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GENETIC RELATIONSHIP BETWEEN NEUROPTERAN FAMILIES (INSECTA, NEUROPTERIDA, NEUROPTERA) BASED ON CYTOCHROME OXIDASE-I SEQUENCES

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The studied specimens belonged to five species of three families of Chrysopidae, Myrmeleontidae and Ascalaphidae (order Neuroptera). They were collected from Shush and Dezful, south of Iran. These insects are useful insects in biological control as the important predators of aphids, psyllids, caterpillars, ants and other insects. The Sequence alignment of parts of Cytochrome Oxidase-I (COI) gene of those species was studied. The insect bodies were entirely ground in a microtube. The PCR products of COI were sequenced. Pairwise alignment of nucleotides sequences belonging to five species of Neuroptera was carried out using MegAlign and EditSeq softwares. The sequencing results in *Palpares solidus* and *Creoleon remanei* showed the mutation potentials in locations of 699, 702, 726, 735 and 750 of COI gene of mtDNA. According to COI gene sequences, *Chrysopa pallens* and *Palpares solidus* species showed the maximum genetic similarity (98.2%). There was the minimum genetic similarity (75.9%) between *Chrysopa viridana* and *Creoleon remanei* species.

Key words: Cytochrome Oxidase-I (COI), Genetic Relationship, Neuroptera

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INTRODUCTION

In the past twenty years, molecular techniques were used to acquire more informations about different living organisms from viruses and bacteria to humans. The use of DNA of insects for taxonomical studies is a better and more precise method to know the relationship between different orders or families of insects.

The study of biological polymorphism is dependent to the study of biodiversity, phylogenetics and evolutionary sciences (BROOKS, 1997; MIRMOAYEDI *et al*., 2012,). Neuroptera is the name of an order of holometabolous insects belonging to super order Neuropterida. This superorder consists of three orders; Neuroptera, Megaloptera, Raphidioptera (BROOKS and BARNARD 1990; FARAHI *et al*.,2009).

Green lacewings (*Chrysopidae*) and brown lacewings (*Hemerobiidae*) are important predators of aphids, psyllids and caterpillars (MIRMOAYEDI 2003; GHAHARI *et al*., 2010) and *Coniopterygidae* are predators of mites. Therefore these insects are useful insects in biological control. Other Neuropteran families such as *Myrmeleontidae* (antlions) are predators of ants and other insects. The larvae of *Ascalaphidae* (owlflies) are also predators of ants and resemble very much to antlion larvae. The adults make themselves hidden by pressing their legs and body to the long axis of plant twigs such as wheat shoots in wheat field in daytime, besides adult antlions are active when it is dawn or dusk (MIRMOAYEDI, 2003; KRIVOKHATSKY, 2011).

For rapid identification of different insect species, DNA based methods are increasingly used and the use of mitochondrial Cytochrome Oxidase1(mt CO1) gene, is mostly used in insect species identifications. For showing the importance of mt CO1, we mention the works of some of the authors which used this method here. The relative lack of diagnostic morphological characteristics in Aphidina *(Insecta,Hemiptera, Aphididae)* caused the identification of species in this group to be difficult and erroneous, so some authors have used mt CO1gene for identification of this subtribe of Aphididae with success. Thirty-six species of Aphidina were identified in a neighbor-joining tree. Mean intraspecific sequence divergence in Aphidina was 0.52%, with a range of 0.00% to 2.95%, and the divergences of most species were less than 1%. The mean interspecific divergence with Aphidina was 6.80%, with a range of 0.68% to 11.40%, and most genera were in the range of 3.50% to 8.00% (WANG *et al*.,2011).

Patterns of amino acid variation suggest convergent or parallel evolution at the protein level connected to the transition into a parasitic life style. Denser sampling of two diverse insect taxa revealed that the beetles (Coleoptera) show more amino acid variation than the butterflies and moths (Lepidoptera), indicating fundamental difference in patterns of molecular evolution in COI. Several amino acid sites were found to be under notably strong purifying selection in Lepidoptera as compared to Coleoptera (PENTINSAARI *et al*.,2016).

In Thailand, a 658 bp fragment of COI was amplified from 145 adult horse flies belonging to 48 morphologically distinct species and sequenced. Sequence analysis revealed an intraspecific divergence of 0.0%–4.4% (CHANGBUNJONG *et al.*,2018).

As a goal of our study we wanted to understand the family relationship among three families of Neuroptera, so we used the mitochondrial Cytochrome Oxidase-I (COI) genes to compare DNA nucleotides. In the past fifty years, there were some studies on systematics of different families of *Neuroptera* (such as *Chrysopidae, Coniopterygidae* and *Mantispidae*) in Iran, however most of the species recorded for Iran were identified only by the use of morphological characters of the specimens. Although in recent years there were some efforts between the Iranian entomologists to study the phylogenetic and family relationship between

some orders and families of insects. We have already used RAPD-PCR methods for the study of *Chrysopidae* and *Myrmeleontidae* families (MIRMOAYEDI, 2006; MIRMOAYEDI *et.al*.,2012; MIRMOAYEDI *et al*.,2013) and there were also other authors which used RAPD-PCR for molecular studies of other insect families (GADELHAK and ENAN, 2005).

