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Original scientific article

GENETIC DIVERSITY STUDY OF *Chrysoperla carnea* (Neuroptera: Chrysopidae) POPULATIONS VIA MOLECULAR MARKERS

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The objective of this study was to determine the genetic diversity among *Chrysoperla carnea* samples collected from different locations of Iran (including, East-Azerbaijan, West-Azerbaijan, Isfahan, Kerman, Kermanshah, Lorestan, Mazandaran, Gilan, Hormozgan and Hamedan provinces) using the *Inter simple sequence repeat* **(***ISSR***)** and mitochondrial (Cytochrome Oxidase I – COI) molecular markers in 2016-2018. The results showed that a total of 64 bands were produced by ten primers of ISSR markers which among them 43 bands were polymorphic. The highest and lowest polymorphic percentages belonged to primer UBC-809 (88.88%) and primer UBC-886 (33.33%), respectively. The results of cluster analysis based on ISSR marker data divided the samples into three separate clusters. This grouping was also confirmed by analysis of molecular variance. According to the results of the analysis of molecular variance diversity within and among groups was about 84% and 16%, respectively. In the present study five haplotypes were obtained. The first haplotype (H1) was common in all populations which can be considered as the ancestral haplotype, the other haplotypes have been evolved from it. The novelty of this study is that we report the first time genetic diversity analysis of family Chrysopidae using ISSR and CO1 markers covering more than ten provinces and thirty cities of Iran with a full picture of its genetic diversity. Genetic distance matrix based on Jaccard index indicated low genetic distance of

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populations. The results showed that ISSR and CO1 markers have high efficiency in study of genetic diversity in the family Chrysopidae.

Key words: Chrysopidae, Genetic diversity, Iran, ISSR, CO1 Marker

INTRODUCTION

The family Chrysopidae is composed of approximately 1200 species in 80 genera (MCEWEN *et al*., 2001). Forty-five species of family Chrysopidae have been recorded in Iran until 2002 (MIRMOAYEDI, 2002 a). These predators are important agents for insect biocontrol due to their host domain, geographical dispersion, resistance against some pesticides and their potential to be produced in mass (MIRMOAYEDI.,2002b).There were also many molecular systematics studies of Chrysopidae in Iran between them MIRMOAYEDI *et.al* 2012,2017 ; YARI *et.al*,2017 and HENRY *et.al*, 2018.During the past century human activities had a negative impact on dispersion, abundance and population size of many species of Chrysopidae. Extinction of species, artificial reproduction or the transfer of species in other places has become a big problem for biocontrol agents and also there was little knowledge of how these events could affect the dispersion of individuals and the genetic diversity of populations (WINTERTON and DE FREITAS, 2006). Human activities and artificial mass production have changed the structure of population genetic which has led to genetic homogenization of populations. Therefore its of utmost importance to have information on the natural composition and genetic structure of the green lacewings populations in a variety of ecosystems (COSTA *et al*., 2010). Populations are a diverse set of genes. Reduced genetic distance, adverse climatic conditions and habitat loss can led to a decrease in the effective size of populations. Reducing genetic diversity can increase the risk of species extinction. Population-genetic studies are extremely important because the genetic variability of a species is directly associated with its ability to withstand different conditions when introduced into new environments (BARBOSA *et al*., 2014; HUFBAUER and RODERICK, 2005).

Chrysoperla carnea as a predator is widely used in commercial biological control programs. It is also of great economic importance in biological control of pests. So, it is very important to use necessary conservation programs to manage its natural populations. Previous studies have used morphometric traits to evaluate the genetic structure of *Chrysoperla carnea*. By the development of biological sciences and especially molecular markers, these markers have been used to identify their genetic structure. ISSR marker is a suitable tool in genetic diversity studies. A large number of microsatellite markers have been used to detect genetic polymorphisms among races (BARBOSA *et al*.,2014).Mitochondrial DNA (mtDNA) has many advantages for species identification and mapping of a phylogenetic relationship such as greater number of copies per cell, smaller size, maternal heritability, lack of recombination and lack of conserved regions (BRUFORD *et al*.,2003). Today molecular methods such as mitochondrial genome sequencing are among the most applicable methods to determine the phylogenetic relationship between populations and closely related species. The mitochondrial cytochrome oxidase (COI) gene has been used in several insect population studies on genetic diversity, population structure, phylogeny, phylogeography and identification of different insect species (ASOKAN *et al*., 2007). Expanding management plans and genetic resource modification is useful when the genetic diversity of the populations is clear. This information is necessary for the selection of gene donor populations in artificial reproduction, for the structure of the population and restoration of

resources (HELMI *et al*., 2011; HOOPER *et al*.,1993) comparison between Iranian populations of family Chrysopidae using DNA molecular markers was not studied so far therefore the objective of this study was to investigate the genetic diversity and populations structure of green lacewing *Chrysoperla carnea* from different regions of Iran using ISSR molecular markers and the mitochondrial COI gene.

