EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
ENTOMOLOGY

A

ISSN
1687-8809

WWW.EAJBS.EG.NET

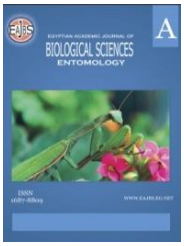
**Vol. 14 No. 4 (2021)**

**Citation**: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 14(4) pp.79-90 (2021)*
**DOI: 10.21608/EAJBSA.2021.202428**

**Egypt. Acad. J. Biolog. Sci., 14(4):79-90(2021)**

**Egyptian Academic Journal of Biological Sciences**
**A. Entomology**

**ISSN 1687- 8809**
**http://eajbsa.journals.ekb.eg/**



# Sequences Comparison of Cytochrome Oxidase I Gene of Certain Species of Insects in Kurdistan with Other Locations in The World

**Janati, S.[1]., Kahrizi, D.[2]., Mirmoayedi, A.[2]. and Yari, K.[3]**

1- Department of Plant Protection, Razi University, Kermanshah, Iran.
2- Department of Plant Production and Genetics, Razi University, Kermanshah, Iran.
3- Medical Biology Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran

E-mail* : dkahrizi@yahoo.com

**ARTICLE INFO**

**Article History**
Received:30/9/2021
Accepted:1/11/2021
**Keywords**:
Certain insects, COI sequences, Phylogeny, Kurdistan

## ABSTRACT

In the present study, sequences of COI gene of insects belong to five orders collected from seven cities in the Kurdistan province of Iran with other locations in the word was studied. Genomic DNA was extracted using the CTAB method, Cytochrome Oxidase I gene was amplified by polymerase chain reaction (PCR) using primer pair and then the sequences were analyzed. The results of the dendrogram show that a specimen of *Periplaneta americana* from Barcelona, Spain was in the same clade as a specimen from Nanjing, China, but *Shelfordella latralis=Blatta lateralis* has made a clad with PE-B *Periplaneta americana* specimen collected in Kurdistan, with more distance from two former specimens of *Periplaneta americana* from Spain and China. Also, *Periplaneta*, two genera *Shelfordella* and *Blatta* have composed the same clade. Two specimens of sunn pest *Eurygaster integriceps*, Heteroptera, Scutelleridae which made a common clade together and made another neighbor clade with *Hypseloecus* sp. Although, Heteroptera belonged to the Miridae family. *Chrysopa pallens*, Chrysopidae, Neuroptera, *Acanthaclisis occitanica*, Myrmeleontidae and Neuroptera both collected in Kurdistan have many genetic similarities in common and have made a clade near to clade of sunn pest but more distant from clades made by *Periplaneta americana* of different regions of the world. Two samples of Mediterranean flour moth, Ephestia *kuehniella*, that collected from Kurdistan province of Iran have made a common clade with each other, and they formed a neighbor clade with a specimen of *Ephestia kuehniella*, from Luebeck, Germany.

## INTRODUCTION

In recent years the Cytochrome Oxidase I (COI) gene sequences have been used for the study of mitochondrial genes of animals. Among them, insects occupy a lot of research to find the evolutionary trends that exist between them. Ease of access, high copy number, and lack of recombination makes many researchers choose to use the mitochondrial COI method. It is the terminal catalyst in the mitochondrial respiratory chain, and its size and structure are conserved among many organisms, making it an ideal evolution tool. Some authors have used the COI gene of a locust *Chlorothippus parallelus*

and eight other insect species to reveal a phylogenetic relationship between them (Lunt, *et al.*, 1996). COI genes were used for differentiating and identification purposes of two near species (*Helicoverpa armigera* and *H.assulta),* so The results showed that the two species are very close in terms of phylogenetic relationships(Li, *et al.,* 2011). when COI genes were used to the barcode of 14 species of Sweden mosquitoes, it was found that exact phylogenetic relationships between mosquito taxa were preserved at shorter evolutionary distances, but at deeper levels, they could not be inferred with confidence using COI gene sequence data alone (Engdahl *et al.,* 2013). Some authors created three profiles of COI one for seven dominant phyla of animals, one for eight orders of insects and another for 200 closed species of Lepidoptera. They have found that among the four major orders of insects, species of Diptera and Lepidoptera showed much fewer variations in their amino-acid sequences than did the Hymenoptera, while the Coleoptera showed an intermediate level of divergence (Herbert et al, 2003a) and between four orders (Coleoptera, Diptera, Lepidoptera and Hymenoptera) which were studied using Cytochrome-C oxidase divergences in the insects as a group was less than those in the other two dominant groups of arthropods, the chelicerates and crustaceans (Herbert *et al.,* 2003b). In Turkey, COI was used for barcoding geometrid moths which were helpful to distinguish between two synonymous species, so a new species was emerged and named *Scopula drenowskii* Sterneck, 1941 from the synonymy of *Scopula decorata* to species rank (Can, 2009). RAPD technique was used to classify twelve species of Myrmeleontidae, and results revealed that molecular techniques could find genetic similarity between some of the species different from those already existed for long years for classification of antlions based on the use of morphological characters (Mirmoayedi *et al.,* 2013).

