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Research Article

Neochrysocharis nr. *diastatae* (Howard) (Hymenoptera: Eulophidae) parasitic on eggs of *Letana* Walker (Orthoptera: Tettigoniidae) in India: first record of host association

ANKITA GUPTA[*], H. M. YESHWANTH[1], CHRISTER HANSSON[2] and T. VENKATESAN

ICAR-National Bureau of Agricultural Insect Resources, Post Bag No. 2491, H. A. Farm Post, Bellary Road, Hebbal, Bengaluru 560 024, Karnataka, India.

[1]Department of Entomology, University of Agricultural Sciences, GKVK, Bengaluru 560 065, India.

[2]Scientific Associate, the Natural History Museum (Entomology), Cromwell Road, London SW7 5BD, United Kingdom.

[*]Corresponding author E-mail: drankitagupta7@gmail.com

ABSTRACT: Species of *Neochrysocharis* Kurdjumov (Hymenoptera: Eulophidae) are potential biocontrol agents for many important agricultural pests. This paper reports, for the first time, gregarious parasitism of *Neochrysocharis* nr. *diastatae* (Howard) on eggs of *Letana* sp. (Orthoptera: Tettigoniidae) on the host plant *Pterospermum reticulatum* Wt. & Arn. (Malvaceae) in Karnataka, India. The eggs of *Letana* sp. were observed along the margin of the leaves of *P. reticulatum*, visually distinguished as oval shaped structures. This is the first record of a host-parasitoid association between a species of *Neochrysocharis* and eggs of a Tettigoniidae grasshopper. DNA analysis of the parasitoid confirms a 100% match with a record of an unidentified species of *Neochrysocharis* from Canada. The geographical distribution and host record indicates that there may be a "*N. diastatae*" complex that this species actually belongs to, rather than being *N. diastatae* itself as *N. diastatae* is a Nearctic species and mainly known from agromyzids.

KEY WORDS: Grasshopper, gregarious egg parasitoid, *Pterospermum reticulatum*, Oriental region

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INTRODUCTION

The genus *Neochrysocharis* Kurdjumov (Hymenoptera: Eulophidae: Entedoninae), with revised status (Burks *et al*., 2011), comprises more than 50 species from different regions of the World (Noyes, 2015). The species are mainly endoparasitic in immature stages of various phytophagous insects (Hansson, 1990). They are reported as potential biocontrol agents for many important agricultural pests *viz*. *Liriomyza trifolli* (Burgess) (Diptera: Agromyzidae) (LaSalle & Parrella, 1991), *Dicladispa armigera* (Olivier) (Coleoptera: Chrysomelidae) (Polaszek *et al.*, 2002), *Ferrisia virgata* (Cockerell) (Hemiptera: Pseudococcidae) (Gaona *et al.*, 2006), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) (Legaspi *et al*., 1999) and many others, having an extensive host range from leaf/stem miners, eggs of sawflies, to hyperparasitoids of ichneumonids and braconids on Lepidoptera. In this paper we record, for the first time, egg parasitism of a grasshopper (*Letana* sp.) by a species of *Neochrysocharis* (*N.* nr. *diastatae* (Howard)).

COLLECTION DETAILS AND METHODOLOGY

The grasshopper eggs were found along the margin of leaves of *Pterospermum reticulatum* Wt. & Arn. (Fig. 1A) in December, 2014 in GKVK, Bangalore, India, 12°58'N, 77°35'E, located at 937 m altitude with a typical tropical climate. The leaves with grasshopper eggs were collected and kept in the laboratory in 25± 2°C temperature in a meshed cage. The emergence of *Neochrysocharis* wasps was observed on December 29, 2014. Photos of eggs and grasshoppers were obtained in macro mode with a digital camera (Canon 7D, 100mm macro lens). Photographs of the wasps were taken using Leica M 205 A stereozoom microscope with Leica DC 420 inbuilt camera. Some specimens were slide mounted in order to emphasize the microsculpture. These specimens were first cleared in 10% KOH with overnight immersion, later washed in distilled water and were subsequently exposed to normal dehydration process keeping 15 minutes each in 50%, 70%, 90%, and 100% alcohol. Later, specimens were transferred to 100%

alcohol+ Terpineol in the ratio 50:50 for 15 minutes and finally mounted in natural Canada balsam. The slide images were captured using a compound microscope, Leica DM 1000.