MATERIALS AND METHODS

Sampling:

The samples were collected from different regions of Iran including East-Azerbaijan, West-Azerbaijan, Isfahan, Kerman, Kermanshah, Lorestan, Mazandaran, Gilan, Hormozgan and Hamedan provinces from May to November 2016 (Table 1). Sampling was made by sweeping alfalfa plantations by a sweep net and collecting of samples was done by pendulum swings of the net throughout the day and by using of light trapping at the night. In this experiment, 2000 samples were collected from 10 provinces and 30 samples from each province (300 samples in total) were selected for molecular studies. The collected samples with light trapping were kept in 70% ethanol and some of them were kept dry in double-layered cotton pads.

Table 1. Sampling localities and geographical positions in this research

Province	City	latitude	Longitude	Height	Code
	Urumiva	37.55321585	45.07627659	1348	1
Western Azerbaijan	Bukan	36.51512233	46.20708472	1340	2
	Khoy	38.55179431	44.95974594	1136	3
	Khorramabad	33.48868134	48.35929003	1188	4
Lorestan	Borujerd	33.89813714	48.7501026	1572	5
	Kuhdasht	33.52941544	47.60903406	1192	6
	Isfahan	32.67210698	51.67157274	1575	7
Isfahan	Khansar	33.22605745	50.31752524	2215	8
	Shahreza	32.00734554	51.85222690	1833	9
	Rudbar	36.82289358	49.42928867	212	10
Gilan	Rasht	37.28079786	49.59250067	+ 3	11
	Astara	38.42208367	48.86927386	-24	12

	Sary	36.56611747	53.05876297	42	13
Mazandaran	Amol	36.46981559	52.35124183	94	14
	Chaloos	36.65428442	51.42130387	29	15
	Kermanshah	34.32365879	47.07410791	1351	16
Kermanshah	Kangavar	34.50332392	47.96530129	1468	17
	Sarpol-zahab	34.45758373	45.86860067	556	18
	Hamedan	34.79881288	48.51516762	1818	19
Hamedan	Malayer	34.29290641	48.82193253	1748	20
	Kabudarahang	35.20000154	48.71226525	1672	21
	Maragheh	37.38950217	46.23764728	1451	22
East Azerbaijan	Tabriz	38.07847882	46.30374328	1402	23
	Marand	38.43060508	45.77383802	1331	24
	Kerman	30.28451186	57.07261258	1764	25
Kerman	Baft	29.23494228	56.59894493	2275	26
	Kuhbanan	31.41029769	56.28292425	1990	27
	Minab	27.14554001	57.07321481	40	28
Hormozgan	BandarAbbas	27.19463488	56.30798292	17	29
	Rudan	27.44130894	57.19104800	196	30

DNA extraction

Total genomic DNA was extracted from the head and thorax sections of each *Chrysoperla carnea* sample using the salting out method with slight modifications and then stored at −20 ºC (AWASTHI *et al*.,2004).

PCR amplification (ISSR markers)

DNA samples were amplified using 10 ISSR primers (Table 2). Polymerase chain reaction (PCR) reactions were performed in 25µL volumes containing 60 ng template DNA, 400 µM of each dNTPs, 1X PCR buffer (100 mM Tris-HCl, 50 mM KCL, 0.01% gelatin, and 0.25% tween 20), 1 µM of each primer pair, 1 units/µl *Taq* DNA polymerase, and MgCI2 (0.9–1.5 mM) described by (AWASTHI *et al*.,2004) with minor modifications. Amplification was performed in a thermocycler in the following manner; one denaturation step at 94 ºC for 5 min, followed by 35 cycles of denaturation at 94 °C for 48 s, annealing at 55° C (depending on the primer) for 60 s, extension at 72 °C for 110 s, and a final extension at 72 °C for 10 min. Thermocycler was run to work following the program described above and at the end the PCR product was obtained and was poured on an 2% agarose gel in a 1X TBE buffer and electrophoresed for about one hour at 80 Watts and afterward the gel stained with Ethidium Bromide.

Table 2. ISSR -marker primers used by us in the study (BARBOSA et al.,2014)

Primer	Sequence (5' → 3')	Annealing temperature (ºC)
UBC-809	5′-GAGAGAGAGAGAGAGG-3′	51
UBC-820	5′-GTGTGTGTGTGTGTGTC-3′	55
UBC-836	5′-AGAGAGAGAGAGAGAGCA-3′	48
UBC-849	5′-GTGTGTGTGTGTGTGTCA-3′	55
UBC-856	5′-ACACACACACACACACTA-3′	55
UBC-880	5′-GGAGAGGAGAGGAGA-3′	88
UBC-886	5′-CTCCTCCTCCTCCTCGT-3′	46
UBC-891	5′-GAGCTCTCTCTCTCTCT-3′	50
UBC-810	5′-GAGAGAGAGAGAGAGAT-3′	48
UBC-812	5′-GAGAGAGAGAGAGAGAA-3′	51

Data analysis

The banding pattern of ISSR markers were scored based on the presence or absence of a band in samples. The POPGENE software version 1.31 was used for estimating population genetic structure. The NTSYS-pc version 2.02 was used to cluster analysis, and GenAlex software was used for the analysis of molecular variance (AMOVA) (ROYCHOUDHURY and NEI, 1988).