## MATERIALS AND METHODS

Insects belonging to 5 orders were collected by the use of hand net and light trap and used for molecular studies. The species names of insects were as follows; *Eurygaster integriceps*(Hemiptera order), *Ephestia kuehniella* (Pyralidae, Lepidoptera order), *Periplaneta americana*(Blattidae, Blattaria order), *Blatta orientalis* (Blattidae, Blattaria), *Acanthaclisis occitanica* (Myrmeleontidae, Neuroptera order) and *Chrysopa pallens* (Chrysopidae, Neuroptera order). The cities of Kurdistan province from which the specimens were collected are as follows; Sanandaj, Marivan, Ghorveh, Divandareh, Bijar, Saghez, and Baneh cities. *Ephestia kuhniella* specimens were reared on the wheat flour in the laboratory and used for this research. Cockroaches and Sunn pests were picked with hand, and a hand net was used to collect neuropteran specimens

**Preparation of Insect Specimens:**

Samples collected from different parts of Kurdistan were identified by the use of different keys such as Triplehorn and Johnson, 2005 and Aspoeck *et al,* 2000. Samples are stored in ethanol (97 %) and kept at –20 ºC until the DNA extraction process. Total genomic DNA was extracted from whole-body specimens using the CTAB methods with small corrections and then stored at −20 ºC. The quantity and quality of extracted DNA were evaluated by a Nanodrop spectrophotometer (ND1000, USA) and 1% agarose gel electrophoresis (Eppendorf BioPhotometer).

**Polymerase Chain Reaction (PCR):**

The COI gene sequences used in this study are shown in Table 1. PCR reactions were performed in 25µL volumes containing 2.5 µl of PCR buffer, 0.5 µl dNTP, 0.75 µl mgCl2, 1 µl of Taq DNA polymerase, 1 µl of each primer pair. Amplification reactions were performed in an Eppendorf Thermo Cycler (Eppendorf, Germany) under the following conditions: initial denaturation step at 94 ºC for 5 min, followed by 38 cycles of

denaturation at 94 °C for 35 s, annealing of primer 72 ºC for 45 s, extension at 72 °C for 120 s, and a final extension step at 72 °C for 7 min.

**Table 1:** COI gene primer sequences.

| Primer name | Sequence |
|---|---|
| COI A | 5' CAA CAT TTA TTT TGA TTT TTT GG 3' |
| COI B | 5' TGG AAA TGT GCA ACT ACA TAA TA 3' |

**COI Gene Sequencing:**

Forty microliters of PCR products of COI sequences were sent to Tekapozist Co., Tehran for sequencing. The sequencing process was done both in forward and reverse directions for compared segments of DNA of every species. To be ensured of sequencing results, we have compared the sequences obtained for our species with those accessed from the Gene bank of NCBI.

**Analysis of Molecular Data:**

Megalign software was used to evaluate genetic distances and replacements of nucleotides between nucleotide sequences of different species. Also, Editseq was used to save sequences with a special suffix, and SPSS version 22 was used for calculating the coefficient of cophenetic.

## RESULTS

We have compared between (103- 108) nucleotide sequences of Cytochrome oxidase I (COI) gene of *Blatta orientalis* which we have found in Kurdistan with the same nucleotide sequences of the same species living in different locations of the world (Fig. 1). The results showed that the first specimen in Kurdistan (Bo-A) had nucleotides AGGTAT, corresponding with two amino acids of Serine and Methionine while the second sample of Kurdistan in the same nucleotide sequences had the nucleotides TTTATG, corresponding to Lysine and Tyrosine amino acids. The third row of sequences nucleotides in Fig. 1 is that of Tabriz (Iran) with (An: JQ267492), the sequences were CGTGTA corresponding to amino acids Arginine and Histidine. The three following sequences were from Maryland (USA) which we have got from NCBI with accession numbers of (An: KC617798), (An: KC617795), (An: JX402723) and successively the nucleotides of TGACCA, and amino acids Threonine and Glycine, ACGCTT, Cysteine and Glutamic acid and AATGAT, Leucine, Leucine and the seventh row is a specimen from Barcelona, Spain, (An: AM114926) and GCTCAC, Arginine, Valine. The eighth row is the nucleotide sequences obtained from NCBI, (An: JQ435812) Bucharest, Romania; the DNA nucleotides sequences are AATACC and the amino acids Leucine and Tryptophane. Finally, the last row is from NCBI (An=JN 615390) London England, AATTTT, Leucine, Lysine. Between these nine specimens of *Blatta orientalis* compared from different parts of the world, the specimen from Maryland (An: JX402723), the specimen from Bucharest, and London have the first amino acid, Leucine similar but the second amino acid was different. All other specimens compared have not had similar amino acids.

Comparing between (103- 108) nucleotide sequences of *Periplaneta americana* in different specimens copied from NCBI, we saw that the specimen of Kurdistan had nucleotide sequences of TCTTTG , Arginine and Asparagine as amino acids (Fig. 2), but the specimens of Maryland (An=KC617846) and New York (An=KM577156) had similar sequences of nucleotides TGATCA and relevant mRNA was ACUAGU and corresponding amino acids Threonine and Serine and the rows 4, 5, 6, 7 of Fig. 2, all from different cities

of Iran had the same nucleotides sequences of GCTGTA and relevant mRNA CGACAU and translated to amino acids Arginine and Histidine. We saw that our specimen of *Periplaneta americana* from Kurdistan in its nucleotide sequences of ( 103-108) had the nucleotides different from nucleotides of specimens from Maryland and New York, but although the first three-nucleotide of the Kurdistan specimen were TCT, different from nucleotides GCT common and identical in specimens from Tehran (An=JQ267482), Tabriz (An=JQ267481), Esfahan (An=JQ267484), Kashan (An=JQ267476), but the first triplet mRNA nucleotides of specimen from Kurdistan was AGA and first triplet mRNA nucleotides from specimens of Tehran, Tabriz, Esfahan, Kashan was CGU all of the code for Arginine, although the second nucleotides triplet in Kurdistan was different from GTA which was the second triplet nucleotides in specimens of *Periplaneta americana* collected in other cities of Iran, the TTG sequences of second triplet in Kurdistan corresponds to triplet mRNA nucleotides AAC which codes to amino acid Asparagine, while the second triplet of GTA with the corresponding mRNA codon of CAU codes for Histidine in specimens of other cities of Iran .