Host- parasitoid identification, percent parasitism and DNA analysis

The grasshopper was identified as *Letana* sp. (Orthoptera: Tettigoniidae) (Fig. 1E) based on morphological characters given in Ingrisch (1990). The genus *Letana* is clearly separable from other genera of Oriental Phaneropterinae by the highly modified subgenital plate in the male, which is bilobate and arcuate; adults green in colour; hind wings surpassing the tegmina for less than twice the length of the pronotum, and ovipositor with a very acute apex.

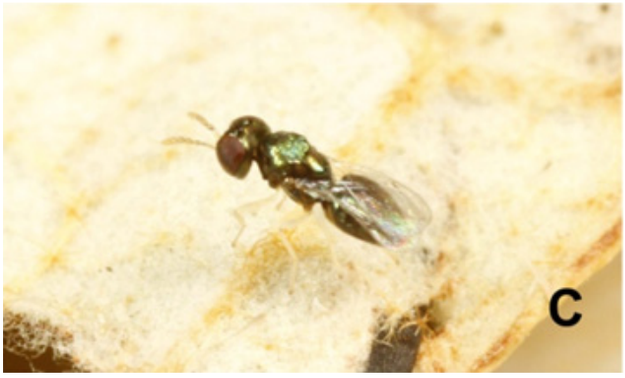

Fig. 1. A. Eggs of *Letana* sp.; B. Eggs with emergence hole of wasp; C. *Neochrysocharis* nr. *diastatae* (Howard) adult female; D. Nymph of *Letana* sp.; E. Adult of *Letana* sp.

The egg parasitoid was identified as *Neochrysocharis* nr. *diastatae* (Howard) (Fig. 2A) based on morphological characters given in Hansson (1995). Female. Coxae dark and metallic (concolourous with body except extreme apices) and femora pale. Costal cell of forewing narrow (Fig. 2B); antenna with long setae, apical three segments fused, clava longer than combined length of funicular segments and slightly wider than funicular segments, first funicular segment not distinctly wider than pedicel; two anelli (distal anellus large) (Fig. 2C). Interscrobal ridge reaching horizontal line, occipital margin rounded. Mesosoma convex, with fine reticulation, notauli not clearly delimited, midlobe of mesoscutum with two pairs of setae (Fig. 2E), one pair on midlobe of mesoscutum and one pair laterally to these on the sidelobe; scutellum reticulate with sides and apex smooth and shiny (Fig. 2F). Fore wing 1.7× as long as wide. Transepimeral sulcus weakly curved. Marginal vein long; postmarginal vein 0.79× as long as the stigmal vein. Propodeum short and transverse, smooth and shiny; petiole small. Gaster 1.05× as long as mesosoma.

COMMENTS

In *N. diastatae* reported by Hansson (1995), the postmarginal vein is slightly shorter than the stigmal vein (ratio of postmarginal vein to stigmal vein = 0.80). In this Indian species, the postmarginal vein is 0.79× as long as the stigmal vein. The distributional record for the Indian species is odd (for *N. diastatae*, a Nearctic species), and also the host might be considered odd, as *N. diastatae* are mainly known from agromyzids. There are other host records for *N. diastatae* as well but these might also be considered odd and possibly indicate that this is actually a complex of species.