Estimation of evolutionary distances between protein and DNA sequences is important to construct phylogenetic trees, dating species divergencies and understanding the mechanisms of evolution of genes, proteins and populations (WILLIAMS *et al*.,1990; TAKEZAKI *et al*., 1995; RAHIMI *et al*.,2014). To estimate the divergence times of species or species groups with molecular data, a linearized tree under assumption of a molecular clock could be constructed (TAKEZAKI *et al*., 1995). Some authors have shown that in phylogenetic inference simple methods are often as efficient as complex ones when the bootstrap test is used (PHILLIPS and SIMON, 1995).

The present study is a new approach to the systematic study of *Neuroptera*. As it compares the relationship between three families of *Neuroptera* (*Chrysopidae, Myrmeleontidae* and *Ascalaphidae*) by use of COI gene sequences. *Deleproctophylla variegata* is a species which belong to *Ascalaphidae* family and we wanted to determine for the first time the genetic similarity or distance and phylogenetic relationship of this species versus the other species belonging to the families of *Chrysopidae* and *Myrmeleontidae*.

MATERIALS AND METHODS

Shush(32°11'21.19" N, 48°15'28.03" E) and Dezful(32°22′52″ N , 48°24′20″ E) are two cities in Khuzestan province in south Iran, a distance of 34 kilometers separates them from each other. The Neuropteran specimens of three families *Ascalaphidae, Chrysopidae* and *Myrmeleontidae* were collected from these two locations.

The *Palpares solidus* and *Creoleon remanei* of Myrmeleontidae family, *Deleproctophylla variegata* of Ascalaphidae family and *Chrysopa viridana* and *Chrysopa pallens* of Chrysopidae family were the species collected and used for molecular assays. The determination of species was done using the male genitalia. The DNA extraction, electrophoresis and other molecular experiments performed in the Zagros Bioidea Co., Razi University, Kermanshah, Iran.

DNA purification

The buffer preparation and DNA extraction was carried out according to previous reports (21, 22). At first the CTAB (Cetyl Trimethyl Ammonium Bromide) buffer was prepared for 40 specimens.

The wings of specimens were separated and using 50 µl of extract buffer (Tris EDTA, NaCl, CTAB, β-mercaptoethanol) the insects bodies was entirely ground in 1.5 mL sterilized microtubes and 550 µl of buffer was added simultaneously. Then samples were incubated in temperature of 65°C for 60 min. The samples were centrifuged in 13000 rpm for 7 min to precipitate proteins and polysaccharides.

The supernatant was pipetted out, then 550 µl of cold chloroform was added and was vortexed slowly. After re-centrifuging and pipetting out of supernatant it was transferred to a new 1.5 ml microtube and 750 µl of cold isopropanolol alcohol was added.

Then samples were kept in a freezer for 30 min. Supernatant was centrifuged and pipetted out once again. Then 70% cold ethanol was added to rinse pelleted DNA and the tubes were centrifuged at 7000 rpm.

The supernatant pipetted out and was poured into opened cap microtubes to let the pelleted DNA to be air dried. Then the dried pelleted DNA was dissolved in 50 µl. deionized water and was preserved for further investigations in (-20 °C) in a deep freeze freezer (23).

Polymerase chain reaction (PCR) and electrophoresis

The two following primers were used for PCR:
Forward primer (5'-CAACATTTATTTTGATTTTTTGG-3') and
Reverse primer (5'-TCCATTGCACTAATCTGCCATATTA -3').

For each DNA sample, a 25 µL of PCR mixture (MgCl2, PCR-buffer, dNTPmix (Bio Flux), primers, DNA, *Taq* DNA polymerase was added and PCR was done as follows; initial denaturation, one cycle, 5 min at 94°C, 38 cycles of (denaturation- 35 s at 94°C, annealing- 45 s at 36°C, primers extension- 2 min at 72°C), and final primers extension one cycle at 72°C, for 5 min. 1.5 % Agarose gel was used for electrophoresis, staining of the bands were done using a 0.5 l g/mL ethidium bromide, and finally UV Rays by (Bio-Rad Gel Doc 2000) was used to make the bands visible, and ready for final photography.

DNA Sequencing

The PCR products of COI sequences were sent to Tekapozist Co., Tehran for sequencing. The sequencing was done both in forward and reverse directions to compare segments of DNA of every two species. Then we have compared the sequences obtained for our species of neuropterans with those which was accessible in Gene bank of NCBI.

Analysis of Molecular Data

MegAlign software (6th edition May 2001, DNASTAR® Inc. USA) was used to evaluate genetic distances as well as the replacements of nucleotides between nucleotide sequences of different species.

