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Occurrence of *Chrysoperla zastrowi arabica* Henry *et al.* (Neuroptera: Chrysopidae), a cryptic song species of *Chrysoperla* (*carnea*-group), in India

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ABSTRACT: **The *Chrysoperla* species (*carnea*-group) widely used in India in augmentative biological control programmes was found to be *Chrysoperla zastrowi arabica* Henry *et al.* The acoustic profile of the mating song of this species was analyzed and found to match that of *C. zastrowi arabica*, the song species characterized earlier as '*Cc5* generator'. The correct taxonomic name for this species remains to be determined as it is morphologically identical to *C. sillemi* (Esben-Petersen), a species already known from India. Molecular characterization of the species was also done and the DNA sequence of the ITS-2 region was deposited in GenBank (Accession No. DQ 825504, as that of *C. sillemi*). The implications of this finding are discussed.**

KEY WORDS: Chrysopidae, *carnea*-group, *Chrysoperla zastrowi arabica*, *C. sillemi*, India, molecular characterization, song analysis

INTRODUCTION

A vast majority of the published literature on lacewings (Neuroptera: Chrysopidae) is on the common green lacewing, *Chrysoperla carnea* (Stephens). Once considered a single Holarctic species, *C. carnea* has been recently shown to be a complex of many cryptic, sibling species, the *carnea* species group, whose members are reproductively isolated by their low-frequency, substrate-borne vibrational songs, identically expressed in both sexes. Presently, some of the species in the complex can be diagnosed principally or only by their song phenotypes and not by morphology (Henry *et al.*, 2002, 2003). The species are often widely distributed (2500-3000 km east-west), broadly sympatric, and syntopic. Songs are nearly uniform over a species' geographical range, but adult and larval anatomy and coloration vary within and between populations (Johnson *et al.*, 2003). The *carnea*-group contains at least 15-20 partly cryptic song species (Duelli, 2001; Johnson *et al.*, 2003).

In most of the published work from the Indian subcontinent, unidentified sibling species of the *carnea*-group from various crops have been widely reported under the name *C. carnea* sensu lato

(Duelli, 2001), though the true *C. carnea* does not occur in India. These species have not been systematically characterized so far using modern techniques, particularly analysis of courtship song profile. Hence, the laboratory population of '*C. carnea*' maintained at the Project Directorate of Biological Control, Bangalore, was acoustically analyzed to establish its correct identity and the DNA of the internal transcribed spacer (ITS-2) of this species was sequenced and the results are presented in this paper.

MATERIALS AND METHODS

The laboratory culture of '*C. carnea*' maintained for over a decade at the Project Directorate of Biological Control (PDBC), Bangalore, was used for the studies. This culture has been supplied to several commercial insectaries and institutions in India as '*C. carnea*' as nucleus culture and also widely used for augmentative releases on several crops throughout India.

1. Analysis of courtship song

Live cocoons of '*C. carnea*' (Bangalore population) from PDBC culture were sent to Dr. Peter Duelli, Swiss Federal Research Institute, Switzerland, and Dr. Charles S. Henry, University of Connecticut, USA, as live specimens of both sexes are required for analyzing the mating signals. The mating song of the Bangalore population was acoustically analyzed as detailed by Henry *et al.* (2002).

2. DNA sequencing

The head, abdomen and other appendages of single individual adults were removed using sterilized scissors and the thorax was used for DNA isolation and sequencing. The thorax was homogenized in 100 µl lysis buffer (200 mM Tris-HCl, pH8.0; 70 mM EDTA; 2M sodium chloride; 20 mM $Na_2S_2O_5$) and to the above 35 µl of sodium lauryl sarcosine (5%) was added, and incubated at 55°C for 2h. The homogenate was spun at 15000rpm for 15min and to the supernatant 10 M ammonium acetate (13.5 µl) and isopropanol (135 µl) were added and incubated at –20° C overnight. DNA was pelleted at 20000 rpm for 20min at 4 °C and washed with ice-cold 70% ethanol and resuspended in TE buffer (Tris 10mM, EDTA 1mM, pH 8.0).

Polymerase chain reaction (PCR) was performed in 50µl reaction volume containing 1unit of Taq, 0.2 mM deoxynucleoside triphosphates, 1.5 mM $MgCl_2$, 1X PCR buffer, 10 picomoles of forward and reverse primers, and 5-50 ng of DNA template. The primers used to amplify the ITS2 region were 5'-TGTGAACTGCAGGACACATG- 3' (forward) and 5'-GTCTTGCCTGCTCTGAG-3' (reverse). The following were the cycling parameters used in this study: initial denaturation at 95°C for 5min followed by 30 cycles of denaturation at 95°C for 1min., annealing at 55°C for 1min., extension at 72°C for 2 min and a final extension at 72°C for 10 min. The PCR amplified product was resolved in 1.2% agarose gel and stained with ethidium bromide (10µg/ml). The stained PCR band was eluted using gel extraction kit and sequenced using ITS2 specific primers both in forward and reverse directions in an ABI PRISM 377 automated DNA sequencer.

