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Genetic Diversity of *Chrysoperla* sp. at East of Red Sea Using Cytochrome Oxidase Subunit I (COI) Gene

Akram Alghamdi

Department of Biology, Faculty of Science, Taif University, Taif, 888, Kingdom of Saudi Arabia

Abstract: ***Genus Chrysoperla (Neuroptera: Chrysopidae) contains 36 species. Chrysoperla carnea Stephens is a widespread predator in agroecosystems with great potential in biological pest control. C. carnea contains several cryptic species comprising the so-called carnea group. The species morphological similarity makes it difficult to differentiate them. Therefore, it is important to differentiate them through molecular methods. In this study, the cytochrome C subunit I (COI) gene have been partially amplified and sequenced for the same taxa of Genus Chrysoperla (Neuroptera: Chrysopidae) inhabiting Jeddah and Tabouk (Saudi Arabia) in order to identify and examine their molecular diversity. The sequences (383 bp) have been manipulated by three analytical method, neighbor-joining producing. The tree topology divided, with strong statistical supports, Chrysoperla into two clusters. Jeddah samples clustered (BP = 62%) with Chrysoperla carnea Stephens while Tabouk samples clustered (BP = 64%) with Chrysoperla nipponensis Okamoto. Both pairwise genetic distances obtained from the concatenated data and amino acid substitution in COI gene supported this division.***

Keywords: *Chrysoperla*, Saudi Arabia, Cytochrome Oxidase I, Diversity

1. Introduction

The green lacewings (Chrysopidae) belong to the Order Neuroptera and are described as voracious predators in the larval stage and sometimes also in their adulthood. They are an important group used in integrated biological control in field and horticultural crops [1].

The family Chrysopidae contains over 1,200 species and subspecies divided into at least 80 genera and subgenera distributed around the world on all continents, except Antarctica and the genus *Chrysoperla* contains 36 species [2, 3, 4]. *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) is a widespread predator in agroecosystems, with great potential in integrated pest management [5, 6]. *C. carnea* adults eat pollen and honeydew and are not predatory but the larvae have been recorded as feeding on seventy different prey species in five insect orders. The preys are mostly from the order Homoptera and are predominantly aphids on low growing vegetation [7]. On crops, the larvae have been reported as attacking several species of aphids, red spider mites, thrips, whitefly, the eggs of leafhoppers, leafminers, psyllids, small moths and caterpillars, beetle larvae and the tobacco budworm. They are considered to be important predators of the long-tailed mealybug under glass. *C. carnea* occurs naturally in many growing regions of the northern hemisphere. It is an important group used in integrated biological control in field and horticultural crops[8, 9].

Molecular data – nuclear or mitochondrial genes – have been used to study phylogeny of both Chrysopidae family and *Chrysoperla* species [10, 11, 12, 13]. It has been suggested that *C. carnea* contains several cryptic species [12, 13, 14, 15, 16], comprising the so-called *carnea* group. The species morphological similarity makes it difficult to differentiate them. Studies on the courtship behavior of the cryptic species in the *carnea* group have demonstrated that the unique courtship song of each cryptic species ensures reproductive isolation [17, 18, 19, 20]. The Japanese green lacewing has until recently been recognized as conspecific to the Eurasian *C. carnea* [21]. However, it revised to *C. nipponensis* (Okamoto) (Neuroptera: Chrysopidae) based on external morphological differences such as the color of the gradate cross veins of the wings, which are black in *C. nipponensis* and green in *C. carnea* [22].

At the DNA level, types of genetic variation include base substitutions (single nucleotide polymorphisms (SNPs)), insertions or deletions of nucleotide sequences within a locus, inversion of a segment of DNA within a locus and rearrangement of DNA segments around a locus of interest [23]. The mtDNA region of the large subunit ribosomal RNA (16S rRNA) and the cytochrome *b* oxidase (cytb) genes are the fastest variable mitochondrial genes[24].