PCR amplification (mtDNA (CO1))

Generic primers were used to amplify genomic DNA. Two sets of primers were used for amplifying COI gene region. . These were C1-J-2183 (5'CAACATTTATTTTGATTTTTTG G3') and TL2-N-3014 (5'TCCATTGCACTAATCTGCCATATTA3') (BIOEDIT,2019;AVISE *et al*.,1989), PCR was run in a total volume 25 µl of the following reaction mixture: 2.5 µl of 10× reaction buffer with KCl as provided by the manufacturer (Fermentas Life Sciences, Vilnius, Lithuania), 1 mM MgCl2, 0.5 mM of dNTP mix, 1 µM of each primer, 2 U of *Taq* polymerase

and 50 ng of total purified honey bee DNA. For each primer pair, the following reaction profile was used: initial denaturation 94ºC for 4 min, 35 cycles of 94ºC for 30 s, annealing at 55ºC for 1 min, and extension at 72ºC for 1 min, followed by a final extension step at 72ºC for 10 min. PCR products were sent to south Korea and sequenced there using method of GeneAll Combo Kit, South Korea (TAMURA *et al*.,2013) by double stranded assay (ABI3730xl, USA, Macrogen Corporation).

Data analysis

498-bp sequences of part of the mitochondrial genome cytochrome oxidase region of 60 samples from 30 populations of *Chrysoperla carnea* were first visualized by (BioEdit version 7.0.5.3,2019) and sequencing was performed using Clustal X (THOMPSON *et al*.,1997).The sequences were blasted in the gene bank at NCBI site to compare and confirm species identification. Descriptive analyses were performed using DnaSP software version 5.10.01 (LIBRADO and ROZAS, 2009) and the number of polymorphic sites (S), number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π), fixation index (FST) and the number of migrants (Nm) were obtained. The nucleotide substitution rate, frequency of each and the genetic distance between populations were determined by software MEGA version (TAMURA *et al*., 2013) using Kimura 2- parameter method and gamma evolutionary model. The relationship between genetic distance Fst and geographic distance km was studied by Mantel test (MANTEL, 1967). The phylogenetic tree was obtained from Maximum likelihood and Bootstrap using MEGA (Fig 3.).

RESULTS

ISSR

Three hundred *Chrysoperla carnea* genotypes were used as materials for DNA genotyping. All used primers showed polymorphism in amplified loci (Table 3). The allele size range varied from 150 to 1500 (bp). All primers showed polymorphisms among all populations. The total number of bands were 64, of which 43 bands were polymorphic. The number of bands scored for each primer varied from 5 to 9. Primer UBC-809 with eight bands and primer of UBC-886 with two bands showed the maximum and the minimum numbers of polymorphic bands among the used primers respectively. The polymorphism percentage for each primer varied from 33.33% to 88.88% (Table 3). The highest and lowest PIC values belonged to primers UBC-809 and UBC-812 (0.643and 0.302), respectively. The genetic diversity index (Nei's index=He) and Shannon's index (I) were calculated for each of the primers using the results of the ISSR data. The primers UBC-809 and UBC-812 had the highest and the lowest Shannon index respectively (Table 3). Maximum heterozygosity value of an ISSR locus was 0.282. Finally, the average heterozygosity values for all detected loci for each primer were estimated. The average heterozygosity was 0.190, and the range was between 0.149 and 0.282.

In the current study the genetic distance among populations was calculated based on Jaccard's similarity coefficient (Table 4). The lowest genetic distance was found among populations of West and East Azerbaijan provinces (0.0412). The low values of the genetic distance among the studied populations indicated a large similarity among the samples of the studied provinces.

