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# Identification of different *Chrysoperla* (Neuroptera: Chrysopidae) species collected from Ardabil province (Iran) by using claw shape

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**ABSTRACT** The family Chrysopidae has several genera of which the genus *Chrysoperla* is more important of them. These lacewings are beneficial insects when they have high potential for predation on harmful aphids which can be used in their biological control programs. Due to high importance of these lacewings in Ardabil province in northwest zone of Iran, identification of their species was done. By examining the collected specimens with morphological features, wing venation, male reproductive organ, and claws shape (as the best way to identify the species) finally 4 species were identified including *Chrysoperla carnea* (Stephens), *Chrysoperla kolthoffi* Navas, *Chrysoperla lucasina* Lacroix, and *Chrysoperla sillemi* Esben-Petresen.

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## Introduction

The fauna of Chrysopidae from Iran was studied by Hölzel (1966, 1967, 1982). He was the first European entomologist that study Iranian Chrysopidae fauna in detail; but, he never visited Iran; the specimens he studied were collected by A. Vartian and E. Vartian, an Austrian-Armenian couple as amateur insect collectors visiting Iran as tourists. The total number of Chrysopidae from Iran reported by them and the others were 45 species (Mirmoayedi 2002). From 1991 to 2000, Mirmoayedi studied the Neuroptera fauna of Iran, including all families of this order. He studied the Chrysopidae fauna from different regions of Kermanshah province and determined six species of them including *C. carnea* (Stephens), *C. septempunctata* Linnaeus, *C. viridana* Schneider, *C. dubitans* McLachlan, *Suarius nanus* (McLachlan), and *S. fedtschenkoi* (McLachlan) (Mirmoayedi 1993). Later, he reported and identified three more previously unrecorded species from this province, namely *Mallada derbendica* Hölzel, *Suarius paghmana* Hölzel, and *S. vartianae* (Hölzel) (Mirmoayedi 1995). Mirmoayedi and Yassayie (1998) studied the fauna of Neuroptera in Golestan national park in northeastern zone of Iran and reported seven species of Chrysopidae from that region, namely *Anisochrysa amseli* Brauer, *A. flavifrons* (Brauer), *A. prasina* Burmeister, *C. dubitans*, *C. viridana*, *S. nanus*,

and *C. carnea*. Mirmoayedi (1998) published a checklist of Neuroptera in Iran that comprised 39 species which were collected and identified between 1991 to 1996 from different regions, such as Tehran, Guilan, Hormozgan, Markazi, Kermanshah, Khuzestan, and Ilam provinces. These eleven species of Chrysopidae were *C. dubitans*, *C. septempunctata*, *C. viridana*, *C. iranica* (Hölzel), *C. carnea*, *Mallada derbendica*, *M. prasina* (Hölzel), *S. fedtschenkoi* (McLachlan), *S. nanus*, *S. paghmana*, and *S. vartianae* (Mirmoayedi, 1998). One year later, he published a list of seven species of Chrysopidae from Kermanshah and Kurdistan provinces; in this list, *Italochrysa vartianorum* Hölzel was recorded for the first time (Mirmoayedi 1999a). In the same year, Mirmoayedi studied the Neuroptera fauna in Shiraz (Fars province) and identified seven species (Mirmoayedi 1999b).

Hölzel (1966) published records of eleven species from Chrysopidae for the fauna of Iran. One year later, he published a list of Chrysopidae that described the morphological characters of each species, gave identification keys, and recorded data on collecting dates and localities. This list comprised 23 species. Some years later, he described two new Chrysopidae species, *A. mira* (Hölzel) and *S. ressl* (Hölzel). In 1982, Hölzel described a new chrysopid, *S. laristanus* Hölzel, which was collected by Ressler (1970) in southern zone of Iran. A brief literature review regarding the Chrysopidae fauna of Iran (from

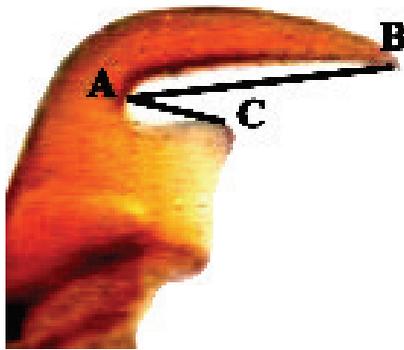


Figure 1. *Chrysoperla* claw and how to measure its different parts.

1961-2000) was carried out by Mirmoayedi (2002). Mirmoayedi et al. (2014) studied new species of Neuroptera from West Azerbaijan. They concluded that between the collected specimens, there were twenty-four species belonging to six families of the Order Neuroptera, and all species (except *C. carnea*) were recorded as the first time for the fauna of this province. Six species were also new recordings for the fauna of Iran.