RESULTS AND DISCUSSION

Mating song analysis

Acoustical analysis of the mating song of the Bangalore population of '*C. carnea*' (maintained at PDBC) confirmed that it belonged to *Chrysoperla zastrowi arabica* Henry *et al.* (2006). This is the song species earlier named as *Cc5*, the song of which was characterized as 'generator' (Henry *et al.*, 1996, 2001). The song species *Cc5*-generator, from the Arabian peninsula, is now considered a subspecies of *Chrysoperla zastrowi* (Esben-Petersen, 1928) from southern Africa and described as *C. zastrowi arabica* by Henry *et al.* (2006). This species is widespread in the near and middle east, apparently associated with a warm, dry climate. Its presence in India suggests a range extension for *C. z. arabica*, east from Oman deep into the Indian subcontinent, where the climate is quite different (Henry, 2008). The correct taxonomic name of this species needs to be clarified as *C. zastrowi arabica* is almost certainly the same species as *C. sillemi* (Esben-Petersen, 1935), which is morphologically indistinguishable from the former, but the name *C.*

zastrowi has priority as it was published earlier (Charles Henry, *in litt.*; Henry, 2008). Henry *et al.* (2006) provided a complete morphological description of *C. zastrowi arabica*. *Chrysoperla sillemi* itself has been listed as present in India (Karakorum) by Ghosh and Sen (1977) in their checklist of Indian Planipennia (spelt as *C. silleme*). Three other species described from India, namely, *Chrysoperla gujaratensis* (Ghosh, 1976a), *C. sanandensis* (Ghosh, 1977) (from Gujarat), and *C. punensis* (Ghosh, 1976b) (from Maharashtra), are presently considered to be synonymous with *C. sillemi* (Oswald, 2007). *C. sillemi* was originally described from China and is also distributed in West Asia (Oman, Israel and Iran). *C. zastrowi arabica* is likely to have a wide distribution in India as the culture from PDBC has been supplied to institutions allover India for research and augmentative releases in various crops. It is essential to extensively collect the field / wild populations of *carnea*-group from different crops and agroclimatic / geographic zones of the Indian region to characterize and quantify their diversity.

Duelli (2001) earlier conjectured that '*C. carnea*' reported in works from India could be *Cc5* or *C. sillemi*. Now this study confirms this. Unfortunately, it is usually difficult or impossible to assign specimens to such acoustically diagnosed species, because the taxonomist needs access to living specimens and special equipment and training at recognizing song phenotypes (Henry *et al.*, 2001). There is a need for further characterization of the species through behavioural, morphological and ecological studies.

DNA profile

Agarose gel electrophoresis showed an expected product size of approximately 550 bp (Fig. 1). Direct sequencing of the PCR amplified product resulted in 538 bp fragment and the sequence (Table 1) was submitted to GenBank, National Center for Biotechnology Information (NCBI) (Accession No. DQ 825504, as *C. sillemi*). At the species and intra-specific levels, the internal transcribed spacers (ITS1 and ITS2) are often used as taxonomic tools in many groups such as fungi (Carbone *et al.*, 1993), plants (Hsiao *et al.*, 1994) and animals (Bowles *et al.*, 1993) including insects (Campbell *et al.*, 1993; Kuprus *et al.*, 1994). Considering the vastness of India and its varied agroclimatic conditions, it is possible that more than one or many song species of *carnea*-group besides *C. zastrowi arabica* / *C. sillemi* may exist here.

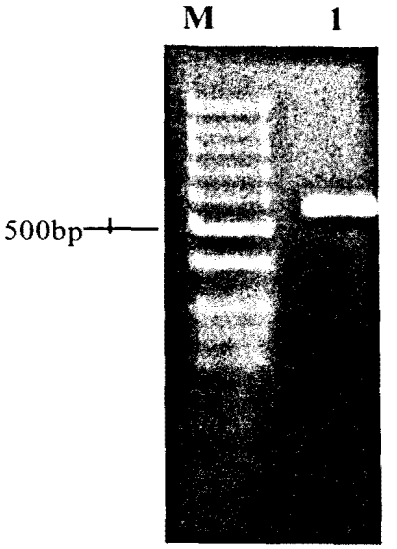

Fig 1. PCR amplification of ITS-2 region of *C. sillemi* (Lane M- 50bp ladder; 1- *C. sillemi*)

The Indian species of *C. carnea*-group need to be characterized by a combination of techniques including bioacoustics, behavior, morphology, biogeography, ecology, ecophysiology, and molecular tools. The practice of depositing voucher specimens of chrysopid research in designated repositories should also be henceforth made mandatory and strictly enforced for Indian researchers before they publish their works to enable future reference and confirmation because the identities of chrysopids reported in most of the published works from India are totally unreliable. These initiatives would help in placing the systematics of Indian chrysopids, particularly the *carnea*-group, on a sound footing and facilitate the work of entomologists, commercial insectaries and practitioners of biological control using green lacewings in selecting the right and effective species for biological control programmes.

Table 1. ITS-2 sequence of *C. sillemi* – 538bp

tgtgaactgc aggacacatg aacatcgaca tttcgaacgc acattgcggt ccacggatct cgttcccgga ccacgcctgg ctgagggtcg tttataaaaa cgaacccgac tgctctctcg caagagagag cgttgatctg ggcgctcgtc tctatctcct acggcgcgct ctttcgagag tgtcgcaggc agtgtgatac gtcgcctcaa acgaaacgca agaaaattga tgaattcgtt cgtctagctg gcgagcgcgc ttaccgcttg gagagtacgc gagtacttcc gatcgttctg cgtcgagtcc cggagctttc tcgcacacga ctactcgtcg cgtcgagcac agcggaccga cgtctagcac acgatcaggc tcgtccatgc atcggtcatt gaatgcgcgc gtgccttgta gttgttgttg ttgttgttgt tgtgtatcaa caacagcagc agcagcagca gaaaaatggc tcgctcgaag catgaacgag tctcttttct cgatcgacga cctcagagca ggcaagac

Gen. Acc. No. DQ825504 (deposited as *C. sillemi*)

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