Table 3. Polymorphism in different populations of Chrysoperla carnea by use of 10 ISSR primers.

name of primers	observed alleles size range of	total number of bands	polymorphic bands number of	Polymorphic (PIC)	Polymorphism percentage	Heterozygosity	Shannon's diversity (I)
UBC-809	150-1400	9	8	0.643	88.88	0.282	0.459
UBC-820	220-1500	8	6	0.615	75	0.231	0.380
UBC-836	280-1200	7	5	0.572	71.42	0.201	0.342
UBC-849	300-1500	6	4	0.464	66.66	0.176	0.307
UBC-856	180-1500	6	4	0.444	66.66	0.164	0.298
UBC-880	170-1400	7	4	0.521	57.14	0.198	0.331
UBC-886	300-750	6	2	0.391	33.33	0.183	0.261
UBC-891	250-1100	5	4	0.320	80	0.167	0.223
UBC-810	300-700	5	3	0.311	60	0.152	0.209
UBC-812	210-1000	5	3	0.302	60	0.149	0.194

Genetic distances of studied populations

Table 4. Genetic distances based on Jaccard's coefficient among C. carnea populations

	1	2	3	4	5	6	7	8	9
E.Azerbaijan(1)									
W.Azerbaijan(2)	0.0412								
Hamedan(3)	0.1310	0.1510							
Kermanshah(4)	0.1241	0.1311	0.0861						
Lorestan(5)	0.1441	0.1311	0.0951	0.0861					
Gilan(6)	0.1698	0.1510	0.1176	0.1070	0.0921				
Mazandaran(7)	0.1587	0.1421	0.1354	0.1065	0.0941	0.0821			
Esfahan(8)	0.0994	0.0912	0.0840	0.0810	0.0791	0.0823	0.0723		
Hormozgan(9)	0.0891	0.0821	0.0721	0.0710	0.0671	0.0569	0.0721	0.0998	
Kerman(10)	0.0852	0.0901	0.0730	0.0921	0.0652	0.0532	0.0891	0.0787	0.0541

Cluster analysis

Cluster analysis based on unweighted pair group method (UPGMA) using Jaccard similarity coefficient divided the studied populations into three different groups (Figure 1.). The cophenetic correlation coefficient was 0.9 indicating of the goodness-of fit of the dendrogram to the original data. Dendrogram showed that the northern provinces of Iran are scattered in the first group and the southern and central provinces in the third group. The grouping largely corresponded to the geographical origin.

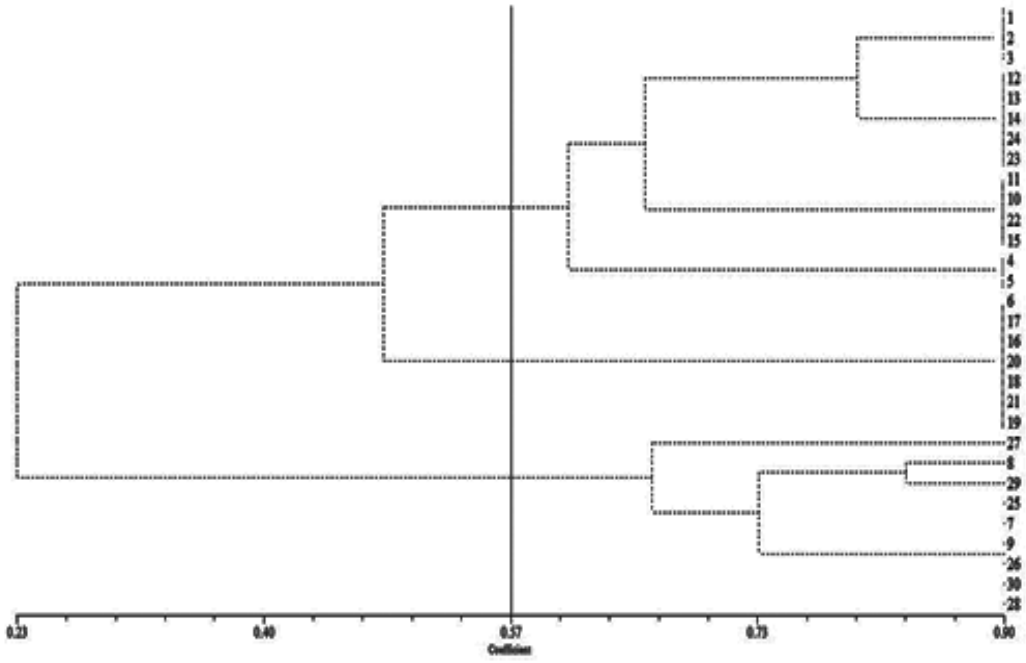

Figure 1. Cluster analysis based on UPGMA using Jaccard similarity coefficient in the studied populations.The numbers 1-30 in the vertical axis of dendrogram corresponds to the cities in Table 1.

Two-dimensional scatter plot of studied samples based on principal component analysis using the Jaccard coefficient are presented in Figure 2. *Chrysoperla carnea* samples were divided into three main groups, confirmed the results of the cluster analysis.