When we compared the sequences of nucleotides of COI in the location of (103-108) of *Ephestia kuehniella* in different specimens from Iran and other locations in the world (Fig. 3), we saw that the specimen from Kurdistan in sequences of (103-108) had ATAACT as nucleotide sequences, the corresponding mRNA of them code for Tyrosine and Tryptophane, while the same sequences of nucleotides COI gene of other specimens of *Ephestia kuehniella* copied from NCBI, Austria (An: KM573100), Austria (An: KF405359), Austria (An: JN820109), Austria (An: JN820109), Canada(An: KF405066), Canada (An: JF859845) all of them TGATCA, the first triplet of which has anticodon of mRNA, ACU, which codes for Threonine and the second triplet TCA with corresponding mRNA anticodon AGU, which codes for Serine.

Concerning *Eurygaster integriceps* (Fig. 4), when looking to the sequences of 104 to 109 nucleotides of mitochondrial COI gene of this species from South Korea copied from NCBI (An=KF966609) we saw ACCAAA, DNA nucleotide sequences which matches with UGGUUU mRNA, code for Tryptophane and Phenylalanine amino acids successively, but when we looked to two specimens of *Eurygaster integriceps* from Bremen Germany(An=KM022776), ( An=KM022883) and two specimens of this species from Bayreuth, Germany (An=KM022659) and (An=KM022602) and a specimen from Tubingen Germany (An=KM022584) all of these five specimens of *Eurygaster integriceps* had GACCAA nucleotides in (104-109) sequences of COI gene, their relative mRNA was CUGGUU, which translated to Leucine and Valine amino acids successively, while as in Fig. 4, the Iranian specimen which was collected in Kurdistan the (104-109) sequences of these nucleotides were empty which signifies the possibility of a deletion of these six nucleotides and means that in the corresponding mitochondrial protein of Cytochrome Oxidase-1 there was an absence of two amino acids which are Leucine and Valine. The eighth row of Fig. 4 consists of nucleotides of sequences (104-108) of COI gene of a specimen of *Eurygaster integriceps* accessed from NCBI(An=Kr105371) and the sunn specimen was collected from Voronezh, Russia, in this case as like as the last case, the nucleotides are GACCAA and the corresponding mRNA, CUGGUU and the corresponding amino acids are Leucine and Valine.

We looked at the nucleotide (103-108) COI gene sequence of the antlion *Acanthaclisis occitanica* in Fig. 5 and we saw that in the first row the Kurdistan specimen Ac-A, the DNA nucleotides were GTGTAC which corresponded to CACAUG of mRNA codons which code for Histidine and Methionine successively, while the second row of that figure *Acanthaclisis occitanica* (Ac-B) collected in Kurdistan the nucleotide sequences were TGCTGT corresponded to ACGACA of mRNA codons which code for Threonine

and Threonine amino acids successively, the third row of that figure, *Acanthaclisis occitanica* (An=NC_025905) from China copied from NCBI, the nucleotide sequences were AATAAA corresponded to UUAUUU of mRNA codons which code for Leucine and phenylalanine amino acids successively,

Concerning comparing of COI nucleotides sequences (103-108) of *Chrysopa pallens* collected in Kurdistan with those of Japan, Germany and China (Fig. 6), we saw that the specimen which we have collected from Kurdistan the nucleotide sequences were TTGTGC and its corresponding mRNA was AACACG which code for Asparagine and Threonine successively. The same site of nucleotides in a specimen from Ibaraki, Japan with Accession number (AB 354061) copied from NCBI was CATGCA the related mRNA was GUACGU which code for Valine and Arginine successively. In the 3rd row in Fig. 6, the nucleotide sequences of a specimen of *Chrysopa pallens* from Munich, Germany accessed from NCBI with(An=JQ 267476) the nucleotide sequences of which was TGATCA and the corresponding mRNA codons, ACUAGU which code for Threonine and Serine amino acids successively. The fourth and the fifth rows in Fig. 6, are from two specimens from Haidian China downloaded from NCBI with (An=JN242032), (An=JN242032) and the six nucleotides of both of the specimens are TGGAAT, the corresponding mRNA sequences are ACCUUA and the amino acids are Leucine and Threonine. The sixth or the last row is a specimen of *Ch.pallens*, from Haenan, China, copied from NCBI (An=GAGF1000016) sequences of nucleotides (103-109) from the COI nucleotides of this specimen were TCACAA the corresponding mRNA codons were AGUGUU which are nucleotides sequences which code successively for Serine and Valine amino acids.

All the cockroaches made a clade (Fig. 9) in which *Periplaneta americana*; (PE-B) was found in Kurdistan, Iran, with those which was found in China (NCBI accession number GU947663) and Spain (NCBI accession number AM114927), *Blatta orientalis* (BA-primer F) which we collected in Kurdistan, *Blatta orientalis* (NCBI accession number JN615390), *Periplanetta Americana* had high similarity with *Shelfodrella lateralis* (= *Blatta lateralis*) (NCBI accession number: JN615393). All of them have clustered in a clade, distinct from another clade near to them, and formed by *Eurygaster integriceps*( Sunn pest) (EUI-A-primer F, EUI-B-primer F) and *Hypseloecus sp*., a Miridae planthopper(accessed from NCBI, accession number: AY253135). *Chrysopa pallens* (Ph) family Chrysopidae and *Acanthaclisis occitanica* (Ac) family Myrmeleontidae both belonged to Neuroptera order clustered in another clade distinct from two others and finally, Mediterranean flour moth *Ephestia kuehniella* (EK-A primer F, EK-B primer F), (KF305832, KF 05832 accessed from NCBI) clustered in a clade distinct from three other clades

The results of our researches showed that in site 104-109 nucleotide sequences of *Blatta orientalis* (Fig. 7) there was the addition of six nucleotides (CCGGTA) which was unique to this species and were not seen in other insects studied by us. The GGC corresponds to mRNA codon for CCG DNA nucleotide which codes for Glycine amino acid and CAU stands for mRNA codon for GTA, DNA nucleotide which codes for histidine.