The EditSeq (6th edition May 2001, DNASTAR® Inc. USA) was used to edit sequences, comments and annotations and SPSS version 16 was used for calculating coefficient of cophenetic.

RESULTS AND DISCUSSION

Although all neuropteran families are predators but they have different strategies to prey on their hosts, for example larvae of Chrysopidae are predators of aphids so they are named as aphidlions and Cannibalism is very intense between larvae of *Chrysoperla carnea* in absence of prey, sometimes reach to 100%. (MOCHIZUKI *et al*., 2006). But the adults lacewings are pollen feeders and not predators and larvae of *Myrmeleontidae* are predators of ants so they are called antlions.The body size of neuropteran are different from a few millimeters in Coniopterygidae to more than 7 centimeters in some of antlion species. Their habitat also are very diverse, while *Chrysopidae* prey on aphids so are generally found in aphid assemblages in plants, shrubs and trees, *Coniopterygidae* larvae should be found in colonies of plant mites. The antlions (family

Myrmeleontidae) have the unique behavior to be sedentary predators among insects, mostly pit building in the soil, they make an inverted cone shaped pit in the soil at the bottom of which the larvae wait for the prey(ants) to fall into the pitfall and the antlion suck the blood of ants by inserting a pair of pointed mandibles to the body of their victims. DEVETAK *et al*. (2005) emphasized that antlions *Euroleon nostras* make their pits with sand particles 0.23-0.54 mm of diameter and these antlions prefer fine sands to coarser sands, they found that larval antlions perceive their preys visually together with their odors and vibrations made by the movements of the walking ants on the sands near their pit.Concerning the ladybirds, they are intense predators of aphids both as larvae or adults, however the adults of many species such as *Coccinella septempunctata* like many other ladybug species are more effective predators of aphids than their larvae, the exception is wingless ladybird *Harmonia axyridis* (RIDDICK, 2017). *Silverflies* (Diptera, Chamaemyiidae) are another group of aphidiphagous and coccidophagous predator insects, which the maximum peak of their population reach in fall and winter, although one species is found in midsummer. *Chamaemyiidae* larvae was seen preying on aphids infesting herbaceous crops, fruit orchards and also neighborhood plants belonging to spontaneous flora, poor synchronization of predator-prey seasonal habits and lack of searching ability of some *Chamaemyiidae* species against a targeted prey are two major weakness of these predators in nature (SATAR *et al*., 2015).The Cecidomyiidae midges=larvae (Diptera) are another predators specially of Tetranychid mites. *Feltiella acarisuga* one of the species of Cecidomyidae is now commercially available in the USA and other countries as a biological control agent of *Tetranychid* mites (ZHANG, 2003).

Pairwise alignments of nucleotide sequences belonging to five species of Neuroptera (Fig-1).Some segments of nucleotides of COI gene of *Palpares solidus* (M1) was compared with *Creoleon remanei* (M2). This comparison showed that differences in COI gene nucleotides sequences were observed in residues of 699, 702, 726, 735 and 750 of *Palpares solidus*. The adenine was changed to thymine in *Creoleon remanei*. Other mutations were the cause of changing guanine to adenine in position 705, cytosine to thymine in position 720, adenine to cytosine in position 724, adenine to guanine in position 729 and guanine to adenine in position 738 of the nucleotide sequences in sequences of COI gene of *Palpares solidus* (Fig.1).

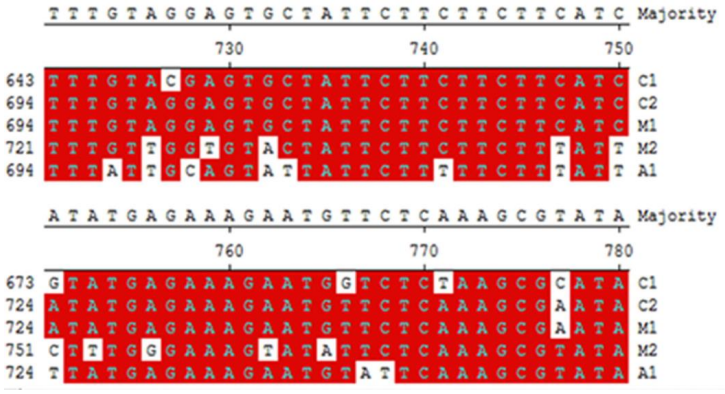

Fig 1.Pairwise comparison of a part of nucleotides of COI in five species of Neuroptera.

The *Chrysopa pallens* and *Palpares solidus* have 98.2% (maximum of similarity), in other words there are the least divergence (1.6%) between them (Table 1). The *Chrysopa viridana* and *Creoleon remanei* have the maximum of distance (20.7%) and the minimum similarity (75.9%).