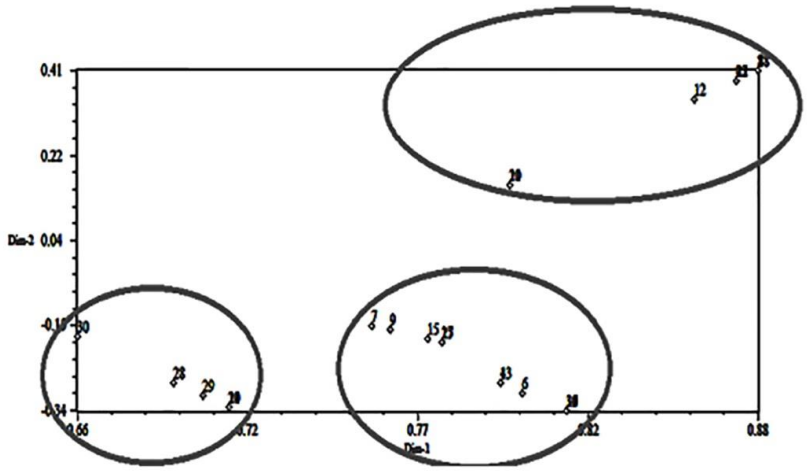

Figure 2. Two-dimensional diagram, the resultant of two components analysis, this graph was drawn, by considering the first and the second components

AMOVA analysis

The results of the analysis of molecular variance (AMOVA) based on ISSR marker data is shown in the table 5. Therfore, according to molecular analysis of variance (AMOVA), intra-population and inter-population genetic diversity were estimated to be 84% and 16%, respectively.

Table 5. Result of AMOVA based on data produced by use of ISSR marker

Source	Df	MS	Variance of each component	Total percentage variance	PHIPT	P-value
Among populations	2	241.540*	1.680	%16	0.019	0.002
Within populations	297	11.038	12.005	%84		
Total	299	252.578	13.685	%100		

CO1

DNA sequences at NCBI site were blast in order to compare and confirm species identification, and high similarity was observed (92-100%) with reported populations from other parts of the world. The average nucleotide composition of 498 bp of COI sequences of thymine, cytosine, adenine and guanine bases were 40.6%, 15.3%, 29.4% and 14.7%, respectively. Five haplotypes were obtained from all populations studied. Among them, Haplotype 1 (H1) was

observed in all populations. Haplotype 2 (H2) was common among all populations except Bandar Abbas, Kerman and Isfahan. Haplotype 3 (H3), Haplotype 4 (H4) and Haplotype 5 (H5) were observed in a populations (Lorestan, Sari and Rasht, respectively) with the highest number of nucleotide differences in the north group (Table 7).

Table 6.Distribution of haplotypes of different populations of C. carnea in Iran

Haplotype	Populations										Total
	1	2	3	4	5	6	7	8	9	10	
H1	3	3	3	1	1	1	2	2	2	2	20
H2				1	1	1	1	1	1	1	7
H3						1					1
H4				1							1
H5					1						1
Total											30

Table 7. Genetic diversity indices of the mitochondrial gene of COI for each population

	N	S	h	Hd	k	pi
Tabriz(1)	3	1	2	0.653	0.653	0.0010
Urumiva(2)	3	1	2	0.653	0.653	0.0010
Hamedan(3)	3	1	2	0.653	0.653	0.0010
Kermanshah(4)	3	1	2	0.653	0.653	0.0010
Khorramabad(5)	3	2	3	1	1.343	0.0020
Rasht(6)	3	2	3	1	1.343	0.0020
Sari(7)	3	2	3	1	1.343	0.0020
Esfahan(8)	3	0	1	0.000	0.000	0.0000
Bandar- Abbas(9)	3	0	1	0.000	0.000	0.0000
Kerman(10)	3	0	1	0.000	0.000	0.0000
Total	30	10	20			

It seems that Haplotype 1 (H1) which is the largest and includes all the studied populations, is considered as the oldest or native haplotype. The less frequent haplotypes include the smaller population are smaller in size and depending on the number of mutations, they bind more closely to the central haplotype. Studies on phylogenetic relationships showed that most populations are very close together and 5 haplotypes obtained are also differentiated on the phylogenetic tree. Analysis of molecular variance (AMOVA) showed that genetic changes within populations was a general occurence. The Mantel test indicated no correlation between genetic and geographic distances ($r = 0.0950$; $P = .198$). The genetic distance between populations was very low and varied from 0.001 to 0.005. The genetic distance between populations was low for both markers.

Table 8 .Nei's genetic distance based on mtDNA (COI) (below diagonal) and geographic distance (km) (above diagonal) of C. carnea populations

	1	2	3	4	5	6	7	8	9	10
Tabriz(1)		146	552	580	797	475	869	891	1801	1503
Urumiova(2)	0.001		531	542	721	614	1008	1030	1940	1642
Hamedan(3)	0.004	0.004		188	246	400	568	508	1418	1120
Kermanshah(4)	0.003	0.004	0.001		189	578	747	619	1596	1299
Khorramabad(5)	0.005	0.004	0.002	0.003		645	726	377	1344	1046
Rasht(6)	0.005	0.003	0.004	0.003	0.003		362	634	1544	1246
Sari(7)	0.005	0.005	0.004	0.004	0.002	0.002		690	1525	1227
Esfahan(8)	0.004	0.002	0.003	0.002	0.002	0.003	0.003		968	671
Bandar- abas(9)	0.003	0.001	0.002	0.003	0.002	0.003	0.003	0.001		486
Kerman(10)	0.002	0.002	0.001	0.002	0.001	0.002	0.001	0.003	0.003	