When we compared the CO-1 genes of the 104-109 nucleotide sequence sites of *Blatta orientalis* with other five species of insects belonging to orders of Lepidoptera, Blattodea, and Neuroptera we have found that this substitution of six nucleotides is due to addition mutation of two amino acids Glycine and Histidine in *Blatta orientalis* which does not exist in another Blattidae species *Periplaneta americana* equally investigated by us in our work.

91TTTTGGAAACCTAGGTATAATTTTTGCTAC 120 Kurdistan Bo-A-PrF COIgene
91CTTTAGGTATAATTTATGCTATAATAACTA 120 Kurdistan Bo-B-PrF COIgene
91AATAGTAATAGTGCTGTAATGGCTACAGAT120 TABRIZ 1(An:JQ267492)COIgene
91ACTAATTGGAGATGACCAAATTTATAATGT 120 MD-USA (An:KC617798)COIgene
91TTGTTACTGCTCACGCTTTTGTTATAATTT 120 MD-USA (An:KC617795)COIgene
91GCAAGGACTGGTAATGATAGTAATAGTAAT120 MD-USA (An:JX402723)COIgene
91GTAATTGTTACTGCTCACGCCTTTGTTATA 120 Bar-Spain (An:AM114926)COIgene
91TTTTTTTATAGTAATACCAATTATAATTGG 120 Buc-Romania(An:JQ435812)COIgene
91AAACCTAGGAATAATTTTTGCTATATTAGC 120Lon-England(An:JN615390)COIgene

**Fig. 1.** Comparison made between (103- 108) nucleotide sequences of *Blatta orientalis* in different locations of the world.

91 ATCTAGGTATAATCTTTGCTATACTAGCAA 120 Kurdistan PE-B COI
91ACTAATTGGAGATGATCAAATTTATAATGT120Maryland USA(AnKC617846) COI
91ACTAATTGGAGATGATCAAATTTATAATGT 120NewyorkUSA(AnKM577156)COi
91AATAATAATAATGCTGTAATAGCTACTGAT 120TehranIran(AnJQ267482)COI
91AATAATAATAATGCTGTAATAGCTACTGAT 120TabrizIran(AnJQ267481)COI
91AATAATAATAATGCTGTAATAGCTACTGAT 120EsfahanIran(AnJQ267484)COI
91AATAATAATAATGCTGTAATAGCTACTGAT120KashanIran(AnJQ267476)COI

**Fig.2.** Compare (103- 108) nucleotide sequences of *Periplaneta americana* in different locations of the world.

91ATTTATGCTATAATAACTATTGGATTATTA 120 KurdistanEK-Pr-A
91TTTAATTGGAGATGATCAAATTTATAATAC 120Austria(An:KM573100) COIgene
91TTTAATTGGAGATGATCAAATTTATAATAC 120Austria(An:KF405359) COIgene
91TTTAATTGGTGATGATCAAATTTATAATAC 120Austria(An:JN820109) COIgene
91TTTAATTGGAGATGATCAAATTTATAATAC 120Canada(An:KF405066) COIgene
91TTTAATTGGTGATGATCAAATCTACAATAC 120Canada(An: JF859845) COIgene

**Fig. 3.** Compare between (103- 108) nucleotide sequences of *Ephestia kuhniella.*

91ATAATCTACGCCA - - - - - - TACTTGCCATT 120Ker.Iran(no Access.no) COIgene E.int
91TTTATTGGGGATGACCAAATTTATAATGTC 120 S.Korea(An:KF966609)COIgene E.int
91ATTTATTGGAGATGACCAAATTTATAATGT 120 Br.Ger(An:KM022776)COIgene E.mau
91ATTTATTGGAGATGACCAAA TTTATAATGT120 Br.Ger(An:KM022883)COIgene E.testu
91ATTTATTGGAGATGACCAAATTTATAATGT 120 Bay.Ger(An:KM022659)COIgene E.mau
91ATTTATTGGAGATGACCAAATTTATAATGT 120Bay.Ger(An:KM022602)COIgene E.testu
91ATTTATTGGAGATGACCAAATTTATAATGT 120Thu.Ger(An:KM022584)COIgene E.mau
91ATTTATTGGAGATGACCAAATTTACAATGT 120 Voronezh Russia(An=KR105371)E.int

**Fig. 4.** Compare between (104- 109) nucleotide sequences of *Eurygaster integriceps.*

91TATTTAGGGATGGTGTACGCGATGGGATCG 120 Kur.Iran(no Access.no)COIgene Ac-A-
91ATGAATAATTTGTGCTGTATTATCTATTGG 120 Kur.Iran(no Access.no)COIgene Ac-B-
91TAGAGCTAATTAAATAAAATTATGCACTAC 120 Ac China(An:NC_025905)COIgene

