTATTTTATTAAATGA  602
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3661  AATCATAGTTTTATACCTATTGTTATTGAAAGAATTCCAATAAATTATTTTATTAAATGA  3720

Query  603   GTTTCTTCTCAATTA  617
             |||||||||||||||
Sbjct  3721  GTTTCTTCTCAATTA  3735
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Figure 3: Multiple sequence alignment of partial mitochondrial COII sequences of *Culex quinquefasciatus* from *Baghdad* compared with *Cx. quinquefasciatus* from USA mitochondrion in GenBank, complete genome Sequence ID: gb|HQ724617.1|

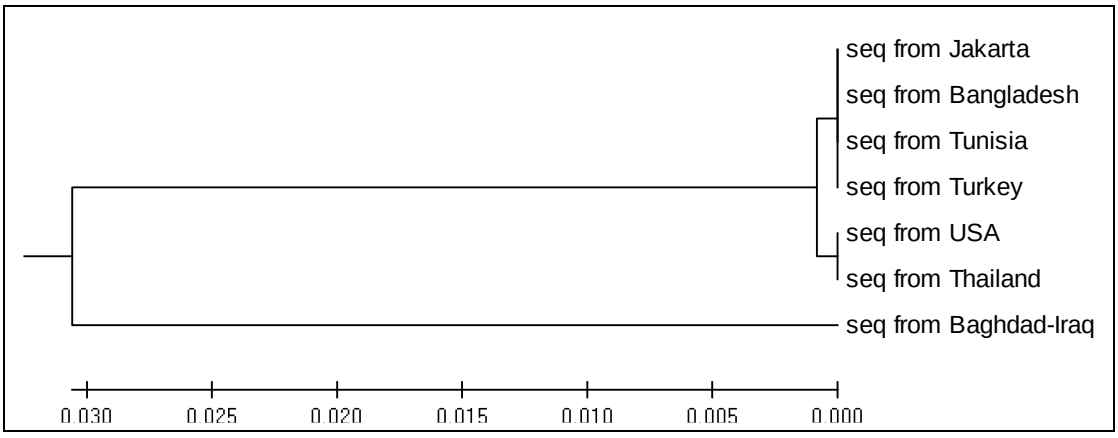

Figure 4: Phylogenetic tree based on genetic distances of the COII gene sequences of mosquitoes prevalent in Iraq.

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