Low genetic distances were also observed by BARBOSA *et al*, 2014, between populations of *Chrysoperla externa* in the São Paulo State in Brazil. The gene flow and the neutrality test Tajima's values was obtained for all studied populations and are successively Nm= 22, Gst= 0.0128 and D= -1.7321. The overall stability index FST was not significant for all populations. FST = -0.09831; p> 0.05), which indicated the absence of genetic structure between these populations. The prominent features of mitochondrial genes especially CO1 such as lack of introns, low number of deletion sites and high rate of evolution (10-1 rate - single copy nuclear gene) makes it a suitable marker not only for differentiation near species but also for phylogeographic studies of species (AVISE *et al*., 1987; BROWER, 1994). The rapid sequencing divergence rate, especially at silent sites of mitochondrial protein coding genes, allows for recently branched lines to be separated (HARRISON, 1989). In the present study we used CO1 gene in order to evaluate the genetic structure and diversity of geographical populations of *Chrysoperla carnea* in Iran. The study of genetic diversity parameters indicated that there is little haplotype and genetic diversity between the populations studied. 5 haplotypes were obtained from COI gene sequence analysis. Analysis of molecular variance (AMOVA) showed that genetic variation within populations was greater than genetic change between populations. The results showed that the high gene flow and low genetic diversity between populations may help to attract different populations.The negative value obtained from neutrality test (Tajima's D = -1.732) indicated the population integration and low frequency of polymorphism. The phylogenetic tree clustered 30 samples in a clade (Fig 3). This phylogenetic tree confirms high

genetic diversity among individuals, although there was not a clear genetic structural change in the population. *Ceraeochrysa lineaticornis* (NCBI accession KR146303) was selected as the outgroup and the confidence level was calculated with the 1000 replicates of the bootstrap test. The results showed that the high gene flow and low genetic diversity between populations could not have a great impact on the change of genetic structure of different populations of lacewings studied by us. In numerous studies, COI sequences have been used to examine the population structure of a species and populations have been subdivided into clades or groups. The study of *Chrysoperla externa* (Neuroptera: Chrysopidae) populations in Brazil and *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) in India (HUNDSDOERFER and WINK,2009) which showed no specific genetic diversity between the studied populations, however these authors blamed migrations and displacing of insects in long distances as two major factors responsible for the change of genetic structure of the studied insect populations. Nucleotides accession numbers was obtained from NCBI by direct submission to NCBI gene bank data base for genes of CO1 in our study and totally 30 accession numbers were obtained as follows; Eight accession numbers MW857150 to MW857157, Eleven accession numbers MW909771 to MW909781, nine accession numbers from MZ376743 to MZ376751, two accession numbers MW856823 and MZ37675.

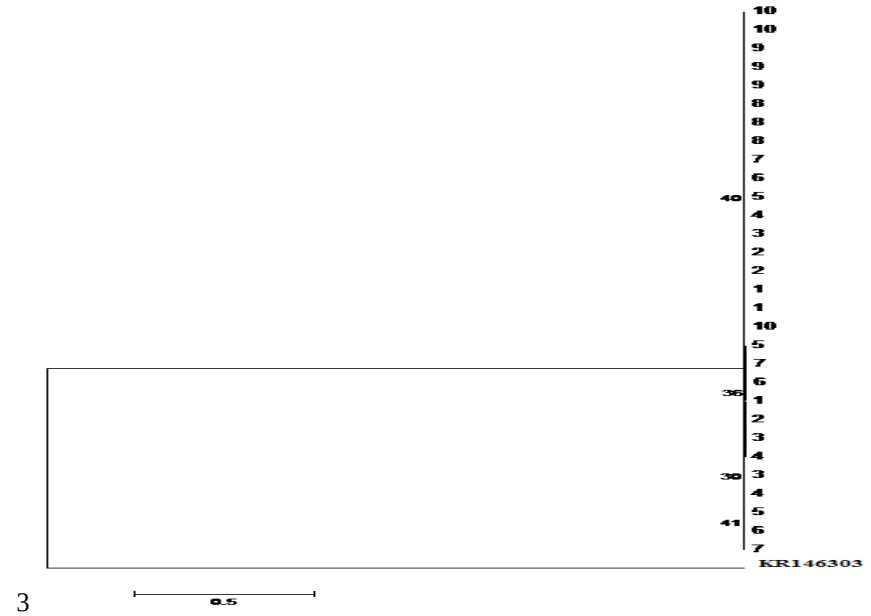

Figure 3. Phylogenetic tree with Maximum likelihood method based on sequences of *Chrysoperla carnea* populations in Iran