**Fig. 5**. Compare between (103- 108) nucleotide sequences of *Acanthaclisis occitanica.*

91ATTAGGAATGATTTGTGCTATATTAGCTAT120Kur.Iran(no Access.no)COIgenePh.
91TCTAGAATTGCTCATGCAGGAGCTTCTGTT120 IbarakiJapan (An=AB354061)COI
91TTTAATTGGAGATGATCAAATTTATAATGT120MunichGermany(An KJ592516)COI
91TATATTTACTGTTGGAATAGATGTTGATAC120 HaidianChina(An=JN242032)COI
91TATATTTACTGTTGGAATAGATGTTGATAC 120 HaidianChina(An=JN242033)COI
91TACGCGCGCTCCTCACAAAAAATTCACTCT 120 Henan China(An GAGF01000016)COI

**Fig. 6.** Comparison between (103- 108) nucleotide sequences of *Chrysopa pallens* Ac=*Acanthaclisis occitanica*, Ph= *Chrysopa pallens*, BO-A-Primer F=*Blatta orientalis*, EK-A-Pr F=*Ephestia kuehniella* specimen A, EK-B-Pr F=*Ephestia kuehniella* specimen B, EUI-A-Pr F=*Eurygaster integriceps* specimen-A, EUI-B-Pr F=*Eurygaster integriceps* specimen-B, PE-B= *Periplaneta americana* .

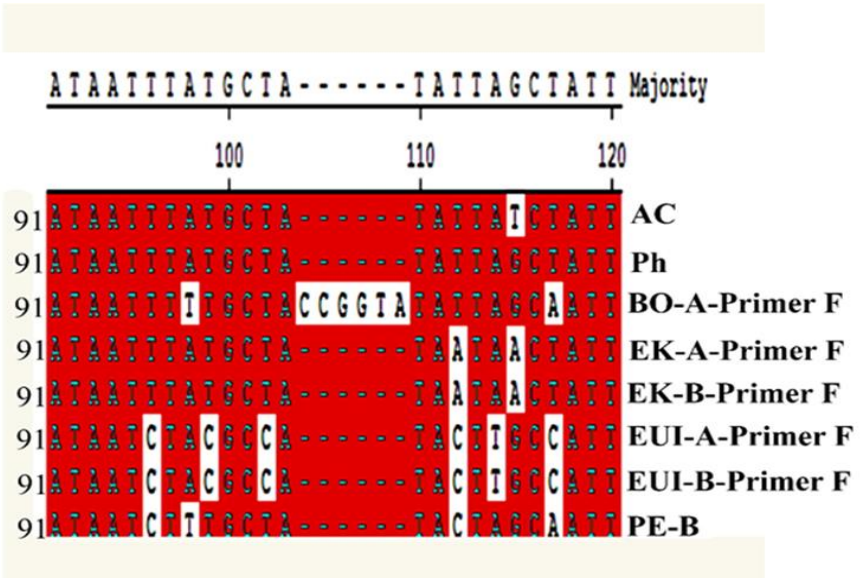



**Fig. 7.** Alignment of DNA sequences of the part of COI gene of six species of insects.

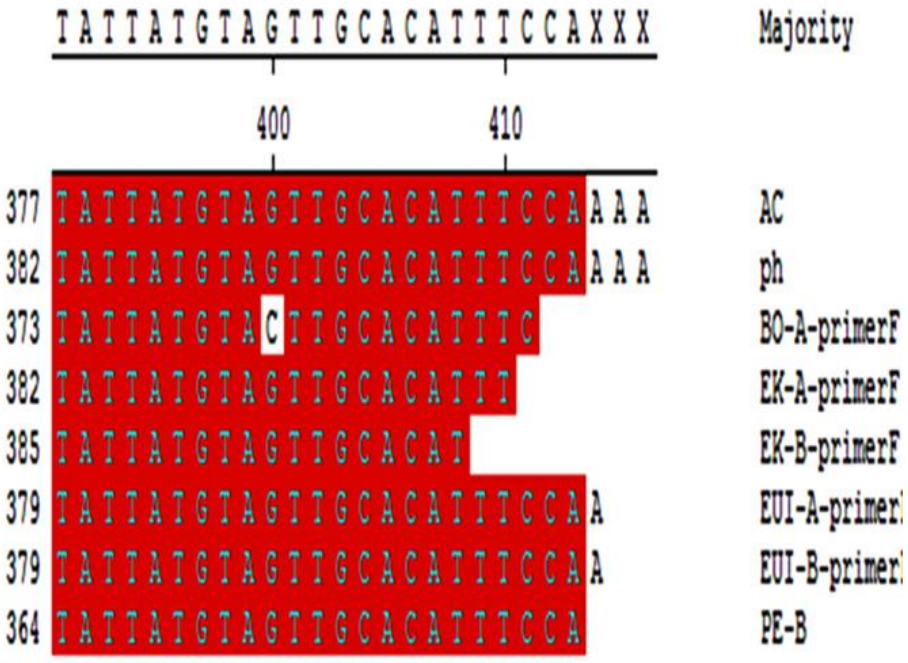



**Fig. 8.** The majority of 26 identical nucleotides sequences belonging to six species and seven specimens of different orders of insects were compared to each other.