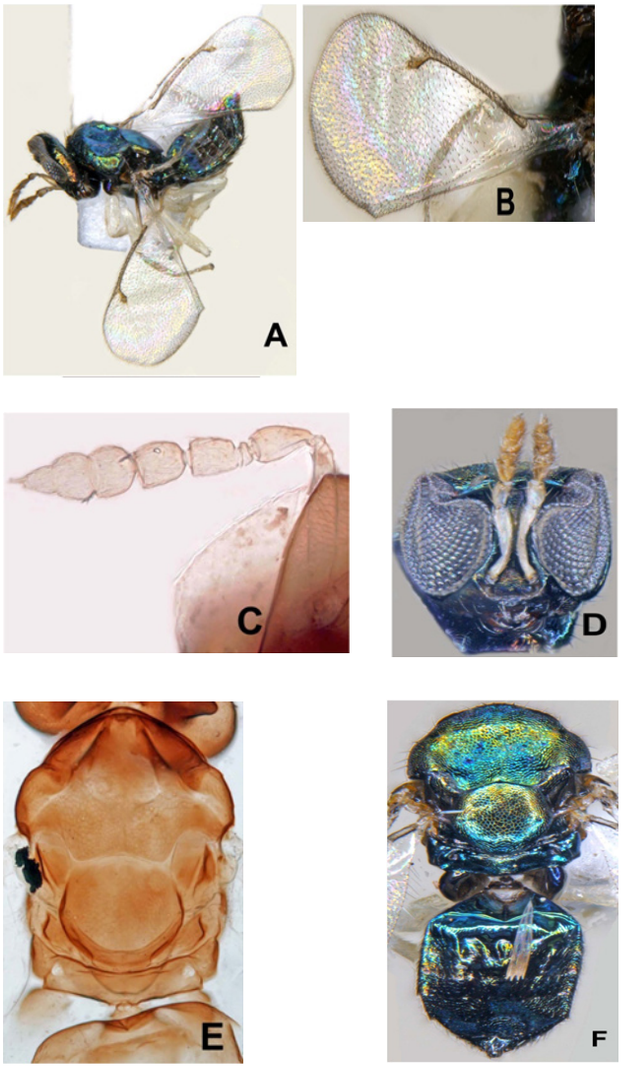

Fig. 2. (A-F) *Neochrysocharis* nr. *diastatae* (Howard) female. A) Profile view; B) Fore wing; C) Female antenna (KOH treated); D) Head in frontal view; E. Mesosoma (KOH treated) in dorsal view; F. Mesosoma and Metasoma in dorsal view.

From the total of 21 *Letana* eggs found, nine eggs were parasitized, i.e. with 42.86% parasitism. Altogether 65 wasps (both females and males) were collected from the parasitized eggs. In addition to the interesting host association of *N.* nr. *diastatae* parasitizing an Orthopteran egg, this parasitoid, which was earlier recorded from other parts of the World (Noyes, 2015), mainly from North America (Hansson, 1995), is documented for the first time from India.

DNA extraction and sequencing

DNA was extracted using Qiagen DNeasy® kit, following the manufacturer's protocols. Amplification, sequencing and sequence analysis closely followed standard methods. PCR amplification of the CO1 gene was done by using the Universal primer. PCR was performed with a total reaction mixture of 50 µl consisting of 10x Taq Buffer (Complete Buffer with $MgCl_2$), 10 mM dNTP mix (Genei), Universal primer HCO1–2198 (20pmol/µl), LCO1–1490(20 pmol/µl) (Folmer *et al*., 1994), template DNA (50ng/µl), Taq DNA polymerase (Genei 1U/µl) and sterile water. The DNA extracted was amplified under the following conditions: Initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation (94°C for 1 min), annealing (45°C for 1 min), extension (72°C for 1 min) and a final extension step at 72°C for 10 min. To detect any possible contamination a negative control was kept in the PCR amplification. The PCR amplification was performed in a thermal cycler (C1000™ Thermal Cycler). The amplified genes by PCR were visualized on 1.8% gel with a low range ladder (Fermentas Mass Ruler 1000bp). The PCR products from which the partial sequences of CO1 region were obtained were sequenced. The species was bi-directionally sequenced and checked for homology, insertions and deletions, stop codons, and frame shifts by using NCBI BLAST. The sequence was uploaded to GenBank and the Barcode of Life Database (BOLD, http://www.boldsystems.org). The following sequence with Accession number KR080478.1 was obtained:

attttatattttatttttggtttatgattnggggtgataggttta
tctttaagaataattattcggatagaattaagaagattaggatca
ttaattggaaatgatcaaatttataattctattgttactgctcat
gcatttattataatttttttttttgtaataccagttataatggga
gggtttggaaattatttaattccaataatttttaggatgtccagat
atagcatttcctcgaataaataatataagattttgattattacct
ccaagattaatattattaatatcaagaatatttattggtcaagga
actggtactggttgaactgtttatccaccattatcattaaattta
tcacatagaagattttcagttgatttatcaatttttttcactacat
attgctggtttatcttcaattataggttcaattaattttatttca
actattttaaatataaaaatttataaaatagaaattattccactt
ttagcttgggctatattattaactgcaattttattattattatca
ttacctgtattagctggggctattacaatattattatttgatcgt
aatataaatacatctttttttgatccagctggt

Surprisingly on comparison with the NCBI database, this species showed 100% query cover with a record (KJ165214.1) submitted from Canada, with Point Pelee

(Ontario) as collection locality. This clearly shows that this species, which is widespread in the Nearctic region, has a very wide host range and is of potential invasive nature and possibly it belongs to a complex of species.

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