This study therefore aimed to investigate the molecular diversity of the green lacewings from different areas in the east of Red Sea using the mitochondrial cytochrome oxidase subunit I (COI) gene and find out the molecular relationship among the distant populations and the interaspecific genetic relationships.

2. Material and methods

2.1 Sampling and locations

The collected individuals were identified according to the morphological characteristics [22]. The selected specimens used in this study were collected in two places during March 2014. All the specimens were sampled in an area containing cultivated vegetation. The two collection places were (Tabuk (28º 17’ 20” N and 36º 44’ 57” N) and Jeddah (21º 36’ 15” N and 39º 22’ 30” N). All specimens were stored in absolute alcohol for molecular analysis.

2.2 DNA extraction, polymerase chain reaction (PCR) and sequencing

Total DNA extraction was done from each individual preserved in ethanol [25]. Extracted DNA concentration and quality were determined spectrophotmetrically at 260/280 nm and was used for Polymerase Chain Reaction (PCR). PCR was conducted in a final volume of 50 µL containing 2 µL DNA template and 2 µL of 10 picomolar forward primer (5- CACCTTGCCGGTATTTCATC-3), 2 µL of 10 picomolar reverse primer (5-TAATAGCAAATACTGCCCC-3), and 25 µL PCR master mix (Promega Corporation, Madison, WI) and 19 µL autoclaved deionized distilled water. PCR was carried out using a PeX 0.5 thermal Cycler with the cycle sequence at 94°C for 5 min one cycle, followed by 35 cycles each of which consisted of denaturation at 94°C for one min, annealing at 52°C for one min and extension at 72°C for one min with a final strand elongation for one cycle at 72°C was done for an additional 5 min. The PCR products were analyzed in 1% agarose gel electrophoresis in TAE buffer (40 mm Tris, 40 mM acetic acid and 1 mM ethylenediamine-tetra acetic acid) with ethidium bromide staining. 100bp DNA ladder (Biolabs) was used as a molecular marker. Then PCR products bands were visualized under UV light and photographed. The PCR products were then excised from agarose gels and purified using spin column (BioFlux, Tokyo, Japan) according to the manufacturer's instructions and sequenced in an ABI PRISM 3730×l sequencer (Applied BioSystems) and BigDyeTM Terminator Sequencing Kits with AmpliTaq-DNA polymerase (FS enzyme) (Applied Biosystems) following the protocols supplied by the manufacturer.

2.3 Phylogenetic Analyses

After reading the targeted gene, the nucleotide sequences have been treated with different software programs (DNASIS, MacClade, PAUP) that enabled us to detect genetic relatedness between different samples. The sequenced fragment of CO1 gene for Chrysoperla samples from Jeddah and Tabouk was aligned separately and manually with its counterpart from *C. nipponensis* (AB282928) and *C. carnea* (AB282923) using MacClade v.4. The same fragment for *Dichochrysa* sp. tD2 (KF936664) was used for rooting the tree. The tree analyses were done by Maximum-Parsimony (MP) and Neighbor- Joining (NJ) methods with PAUP* 4.0b10 [26]by heuristic searches with the TBR branch swapping and 10 random taxon additions, respectively. The bootstrapping replicates were set to be 100 with simple additions for the Neighbor-Joining method.

3. Results and Discussion

The sequenced fragment of CO1 gene for *Chrysoperla* samples from Jeddah and Tabouk was aligned separately and manually with its counterpart from *C. nipponensis* and *C. carnea* using MacClade v.4. The gap-containing sites were deleted so that 383 bp were left for analyses.

The sequenced Co1 gene fragment (383 bp) for the different *Chrysoperla* samples was deposited in NCBI GenBank database with its accession numbers (KP844575 to KP844579). The aligned sequences showed base frequencies of A = 29.7%, C = 11.2%, G = 17.5% and T = 41.6%.