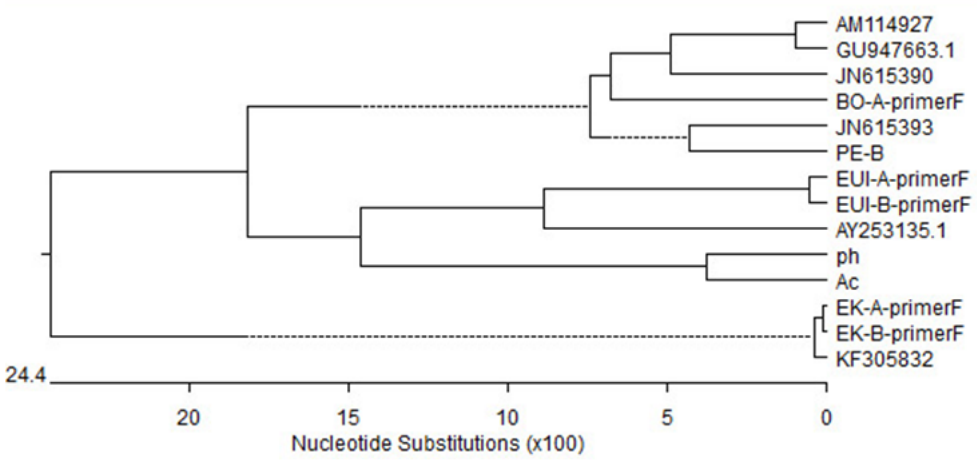



**Fig. 9.** Dendrogram of cluster analysis based on mixing data obtained by us in our specimens with those accessed from NCBI. The meaning of abbreviated codes or names are as follows; AM114927NCBI (Periplaneta americana, Spain, Barcelona), GU947663.1NCBI (Periplaneta americana, Nanjing, China), JN615390 NCBI (London, U.K.), BO-A-primerF (Blatta orientalis, Kurdistan, Iran), JN615393NCBI (Shelfordella lateralis, London, U.K.), PE-B (Peiplaneta americana, Kurdistan, Iran), EUI-A-primerF and EUI-B-primerF (two specimens of Eurygaster integriceps, Kurdistan, Iran.), AY253135.1NCBI(Hypselocus sp., Heteroptera, Miridae, New York, USA.), Ph( Chrysopa pallens, Kurdistan, Iran), Ac (Acanthaclisis occitanica, Kurdistan, Iran.), EK-A-primerF and EK-B-primerF(two specimens of Ephestia kuehniella, Kurdistan, Iran.), KF305832NCBI(Ephestia kuehniella, Luebeck, Germany).

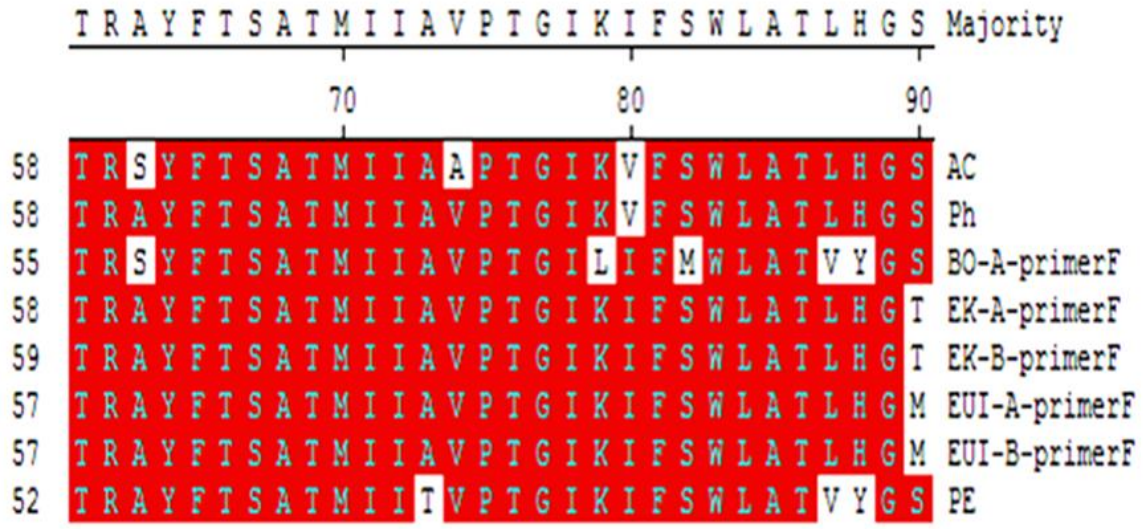



**Fig. 10**- Alignment of aminoacids sequences of a part of COI gene in all of our studied species.

When we looked at the majority aminoacids sequences of 61-90 of COI gene of studied species and compared those sequences individually with each other (Fig 10), we have observed that in *Acanthaclisis occitanica* in sequence no. 60 Alanine was replaced by Serine and in sequence 71, Valine was replaced by Alanine and in sequence 77 Isoleucine was replaced by Valine. In *Chrysopa pallens* similar to *Acanthaclisis occitanica* in sequence 77 Isoleucine was replaced by Valine. In *Blatta orientalis* (BO-A-primer F) in sequence 57, Alanine was replaced with Serine and in sequence 73, Lysine was replaced

by Leucine, in sequence 76, Serine was replaced by Methionine, in sequence 81, Leucine was replaced by Valine and in sequence 82 and Histidine was replaced by Tyrosine. In *Ephestia kuehniella* (specimens A and B, ) all the amino acids in that interval are similar except in sequence 87, of specimen A(EK-A-primer F) and in sequence 88 of specimen B(EK-B-primer F) Serine was replaced by Threonine. In sequence 86 of two specimens of sunn pest *Eurygaster integriceps* (EUI-A-primer F, EUI-B-primer F ) Serine was replaced by Methionine. In sequence 64 of *Periplaneta americana*, Alanine was replaced by Thymine and in sequences 78 and 79 Leucine was replaced by Valine and Histidine by Tyrosine respectively.