Table 1. Coefficient of cophenetic for cluster analysis of aminoacids of mitochondrial COI in five species of Neuroptera (Palpares solidus(M1), Chrysopa pallens (C_2), Chrysopa viridana (C_1), Creoleon remanei (M_2), Deleproctophylla variegata (A_1) in this figure, is equal to 0.702 which is significantly different at 5% probability($0.01 \leq p < 0.05$)

Percentage of identity

		5	4	3	2	1	
Deleproctophylla Variegata	1	81.3	82.9	82.7	79.1		1
Chrysopa Viridana	2	75.9	95.5	96.1		18.8	2
Chrysopa pallens	3	78.8	98.2		4.00	18.3	3
Palpares solidus	4	79.8		1.6	4.2	17.9	4
Creoleon remanei	5		18.9	19.9	20.7	17.8	5
		5	4	3	2	1	

Divergency

We also have compared pairwise alignment of sequences 22-81 of 60 amino acids sequences corresponding to triplets of nucleotides in parts of COI gene of *Palpares solidus*, *Deleproctophylla variegata*, *Chrysopa pallens* with positions of 31-90 amino acids sequences in *Creoleon remanei* and compared them with sequences 5-64 of amino acids corresponding to triplets of nucleotides in COI of *Chrysopa viridana*. Maximum similarities (i.e. 56 AA of 60 AA) have been found in the above indicated sequences; however four of them are different. Then 93% of AA in the interval of 60 above mentioned AA had the same sequences in five species of Neuroptera. As concerning the amino acids which participate in composition of proteins, coded by COI gene (Fig. 2) we have seen that histidine (H) was the amino acid in position of 26 in AA sequences in *Palpares solidus*, *Deleproctophylla variegate*, *Chrysopa pallens* and in position 35 of AA sequences in *Creoleon remanei*.

However it was replaced by Arginine (R) in position of 9 of AA sequences in *Chrysopa viridana*. There is two codons for histidine (CAU and CAC), while the two codons of Arginine are AGA and AGG. So there was a chance of occurrence of a point mutation which changed histidine and replaced it with arginine. In position of sequence 30 in COI of *Palpares solidus*, *Chrysopa pallens*, in position 39 in *Creoleon remanei* and position 13 in *Chrysopa viridana* we saw histidine (H) replaced by glutamine (Q) in *Deleproctophylla variegata* and as the two first letters of two codons for Histidine and glutamine are similar and are CA, but the third letter are different, so there was a possibility that U or C as the third letters of the codons for histidine have had a mutation which changed them to A or G. Glutamic acid in position 36 of sequences of amino acids in *Deleproctophylla variegata* and in position 45 of sequence of amino acids in *Creoleon remanei* and in position 19 of sequences of amino acids in *Chrysopa viridana* was replaced by Glycine (G) in position 36 of sequences of amino acids in *Palpares solidus* and *Chrysopa pallens*. As in this case the two codons for glutamic acid (GAA and GAG) and the four codons for glycine (GGU, GGC, GGA and GGG) and as the first letter of the codons for

both amino acids are similar, so there was a chance of mutation that the second letter (A) in two codons of glutamic acid was changed to (G) in four codons of glycine.

Simultaneously the third codon (A) in GAA for glutamic acid probably had a mutation and was changed to U,C,G in glycine and (G) in the third codon of GAG for glutamic acid was changed to A, C and U in codons which code glycine.

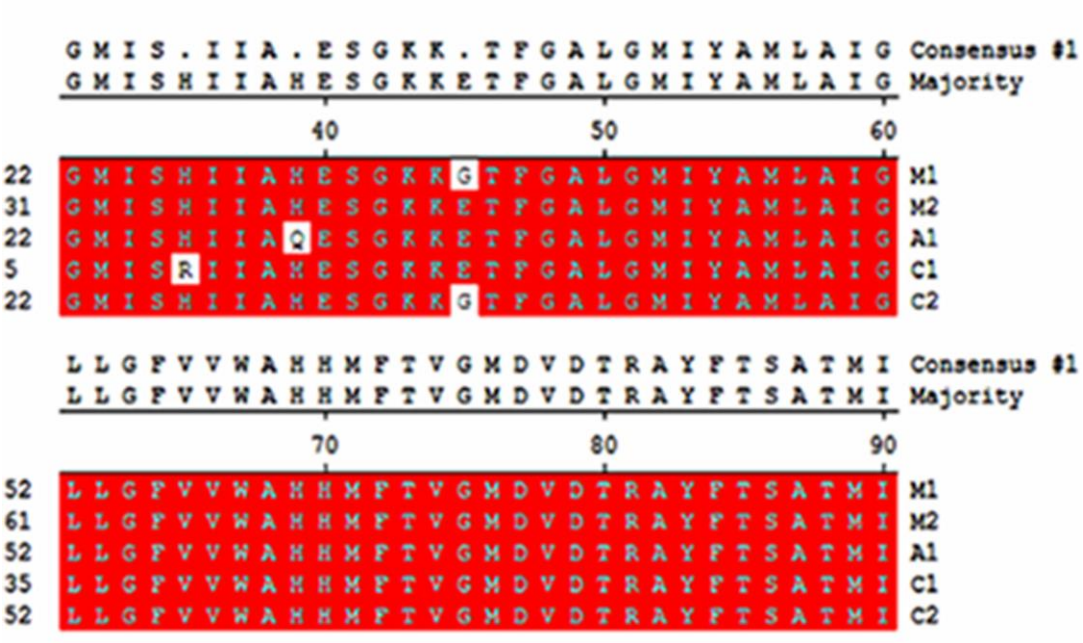

Fig 2. Percentage of pairwise identical sequences of aminoacids of part of chain of polypeptides in COI in five species of Neuroptera