The phylogenetic tree (Figure 1) showed general clustering of the individuals of each locality with each other, which showed a basal relationship with *C. carnea* and *C. nipponensis*. Jeddah and Tabouk populations grouped within the cluster of *C. carnea* and *C. nipponensis* with strong bootstrapping . On the other hand, thirty six sequences with 805 pairs of bases for COI were analysed and the genetic parameters revealed 24 haplotypes for this population, a total of 36 mutations and haplotype diversity of 0.956. the species *Chrysoperla externa* from Jaboticabal, SP, is a single population, without genetic structure neither due to the area of origin nor to the seasons of the year [1].

In the present study, the pairwise genetic distances among the studied populations are shown in Table (1). The genetic distance showed the smallest values between Jeddah and Tabouk populations (D=0.003) and between each of them and either *C. carnea* or *C. nipponensis* (D= 0.01). Six of mutations were in the different position with four amino acids changes only (Table 2). On the other hand, *C. carnea* is a well-supported, separate taxon, but that other taxa of the complex are not consistently differentiated by DNA data [27]. Moreover, a survey of 1,033-bp fragment of the cytochrome oxidase subunit I (COI) sequence to look for a useful diagnostic marker for discrimination between the Japanese cryptic species *C. nipponensis* (types A and B) and the commercially introduced *Chrysoperla carnea* s. str. from Germany [28]. Phylogenetic analysis showed four distinct haplotype groups: one group corresponded to *C. nipponensis* type B and one group to *C. carnea* s. str., and the remaining two distinct groups, A1 and A2, corresponded to the same song phenotype, *C. nipponensis* type A. A2 was linked with the group of *C. carnea* s. str. and A1 was linked with the group of *C. nipponensis* type B. Cross-testing between A1 and A2 revealed their reproductive compatibility.

In conclusion, The phylogenetic analyses of the collected green lacewings individuals showed that the species in Tabouk is *C. nipponensis* while the species in Jeddah is *C. carnea* .The importance of the genetic diversity is derived from the well known fact that the adaptability of any given population is dependent on its phenotypic and ecological plasticity. In this case, green lacewing, is directly dependent on the population chosen to be released in the field.

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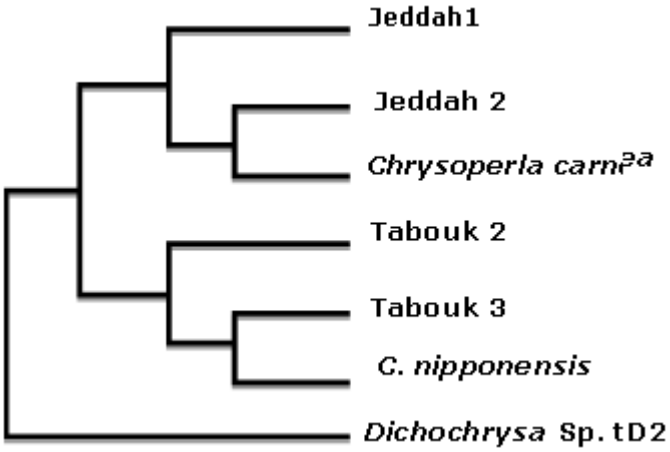

Figure 1: Neighbor-joining tree constructed based on 383 bp sequenced from CO1 gene for the diiferent samples of *Chrysoperla*. Bootstrap values were shown on branches when they were over 50%.

Table 1: Pairwise genetic distances among the different *Chrysoperla* populations as calculated from the sequenced CO1 gene fragment

	Jeddah1	Jeddah2	Tabouk1	Tabouk2	*C. nipponensis*	*C. carnea*
Jeddah1		0.000	0.003	0.008	0.008	0.008
Jeddah2			0.003	0.008	0.008	0.003
Tabouk1				0.006	0.006	0.006
C. nipponensis						0.01

Table 2: Single Nucleotide Polymorphisms of COI fragment sequences in two population of *Chrysoperla* sp.

Base No.	origin	Modified to	Specimen No.	Position in Amino acid
550	T	C	2	1
906	G	A	1, 2, 3	3
940	G	A	6	1
960	A	G	5	3
971	G	A	5	2