## DISCUSSION

For comparing between (103-108) nucleotide sequences of CO1 genes of two samples of *Blatta orientalis* collected in Kurdistan with the identical nucleotide sequences, we have copied the sequences of seven specimens of *Blatta orientalis* from NCBI. The specimen from Bucharest and London has the first similar amino acid Leucine, but the second amino acid was different. All other specimens compared have not had similar amino acids (Fig-1). *Blatta lateralis* was collected in London, UK, and its COI sequences were retrieved from NCBI. The first triplet DNA sequence (AAT) was similar to that of specimens from the USA and Rumania. This species is rapidly replacing *Blatta orientalis* in the southern USA (Kim and Rust, 2013).

We have collected another cockroach *Periplaneta americana* from Kurdistan and retrieved from NCBI six other identical (103-108) nucleotide sequences of the same species, and compared their sequences with each other. We saw that the first triplet mRNA nucleotides of the specimen from Kurdistan were AGA and the first triplet mRNA nucleotides from specimens of Tehran, Tabriz, Esfahan, Kashan was CGU all of them coded for Arginine although the second triplet of nucleotides of the specimen from Kurdistan coded for Asparagine while the specimens of other cities of Iran had nucleotide sequences which coded for Histidine.

Concerning the Mediterranean flour moth, *Ephestia kuehniella*, we have compared the nucleotide sequences (103-108) of a specimen collected in Kurdistan with four other specimens from Austria and two specimens from Canada all retrieved from NCBI(Fig-3). All of these six specimens had their (103-108) nucleotide sequences coding for amino acids Threonine and Serine successively different from the nucleotides sequences (103-108) in Kurdistan specimen which coded for different amino acids Tyrosine and Tryptophane.

Concerning the specimens of sunn pest, *Eurygaster integriceps*, the CO1 sequences of five specimens of *Eurygaster integriceps* from Germany and one from Russia were retrieved from NCBI and their (104-109) nucleotide sequences were compared with the specimen that we collected in Kurdistan. The German specimens plus one Russian specimen had GACCAA nucleotides in (104-109) sequences of COI gene, their corresponding mRNA was CUGGUU which corresponded to Leucine and Valine amino acids successively while as in Fig-4 the Iranian specimen which was collected in Kurdistan, the sequences (104-109) of its' nucleotides were empty which signifies the possibility of deletion of these six nucleotides and means that in the corresp[onding mitochondrial protein of Cytochrome Oxidase-1 there was an absence of two amino acids which are Leucine and Valine. Concerning the specimen from South Korea, its nucleotide sequences (104-109) coded for two amino acids Tryptophane and phenylalanine. Some authors using COI and COII and rRNA 16S for their experiments found that Blaberoidea was a sister group to the remaining Blattodea(Djernes, et.al., 2012).

We have looked at nucleotide sequences (103-108) CO-1 gene of the antlion

*Acanthaclisis occitanica* (Fig-5) and the DNA nucleotides for the first and second Iranian samples were GTGTAC and TGCTGT which coded successively for amino acids Histidine and Methionine and two molecules of Threonine. While the Chinese specimen of the same species of antlion copied from NCBI had AATAAA at the (103-108) nucleotides sequences of its CO-1 gene which coded for amino acids Tryptophane and Phenylalanine. So neither Iranian specimens nor the Chinese ones had identical nucleotide sequences or amino acids. We have compared COI nucleotides sequences (103-108) of *Chrysopa pallens* collected in Kurdistan with those of Japan, Germany and China accessed from NCBI (Fig-6), and the only equal sequences of nucleotides in position(104-109) were two specimens from Haidian, China, the DNA nucleotide sequences were TGGAAT, and the corresponding mRNA sequences were ACCUUA, and the amino acids were Leucine and Threonine, the other specimens from Kurdistan (Iran), Ibaraki (Japan), Munich(Germany) and Henan(China) had different nucleotides sequences which coded for different amino acids. Comparison between(104-109) nucleotide sequences of mitochondrial cytochrome oxidase-1 of specimens of different orders of insects (Fig-7) the whole sequences 91-120 was utilised. The comparison was made between *Acanthaclisis occitanica*, *Chrysopa pallens*, *Blatta orientalis*, *Ephestia kuehniella* specimen A, *Ephestia kuehniella* specimen B, *Eurygaster integriceps* specimen-A, *Eurygaster integriceps* specimen-B and *Periplaneta americana*. The only insect which had six nucleotides in(104-109) sequences was *Blatta orientalis* specimen which was collected in Kurdistan and the DNA nucleotides of the COI gene was CCGGTA, the corresponding mRNA nucleotides are GGCCAU, which the 1$^{st}$ and 2$^{nd}$ triplet of it successively code for Arginine and Histidine, the other specimens had not nucleotides in these sequences.

Concerning (Fig-8) twenty-six nucleotides were composing the majority of specimens had sequences (TATTATGTAGTTGCACATTTCCAXXX), the only insect which had a different nucleotide in the sequence of 400 was *Blatta orientalis* which Cytosine was replaced to Guanine.