We have used pair alignment of DNA nucleotides of a section of COI genes for comparison between studied Neuroptera species. We observed that in species *Palpares solidus* (M_1), *Chrysopa pallens* (C_2), *Chrysopa viridana* (C_1), *Creoleon remanei* (M_2), *Deleproctophylla variegata* (A_1), the sequences of their mitochondrial COI genes have many totally conserved parts (Fig.1), the sequences of 60 nucleotides (694-753) of *Palpares solidus* (M_1) and *Chrysopa pallens* (C_2) were compared. There were 100 % (maximum genetic similarity) between them. The sequences of 60 nucleotides between 643 and 702 of a part of mtDNA genes coding for COI in Japanese *Chrysopa viridana* sequenced by Haruyama *et.al*., (2008) were compared with the same sequences of our specimens of *Chrysopa viridana* COI nucleotides collected from Dezful in Khuzestan, Iran. Those sequences are as follows:

Dezful:

643:TTTGTACGAGTGCTATTCTTCTTCTTCATCGTATGAGAAAGAATGGTCTCTAAGC
GCATA:672

Japan:
643:GTATTAAGGTATTTAGTTGATTAGCTACTCTTCATGGTACTCAATTTACTTATAG
ACCTGCT 672

There were 15 identical nucleotides between sixty nucleotides compared. In other words 25% of them were similar, or we can say 75% of genetic diversity existed between two populations of Iranian and Japanese *Chrysopa viridana* concerning the compared sequences The COI gene is an organelles (mitochondria) gene, some of the nuclear (non organelles) genes are located in nucleus but their product is collected in the organelles. (e. g. the *aro*A gene in plants and bacteria) (MOTAMEDI *et al*., 2011).

Table 2. Certain nucleotide sequences and names of twenty two species of Chrysopidae family accessed from NCBI compared with five species of Neuroptera used by us in our study.

Species name	Family name	Accession numbers
Chrysoperla carnea	Chrysopidae	AB671795
Chrysoperla adamsi	Chrysopidae	AB671779
Chrysoperla agilis	Chrysopidae	AB671786
Chrysoperla johnsoni	Chrysopidae	AB671811
Chrysoperla mediterranea	Chrysopidae	AB671823
Chrysoperla Mohave	Chrysopidae	AB671825
Chrysoperla pallida	Chrysopidae	AB671837
Chrysoperla plorabunda	Chrysopidae	AB671841
Chrysoperla pudica	Chrysopidae	AB671845
Chrysoperla rufilabris	Chrysopidae	AB671851
Chrysoperla zastrowi sillemi	Chrysopidae	AB671853
Chrysopa Formosa	Chrysopidae	AB354054
Chrysoperla lucasina	Chrysopidae	AB671815
Chrysoperla nipponensis	Chrysopidae	AB671830
Chrysopa lezeyi	Chrysopidae	AB354056
Chrysopa nigra	Chrysopidae	AB354058
Chrysopa pallens	Chrysopidae	AB354059
Chrysopa viridana	Chrysopidae	AB354062
Chrysopa excepta	Chrysopidae	DQ414489
Dictyochrysa petereseni	Chrysopidae	DQ 414495
Calochrysa extranea	Chrysopidae	DQ414485
Leucochrysa lancola	Chrysopidae	DQ414503

Certain nucleotides sequences of mitochondrial COI gene of twenty two species of Chrysopidae was downloaded from NCBI and compared with the nucleotide sequences of mtCOI of five species of Neuroptera species used in current study (Table 2), and maximum similarity or divergence between them in (Table 3).

Table 3. Percent of identity and divergence between nucleotides of certain parts of DNA of COI gene of 27 species of Neuroptera. The nucleotides sequences of five species of Neuroptera (A1, C1, C2, M1, M2) are those studied by us, the rest were accessed from NCBI. Red circles denote percent of maximum similarity of nucleotides of some species of Neuropteran studied by us and discussed in the main text.