DISCUSSION

The genetic differentiation in insects may be due to differences in genetic traits such as gene flow, changes in environmental conditions, natural selection and random processes such as mutations, migration and the level of population differentiation.When one or more of these forces are acting in a population, the population violates the Hardy-Weinberg assumptions and evolution occurs (ANDREWS,2010). The genetic differentiation may be due to differences in genetic traits because of changes in environmental conditions and natural selection as well as random processes such as mutations, migrations and the differentiation level of populations which could be estimated by different parameters (NEI, 1972). Gene flow could be considered as one of the responsible cause of the change in genetic structure of the population. Steffler and co-workers used ISSR and GenAlex and considering different climatic types (humid tropical, agreste and semiarid) and have sampled *Aedes aegypti* vectors of yellow fever and Zika in Sergipe,Brazil significant genetic variability but little differentiation was observed among *Ae. aegypti* populations, possibly reflecting intense gene flow mediated by the passive dispersion of mosquitoes due to the intense traffic of vehicles among the cities (STEFLER *et al*., 2016). Rosas and co-workers, studied *Triatoma infestans* the vector of chagas disease in Brazil and expressed that there was not a significant association between geographical distance and genetic differentiation (Φ_{ST}) among sites (Mantel $r = 0.09$, $P = 0.31$) indicating a restricted gene flow among sampling sites where allele frequencies could drift independently from geographical distances separating them (DE ROSAS *et al*.,2017). Chen and co-workers considered differing environments as an impact factor and used ISSR , Cluster analyses and UPGMA to study genetic changes in Chinese populations of gypsy moth(*Lymantria dispar*), they have seen stronger genetic relationships among geographically proximate locations and concluded that more closely related populations share a common origin or evolutionary history and observed a high overall genetic differentiation among populations (AMOVA: FST = 0.2543, $P < 0.001$) which has given further support to the finding of strong genetic structuring among the Chinese gypsy moth (CHEN *et al*.,2014).De Rosas and co-authors studied *Triatoma infestans* the vector of chagas disease in Brazil and expressed that there was not a significant association between geographical distance and genetic differentiation (Φ_{ST}) among sites (Mantel $r = 0.09$, $P = 0.31$) indicating a restricted gene flow among sampling sites where allele frequencies could drift independently from geographical distances separating them (DE ROSAS *et al*.,2017). Hu and co-authors have used ISSR marker for identification of house flies (*Musca domestica*) populations from different cities and regions in China and found differences in genetic diversity , genome and gene types of geographic populations of *M. domestica* from different cities and regions of China (HU *et al*.,2008). Hundsdoerfer and Wink studied separated pairs of the moths *Hyles euphorbiae* and their offsprings in Spain, used ISSR-PCR with 4 primers and found that the bands sharing index did not detect significant difference of genetic variability compared between siblings and individuals of *Hyles euphorbiae* collected from different localities at random (HUNSDOERFER and WINK, 2005). Rahimi and coworkers by using of ISSR and UPGMA have studied the genetic polymorphism between honeybee population in Iran, the authors showed that genetic similarity existed between honey bee populations of six different provinces of Iran, the possible factors were migration of bees to neighboring provinces. Cluster analysis showed that two neighboring provinces of East and West Azerbaijan formed a single group differentiated