Surveys and collections of Iranian Neuroptera fauna have shown that *C. carnea* is a dominant species (Heydari 1995; Sharififard and Mossadegh 2006). However, several reports have indicated that other species such as *C. formosa* Brauer (Daniali et al. 1995; Ghadiri-Rad et al. 2004) and *C. lucasina* (Farahi et al. 2009; Kazemi and Mehrnejad 2011) were more abundant than *C. carnea* in some regions. It is clear that *C. carnea* contains several cryptic species comprising the so-called "carnea group" (Henry 1985; Haruyama et al. 2008). This group comprises a complex of about 20 cryptic species distributed throughout the northern hemisphere and Afrotropics (Henry et al. 2002). The morphological similarities between species make it difficult to differentiate among them. Studies on the courtship behaviors of the cryptic species in the "carnea group" have demonstrated that the unique courtship song of each cryptic species ensures reproductive isolation (Wells and Henry 1992; Henry et al. 2002). Therefore, further studies are required to identify the real species of "carnea complex" in Iran. In this research, we identified the species of this genus based on the claw shape that is a new and easier method than courtship studies which needs more time and cost.

## Materials and Methods

### Collection of samples

Adult insects were collected from farms, orchards, and mountainous areas by hand trap, light trap, and Malaysian



Figure 2. Slide of claws from *Chrysoperla*.

traps. Then, the samples were transferred to the laboratory and males and females were carefully separated based on the abdomen shape. Then, the isolated forewings and hindwings were also carefully drawn by binocular. The end of abdomen from male insects was removed by scalpel and placed inside 10% KOH during 6 h for clarifying. After these stages, reproductive organ was drawn. In the genus *Chrysoperla*, male genitalia were quite similar for all species; therefore, it is not used for species identification; therefore, it was necessary to prepare slides from the claws of left hindlegs when by measuring the scale of the claw (Fig. 1), species was identified (Asadi 2010).

### Preparing of claws slide

Firstly, a total of 36 lacewings (18 males and 18 females) were randomly selected. Then, the claws of left foreleg were carefully separated and put inside ethanol 70% for 2-3 min, then in pure ethanol (100%) for 3-4 min, and

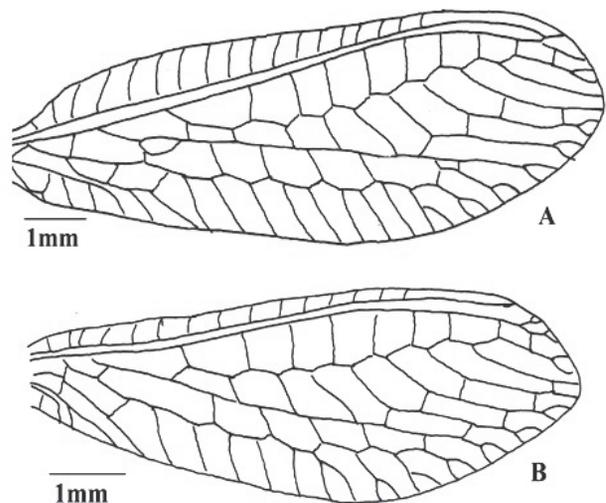


Figure 3. *Chrysoperla* spp., (A): forewing and (B): hindwing.



Figure 4. Male genitalia of *Chrysoperla* spp.

finally in glycerin for 4-5 min. During these processes, the claws were dehydrated well. Then, on a slide, three rows of 6 pieces from glue drops were placed and a claw was placed inside each of them. On the other side of slide, name of locality, relevant area, and slide number were written (Fig. 2). Finally, the slides were kept in oven at 5-50 °C for 4-5 days to dry completely (Asadi 2010).

#### Identification key

Each claw was photographed under a stereomicroscope and measured using AB/AC length. The obtained ratio was compared and species of the genus *Chrysoperla* were identified based on the identification key which is given as follows (Asadi 2010):

AB/AC= 1.93 ± 0.4	<i>Chrysoperla carnea</i>
AB/AC= 2.32 ± 0.2	<i>Chrysoperla kolthoffi</i>
AB/AC= 2.65 ± 0.3	<i>Chrysoperla lucasina</i>
AB/AC= 4.17 ± 0.5	<i>Chrysoperla sillemi</i>

#### Results

Totally, 1166 samples were collected in different locations of Ardabil province. Finally, 4 species of this genus were identified which include as follows:

#### Genus *Chrysoperla* Stienmann, 1964

Head is yellow without any spot; antenna is pale yellow; gena brownish and a dorsal longitudinal yellow line extends from prothorax to the last abdominal segments; coxa, femur, and tibia are green; tarsi claws are yellow-brownish; wings are hyaline with green veins and in forewings first RS cross vein meeting MA, distal to apex of intermedian cell (Fig. 3); apex of abdomen in male of *Chrysoperla* contains ectoproct broad, apex of pseudopenis (arcessus) narrow with or without dorsal striations; tignum present and gonapsis absent (Fig. 4).