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
1		80.6	83.8	94.2	82.5	80.7	81.2	81.0	81.2	80.5	81.2	80.7	81.2	81.5	81.4	80.8	79.7	80.1	78.7	81.1	81.8	82.3	82.6	83.1	83.6	80.7	80.3	1
2	18.8		96.3	95.9	77.6	74.5	74.3	74.9	74.4	74.7	74.4	74.5	74.3	74.1	74.1	74.8	71.7	72.3	71.2	72.1	73.4	77.2	78.6	76.7	78.4	75.0	73.9	2
3	18.3	3.8		98.2	80.4	78.3	78.2	78.8	78.3	78.7	78.3	78.3	78.2	78.4	78.2	78.5	78.7	78.7	77.6	78.6	79.7	80.2	81.9	80.1	81.6	78.5	74.7	3
4	17.9	4.2	1.6		80.9	79.0	78.9	79.5	79.0	79.1	79.0	79.0	78.9	78.9	79.1	79.2	79.7	79.7	78.9	79.2	80.6	81.3	81.6	81.2	81.4	79.2	75.6	4
5	17.8	20.7	19.9	18.9		80.8	80.7	81.2	80.7	80.8	80.7	81.0	80.7	80.4	81.5	81.4	80.1	80.6	80.8	80.8	82.6	78.6	80.3	78.9	79.7	81.4	79.0	5
6	19.2	23.1	22.2	21.2	22.3		98.2	98.7	98.1	98.7	98.1	98.7	98.2	94.0	93.9	98.6	93.4	91.7	90.8	92.8	92.8	87.6	87.7	85.4	87.6	98.8	97.9	6
7	18.4	23.4	22.4	21.4	22.5	1.8		98.0	99.9	98.1	99.8	98.0	100.0	94.5	94.3	98.1	92.8	91.5	90.9	93.0	93.3	88.3	87.7	85.5	88.2	97.9	98.0	7
8	18.7	22.5	21.6	20.6	21.7	1.3	2.0		98.1	98.8	98.1	99.1	98.0	94.5	94.3	99.2	93.0	92.0	91.1	92.8	93.1	87.9	87.6	86.0	88.1	98.9	97.4	8
9	18.4	23.2	22.2	21.2	22.5	1.9	0.1	1.9		98.0	99.9	97.9	99.9	94.6	94.3	98.2	92.8	91.5	90.9	93.0	93.4	88.3	87.8	85.6	88.2	97.8	97.9	9
10	19.4	22.9	21.7	21.1	22.3	1.3	1.9	1.2	2.0		98.0	98.6	98.1	94.6	94.1	98.5	93.0	92.0	90.5	92.8	92.8	87.7	87.5	85.6	87.9	98.5	97.8	10
11	18.4	23.2	22.2	21.2	22.5	1.9	0.2	1.9	0.1	2.0		97.9	99.8	94.7	94.5	98.2	92.8	91.5	90.9	93.0	93.4	88.5	87.8	85.6	88.3	97.8	97.9	11
12	19.2	23.1	22.2	21.3	22.0	1.3	2.0	1.0	2.2	1.4	2.2		98.0	94.0	94.0	98.9	93.3	92.0	91.1	93.1	93.1	87.6	87.2	85.7	87.7	98.9	97.4	12
13	18.4	23.4	22.4	21.4	22.5	1.8	0.0	2.0	0.1	1.9	0.2	2.0		94.5	94.3	98.1	92.8	91.5	90.9	93.0	93.3	88.3	87.7	85.5	88.2	97.9	98.0	13
14	18.1	23.8	22.1	21.4	22.8	6.3	5.8	5.8	5.6	5.6	5.5	6.3	5.8		94.5	94.3	92.3	92.2	90.5	91.4	92.6	87.3	87.0	86.4	87.7	94.0	93.9	14
15	18.0	23.8	22.4	21.1	21.4	6.4	5.9	5.9	5.9	6.1	5.8	6.3	5.9	5.8		94.6	91.5	92.6	91.4	92.5	93.9	88.2	87.2	86.2	88.1	94.3	94.0	15
16	19.1	22.7	21.9	20.9	21.5	1.4	1.9	0.8	1.8	1.6	1.8	1.1	1.9	5.9	5.6		93.4	92.2	91.1	93.1	93.4	88.3	88.0	86.1	88.4	98.8	97.5	16
17	18.9	22.1	21.3	20.0	23.2	6.9	7.6	7.4	7.6	7.4	7.6	7.1	7.6	8.1	9.0	6.9		93.3	92.0	93.4	93.3	85.8	80.0	83.7	82.8	93.3	92.6	17
18	18.7	21.1	21.1	19.8	22.8	8.8	9.0	8.4	9.0	8.4	9.0	8.4	9.0	8.3	7.7	8.3	7.1		91.7	92.6	92.6	87.0	80.9	84.4	83.1	91.9	91.4	18
19	20.9	22.7	22.1	20.3	22.3	9.9	9.7	9.5	9.7	10.2	9.7	9.5	9.7	10.2	9.1	9.5	8.5	8.8		92.0	91.5	85.3	80.0	84.4	82.3	91.1	90.8	19
20	17.7	21.1	20.6	19.8	22.3	7.6	7.4	7.6	7.4	7.6	7.4	7.2	7.4	9.1	7.9	7.2	6.9	7.7	8.5		93.0	86.5	81.5	83.3	83.6	93.1	92.6	20
21	16.6	19.3	19.1	17.9	20.2	7.6	7.1	7.2	6.9	7.6	6.9	7.2	7.1	7.7	6.4	6.9	7.1	7.7	9.0	7.4		86.5	83.4	84.8	83.9	93.4	92.2	21
22	21.1	24.3	23.2	22.4	23.5	9.9	9.1	9.6	9.1	9.8	8.9	9.9	9.1	10.3	9.2	9.1	9.6	8.1	10.2	8.7	8.6		89.2	90.5	91.6	87.9	87.9	22
23	19.8	22.5	20.9	21.2	21.2	9.4	9.4	9.6	9.3	9.7	9.3	10.0	9.4	10.3	10.0	9.1	13.3	12.1	13.3	11.3	8.9	11.7		89.1	90.9	87.2	87.2	23
24	19.6	23.7	22.4	21.6	22.6	12.6	12.5	11.9	12.3	12.3	12.3	12.2	12.5	11.3	11.6	11.8	12.1	11.3	11.3	12.7	10.7	10.2	11.8		90.1	85.5	85.4	24
25	18.6	22.8	21.2	21.5	21.5	9.6	8.9	9.0	8.9	9.1	8.7	9.4	8.9	9.4	9.0	8.6	9.5	9.1	10.1	8.6	8.2	8.9	9.7	10.6		87.7	87.3	25
26	19.2	22.4	21.9	20.9	21.5	1.2	2.2	1.1	2.3	1.6	2.3	1.1	2.2	6.3	5.9	1.2	7.1	8.6	9.5	7.2	6.9	9.6	10.0	12.5	9.4		97.3	26
27	19.2	24.0	22.9	21.6	23.0	2.2	2.0	2.7	2.2	2.3	2.2	2.7	2.0	6.4	6.3	2.5	7.7	9.1	9.9	7.7	8.3	9.6	10.0	12.6	9.9	2.8		27
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	