In a dendrogram, we have compared all the specimens which we have studied (Fig-9). AM114927 a specimen of *Periplaneta americana* from Barcelona, Spain are clustered in the same clad as a specimen of the same species from Nanjing, China), but JN615393, *Shelfordella latralis=Blatta lateralis* (Turkestani cockroach) has made a clad with PE-B *Periplaneta americana* specimen collected in Kurdistan, with more distance from two former specimens of *Periplaneta americana* from Spain and China. Recently, Hashemi Aghdam and Oshaghi, 2015 using COII mitochondrial gene acquired similar results to our findings and their two specimens of *Blatta lateralis* and *Periplaneta americana* collected from north Tehran made a common clade distant from other cockroach species collected by them. Although, Grandcolas and D'Haese, 2001 by using sequences of 12S rRNA have argued that four species of domestic *Periplaneta* and two genera *Shelfordella* and *Blatta* could compose the same clade. Von Beeren, et.al., 2015 by the help of 85 participants collected 285 specimens of *P.americana* from 16 states of the USA and specimens from Argentina, Australia, Belize, Guayana, Spain and Venezuela and 24 sequences from the gene bank of NCBI from China, Iran and Korea, they concluded that all available *P.americana* COI barcodes formed a monophyletic lineage separated from congeneric species. EUI-A-primerF and EUI-B-primerF were two specimens of sunn pest *Eurygaster integriceps*, Heteroptera, Scutelleridae which made a common clade together, and they have made another neighbor clade with *Hypseloecus sp.* Although Heteroptera belonged to the Miridae family. Ph=(*Chrysopa pallens*, Chrysopidae, Neuroptera ) and Ac=(*Acanthaclisis occitanica*, Myrmeleontidae, Neuroptera) both collected in Kurdistan have many genetic similarities in common and have made a clade near to clade of sunn pest but more distant from clades made by *Periplaneta americana* of different regions of

the world. EK-A-primerF and EK-B-primerF two specimens of Mediterranean flour moth *Ephestia kuehniella*, both collected from Kurdistan Iran, have made a common clade with each other and they formed a neighbor clade with KF305832. a specimen of *Ephestia kuehniella*, from Luebeck, Germany.

**Acknowledgements:**

The authors thank the University of Medical Sciences of Kermanshah for providing us an opportunity for doing our researches fulfilled and Dr. Samad Jamali and Dr. Leila Zarei both from the Faculty of Agriculture of Razi university for help in revising the original manuscript

## REFERENCES

Aspoeck.H,, U.Aspoeck, H.Hoelzel, 1980. Die neuropteran europas.Goecke and Evers, Krefeld, Germany

Can.F.(2009). DNA barcoding confirms species rank for a cryptic geometrid species fromTurkey and Bulgaria (Lepidoptera: Geometridae: Sterrhinae). *Zootaxa,* 2314: 63–68 DOI: 10.1111/j.1365-3113.2011. 00598.x

Djernaes, M., K.D.Klass, M.D.Picker, J.Damgaard, 2012. Phylogeny of cockroaches (Insecta, Dictyoptera, Blattodea), with placement of aberrant taxa and exploration of out-group sampling. *Systematic Entomology*, 37: 65–83

Engdahl C, P. Larsson , J. Näslund, M .Bravo , M .Evander , J.O. Lundström , C. Ahlm , G. Bucht .(2013). Identification of Swedish mosquitoes based on molecular barcoding of the COI gene and SNP analysis. *Molecular Ecology Resources*. 14(3):478-88.

Grandcolas, P. and C. D'Haese, 2001. The Phylogeny of Cockroach Families: Is the Current Molecular Hypothesis Robust? *Cladistics*, 17, 48–55 (2001)

Hashemi-Aghdam, S, S., Oshaghi, M.A., 2015. A Checklist of Iranian Cockroaches (Blattodea) with Description of *Polyphaga sp*. as a New Species in Iran. *Journal of Arthropod-Borne Diseases*, 9(2): 161–175

Herbert, P.D. N, Cywinska, A., Ball, S.L. and J.R.deWaard. (2003a). Biological Identification through DNA barcode. *Proceedings of Royal Society of London(B),* 270:313-321.

Hebert, P.D.N., S. Ratnasingham and Jeremy R. deWaard.(2003b). Barcoding animal life: Cytochrome- C oxidase subunit 1 divergences among closely related species. *Proceedings Royal Society London. B (Suppl.)* 270, S96–S99

Kim, T. and M.K.Rust 2013. Life History and Biology of the Invasive Turkestan Cockroach (Dictyoptera: Blattidae). *Journal of Economic Entomology*, 106(6): 2428-2432

Li, G. & Quiros, C.F. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction, its application to mapping and gene tagging in Brassica. *Theoretical and Applied Genetics,* 103:455–461.

Li, Q.Q, D.Y. Li , H .Ye , X.F. Liu , W .Shi , N .Cao , Y.Q. Duan .(2011).Using COI gene sequence to barcode two morphologically alike species: the cotton bollworm and the oriental tobacco budworm (Lepidoptera: Noctuidae). *Molecular Biology Reports,* 38(8):5107-13.

Lunt, D.H., D.X., Zhang, D.M, Szymura and G.M., Hewitt (1996). The insect cytochrome oxidase-I gene: Evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology,* 5(3):153-165.

Mirmoayedi, A., Kahrizi, D., Pani, S., Yari, K. 2013. Molecular genetic diversity within Myrmeleontidae family. *Molecular Biology Reports,* (2013) 40:639–643

Murray M.G and W.F. Thompson (1980). Rapid isolation of high molecular weight DNA. *Nucleic acid research,* 8: 4321-4325.

Philips, A. J. and Simon, C. (1995) Simple, efficient and nondestructive DNA extraction protocol for arthropods. *Annals of the Entomological Research,* 88: 281-283

Triplehorn, C.A. and N.F.Johnson, 2005.Borror and Delong's introduction to the study of insects, 7th edition. Brooks/Cole publishing Co.USA

Von Beeren, C., M.Y.Stoeckle., J.Xia., G.Burke., D.J.C.Kronauer, 2015. Interbreeding among deeply divergent mitochondrial lineages in the American cockroach (*Periplaneta americana*). *Scientific Reports,* 5: 8297. doi:10.1038/srep08297