#### *Chrysoperla carnea* Stephens, 1836

Main materials examined: Ardabil: 90♂, 35♀; 4.4.2017

Step-like (rectangular) basal enlargement of the claws (Fig. 5A); pleural membrane on second abdominal segment without any brown stripe; stipes unmarked laterally or only marked with brown distal spot located near basis of maxillary palpus; abdominal sternite with hyaline or slightly colored setae, rarely associated with sparse brown setae.

#### *Chrysoperla kolthoffi* Navas, 1927

Main materials examined: Namin: 62♂, 40♀; 11.5.2017

Step-like (rectangular) basal enlargement of the claws (Fig. 5B); pleural membrane on second abdominal segment without any brown stripe; costal setae on forewing long; stipes marked laterally with brown longitudinal stripe; abdominal sternite with brown or black setae.

#### *Chrysoperla lucasina* Lacroix, 1912

Main materials examined: Nir: 90♂, 35♀; 18.6.2017.

Step-like (rectangular) basal enlargement of the claws (Fig. 5C); pleural membrane on second abdominal segment with thin dark brown stripe beneath sternite, this stripe often extending to segments 3-6; costal setae is short; stipes marked laterally with brown stripe, sometimes very pale; pronotum and abdominal sternite usually with numerous regularly distributed black setae.

#### *Chrysoperla sillemi* Esben-Petresen, 1935

Main materials examined: Sarain: 13♂, 12♀; 25.6.2017.

Head unmarked or with pale brown stripe on gena and clypeus; maxillary palpus brown dorsally; antenna shorter than forewings; pronotum with yellow or white median stripe, setae short dark or pale; wing venation is green, apex pointed; costal setae short inclined towards wing apex (Fig. 5D).

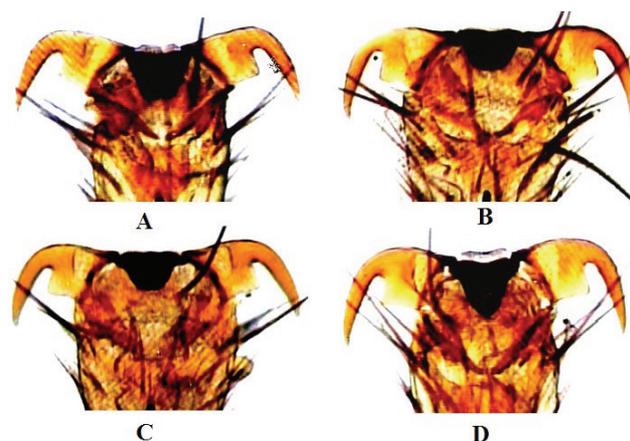


Figure 5: Claw shape of *C. carnea* (A), *C. kolthoffi* (B), *C. lucasina* (C), and *C. sillemi* (D).

## Discussion

There are no sufficient studies have been done on this genus of lacewings and the study based on claw shape is a new work in this field. Henry et al. (2010) investigated important lacewing in the agricultural crops of India, *C. sillemi* as subspecies of *Chrysoperla zastrowi* (Esben-Petersen) while mentioned by comparison of courtship songs when all *C. sillemi* examined conformed acoustically to the subspecies *C. zastrowi arabica* from the Middle Eastern zone. Morphology of adults and larvae confirmed their diagnosis. They also revised carnea group nomenclature from *Chrysoperla* to complete their findings and erect *Chrysoperla zastrowi sillemi* stat. rev. to include populations from India and Middle East regions. Moreover, *C. zastrowi arabica* was a junior synonym from the new subspecies. In another study, Henry et al. (2014) studied a new species of *C. carnea* group from western Asia when mentioned that this new species is similar on morphology and ecology to *C. plorabunda*; but also, to at least four other species in the *carnea*-group that co-occur with it in western Asia. They described *Chrysoperla heidarii* sp. nov. including behavior, ecology, and life history. Moreover, Henry et al. (2018) investigated song phenotypes from two new allopatric species of *C. carnea*-group in Asia including *Chrysoperla shahrudensis* sp. nov. and *Chrysoperla bolti* sp. nov. and mentioned coordinated duets which can be established in the laboratory between individuals of *C. shahrudensis* and recorded songs of either *C. bolti* or *C. adamsi*. Functional songs can determine origin of cryptic species in these lacewings. Morphology, life history, and ecology of larvae and adults from *C. shahrudensis* and *C. bolti* were described. Although, *C. bolti* and *C. shahrudensis* were both from Asia; but, two taxa are distinct and allopatric biological species. Their studies are very valuable; but they are completely different from the research we have done. In conclusion, genus *Chrysoperla* from the family Chrysopidae has a rich fauna with very good species diversity in Ardabil province that this biological potential can be used in various fields for harmful aphid management in agriculture and horticultural crops. Green lacewings have a very high potential as biological control agents on plant pests which can be mass reared and released under biological control programs by accurately identifying the species of this family in different parts of the country and determining their biological characteristics. In the form of field release, used greenhouses or gardens, the purpose of which are to reduce the level of harmful aphids to below the economic injury level (EIL), which is often associated with high success and can control up to 90% of their population.

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