Divergence

A1
C1
C2
M1
M2
AB671795
AB671779
AB671786
AB671811
AB671823
AB671825
AB671837
AB671841
AB671845
AB671851
AB671853
AB354054
AB354056
AB354058
AB354059
AB354062
DQ414489
DQ414495
DQ414485
DQ414503
AB671815
AB671830

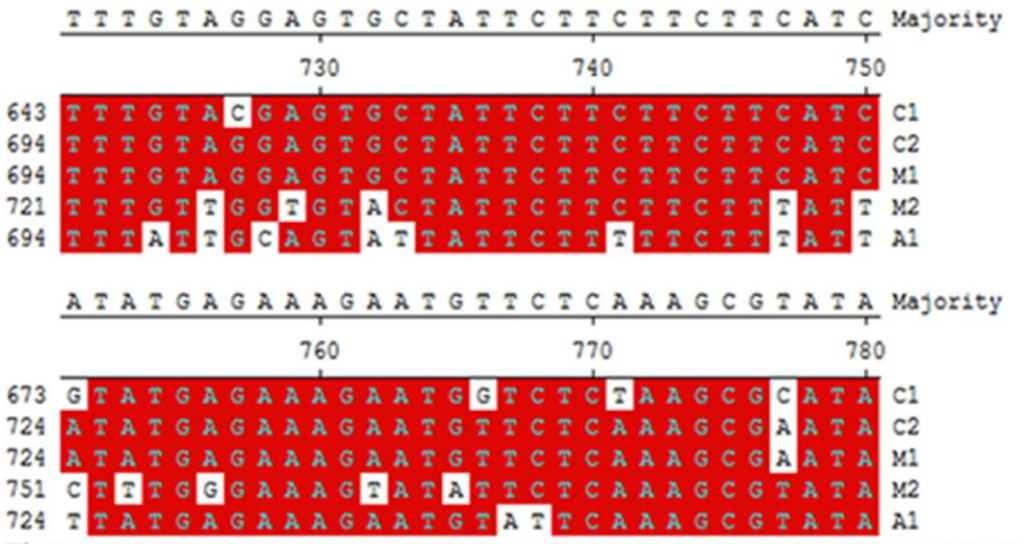

Fig. 3. Nucleotides sequences of COI gene of five species

For this purpose a leading horizontal line representing similar sequences of majority of nucleotides was drawn in upper side of the Fig. 3, beginning with sequence 721and ending to sequence 750.

As sequences of nucleotides 343-372 for mtCOI gene of eleven species of Chrysopidae family (*Chrysoperla carnea*, *Chrysoperla adamsi*, *Chrysoperla agilis*, *Chrysoperla johnsoni*, *Chrysoperla mediterranea*, *Chrysoperla mohave*, *Chrysoperla pallida*, *Chrysoperla plorabunda*, *Chrysoperla pudica* , *Chrysoperla rufilabris* and *Calochrysa extranea*) were proved to be identical, so they should be used for any design of primers for Chrysopidae family in the future researches comparing the evolution of nucleotide changes of mtCOI genes of species belonging to this family.