from the other groups, the existing trade of queens as well as colonies of bees between beekeepers of these two provinces was considered as the factors responsible of genetic similarities between honey bee populations of these two neighbor provinces (RAHIMI *et al*.,2016).The improvement of genetic studies of *Chrysoperla carnea* as a model of green lacewing and its use in breeding programs is directly dependent on the preserving of genetic diversity among their populations. Our study besides the studies of many other authors proved that ISSR markers have a large potential in revealing the existing polymorphism in insects in general (HELMI *et al*., 2011; HU *et al*., 2010; DE ROSAS *et al*., 2017; ABBOT, 2001; HU *et al*.,2007) and specifically in study of polymorphism in green lacewings, *Chrysoperla* spp. populations in particular (BARBOSA *et al*.,2014). Helmi and co-authors have used primers UBC-812 and UBC819 in Hemiptera order (HELMI *et al*., 2011), they have obtained similar results to us. In our study primer UBC-809 with nine alleles (in all populations) had the highest number of observed alleles in studied populations and primer UBC-880 had the highest values for PIC, He and I indices. High levels of genetic diversity were observed for both markers. The results showed that the highest values of genetic diversity appear associated with cities that have the greatest areas of native vegetations. The results of grouping geographical populations showed that the southern and central regions of the country have less haplotypic diversity than the northern regions. By using Mantel's test we have found no relationship between genetic and geographical distances. Such a formation of genetic structure could be the result of the establishing of an uniform farming in ecosystem and the transfer of insects by humans which has led to high gene flow and reduced genetic differences even at long geographical distances. Our study revealed that the samples collected from Gilan, Mazandaran, and Lorestan provinces had a higher number of alleles and genetic diversity indices than samples collected from other locations in Iran. The improvement of genetic status of *Chrysoperla carnea* as a model of green lacewing and its use in breeding programs depends on the preserving of genetic diversity amongst their populations. The current results showed that ISSR markers as a technique has a large potential in study of polymorphism in *Chrysoperla carnea* populations. Some of the studied ISSR primers including UBC-809, UBC-812 and UBC819 in the Hemiptera order have been used previously and show similar results to what obtained by us (ABBOT.,2001). In our study, primer UBC-809 with nine alleles (in all populations) had the highest number of observed alleles in studied populations and primer UBC-880 also had the highest values for PIC, He and I indices. High levels of genetic diversity were observed for both markers. The results showed that the highest values of genetic diversity appear associated with cities that have the greatest areas of native vegetations. The results of grouping geographical populations showed that the southern and central regions of the country have less haplotypic diversity than the northern regions. In Mantel test, no relationship was found between genetic and geographical distances. Such a genetic structure can be the result of the establishment of a uniform farming ecosystem and the transfer of insects by humans leading to high gene flow and reduced genetic difference even at long geographical distances. Our study revealed that the samples collected from Gilan, Mazandaran, and Lorestan provinces have a higher number of alleles and genetic diversity indices. High vegetation cover is one of the main reasons for increasing genetic diversity in these areas. The results of this study are similar to those by other authors on *Chrysoperla externa* (BARBOSA *et al*., 2014). A low genetic distance was observed among the studied populations indicating a high genetic similarity between the

studied provinces. In the same study, Barbosa and coauthors examined the genetic polymorphism of *Chrysoperla externa* populations in Brazil using the ISSR marker and they reported a low genetic distances among studied populations. The genetic differentiation may be due to differences in genetic traits, changes in environmental conditions and natural selection. Random processes such as mutations, migration, and the level of population differentiation also contribute to such differences. Finally Results obtained in our study showed us that ISSR- PCR technique has a reliable capacity to identify polymerphism in the studied populations of *Chrysoperla carnea*, the same results was obtained by different authors using ISSR for study of species of insects belonging to different orders (KURD *et al*., 2020; CHEN *et al*., 2014; LIU *et al*., 2010; SUN *et al*.,2016). Given the positive relationship between the amount of genetic variations and the amount of evolutionary change occurring, there is a similar relationship between the gene improvement efficiency of a breeding population and genetic diversity for the trait in question. Therefore conservation of genetic resources is essential. Understanding genetic diversity in insects could help us to understand the genetic structure, population adaptability as well as succees in finding food for them.

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PROUČAVANJE GENETSKOG RAZNOLIKOSTI POPULACIJA *Chrisoperla carnea* (Neuroptera: Chrisopidae) PREKO MOLEKULARNIH MARKERA

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Izvod

Cilj ove studije bio je da se utvrdi genetička raznolikost među uzorcima Chrisoperla carnea prikupljenim sa različitih lokacija Irana (uključujući provincije Istočni Azerbejdžan, Zapadni Azerbejdžan, Isfahan, Kerman, Kermanšah, Lorestan, Mazandaran, Gilan, Hormozgan i Hamedan) koristeći molekularni markeri inter jednostavne sekvence ponavljanja (ISSR) i mitohondrijalnih (citokrom oksidaza I – COI) u 2016-2018. Rezultati su pokazali da je ukupno 64 trake proizvedeno sa deset prajmera ISSR markera od kojih su 43 trake bile polimorfne. Najveći i najmanji postotak polimorfnosti imao je prajmer UBC-809 (88,88%) i prajmer UBC-886 (33,33%), respektivno. Rezultati klaster analize na osnovu podataka ISSR markera podelili su uzorke u tri odvojena klastera. Ovo grupisanje je takođe potvrđeno analizom molekularne varijanse. Prema rezultatima analize molekularne varijanse diverzitet unutar i među grupama iznosio je oko 84% i 16%, respektivno. U ovoj studiji dobijeno je pet haplotipova. Prvi haplotip (H1) bio je uobičajen u svim populacijama koje se mogu smatrati haplotipom predaka, ostali haplotipovi su evoluirali iz njega. Novina ove studije je da izveštavamo o prvoj analizi genetičke raznovrsnosti porodice Chrisopidae koristeći ISSR i CO1 markere koji pokrivaju više od deset provincija i trideset gradova Irana sa potpunom slikom njegove genetske raznovrsnosti. Matrica genetičke udaljenosti zasnovana na Jaccard indeksu ukazuje na nisku genetsku udaljenost populacija. Rezultati su pokazali da ISSR i CO1 markeri imaju

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