We have marked as red circles in the Table 3 the maximum percentage of genetic similarity between nucleotides of Neuropteran species. So, we have shown that there were 96.3% of genetic similarities between nucleotides of gene of COI in *Chrysopa pallens* (C_2) and *Chrysopa viridana* (C_1), 98.2% of genetic similarities between nucleotides of gene of COI of *Palpares solidus* (M1) and *Chrysopa pallens* (C_2).

While between nucleotides of COI genes of *Deleproctophylla variegata* (A_1) and *Palpares solidus* (M1) there was only 84.2% of genetic similarities. Besides there were 81.8% of genetic similarities between *Chrysoperla viridana* COI gene nucleotides with accession number (AB354062) accessed from NCBI and *Palpares solidus* (M1). However between nucleotides of COI gene of *Creoleon remanei* (M_2) and *Chrysopa nigra* (AB354058) there were 82.6% of genetic similarities. Although there were 98.2% of genetic similarities between COI of *Palpares solidus* (M1), *Chrysopa pallens* (C_2), 96.1% of genetic similarities between COI of *Chrysopa pallens* (C_2), *Chrysopa viridana* (C_1) and 82.9% of genetic similarities between COI of *Deleproctophylla variegata* (A_1) and *Palpares solidus* (M1) (Table 1). Cluster analysis made for our obtained data shows that between the five species of Neuropteran the maximum of genetic similarities were seen between *Palpares solidus* (M1) and *Chrysopa pallens* (C_2)(Fig.4),.

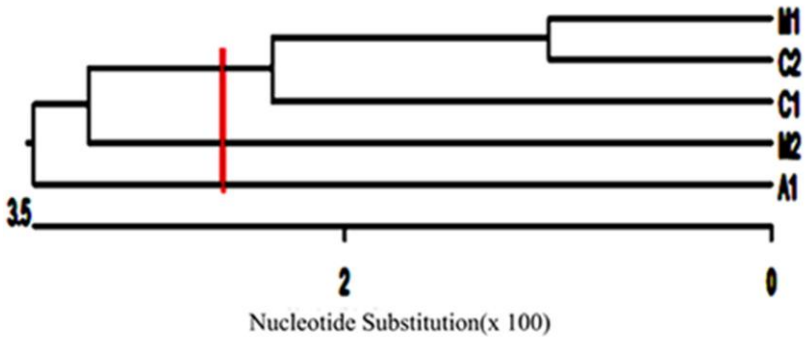

Fig. 4. Cluster analysis of pairwise alignment of proteins of certain parts of mitochondrial COI in five species of Neuroptera**.**

In (Table 4), percent of relative ratio of four nucleotide bases could be seen in five Neuropteran species in a part of genome of mtCOI. The ratio of thymine bases to cytosine bases in nucleotides of five Neuropteran species based on sequences of DNA nucleotides of a part of genome of COI was the maximum and equal to 112, while the ratio observed between Cytosine bases to guanine bases was the minimum and equal to 6.

Table 4. Percent of relative ratio between bases of nucleotides in five Neuropteran Species based on sequences of DNA nucleotides of a part of genome of COI.

	A	**C**	**G**	**T**
A		**46**	**43**	**110**
C	**46**		**6**	**112**
G	**43**	**6**		**11**
T	**110**	**112**	**11**	

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GENETIČKI ODNOS IZMEĐU FAMILIJA NEUROPTERA (*INSECTA, NEUROPTERIDA, NEUROPTERA*) NA OSNOVU SEKVENCI CITOCHROM OKSIDASE-I

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Izvod

Proučavani uzorci pripadali su vrstama iz tri familije *Chrisopidae, Mirmeleontidae* i *Ascalaphidae* (reda *Neuroptera*). Prikupljeni su u Šuši i Dezfulu, u južnom Iranu. Ovi insekti su korisni u biološkoj kontroli kao važni predatori apsida, psilida, gusenica, mrava i drugih insekata. Urađeno je upoređivanje sekvenci delova gena citohrom oksidaze-I (COI) ovih vrsta. Tela insekta su bila u potpunosti usitnjena u mikrotube. PCR proizvodi COI su sekvencirani. Poravnavanje parova nukleotidnih sekvenci koje pripadaju ispitivanim vrstama *Neuroptera* izvršeno je korišćenjem softverskih programa MegAlign i EditSek. Rezultat sekvenciranja *Palpares solidus* i *Creoleon remanei* su pokazali potencijalne mutacije na lokacijama 699, 702, 726, 735 i 750 COI gena mtDNA. Prema sekvencama COI gena, *Chrisopa palen* i *Palpares solidus* su pokazali maksimalnu genetičku sličnost (98,2%). Postojala je minimalna genetička sličnost (75,9%) između vrste *Chrisopa viridana* i *Creoleon remanei.*

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