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Research Article

Cotesia ruficrus (Haliday, 1834) (Hymenoptera: Braconidae) emerging as a common natural parasitoid of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in Indian maize fields

ANKITA GUPTA[1*], S. RAMESH BABU[2] and M. SAMPATH KUMAR[1]

[1]*ICAR-National Bureau of Agricultural Insect Resources, H. A. Farm Post, Bellary Road, Hebbal, Bengaluru – 560024, Karnataka, India*

[2]*Agricultural Research Station, MPUAT, Borwat Farm, Banswara – 327001, Rajasthan, India*

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

ABSTRACT: Field surveys conducted during 2018-2019 in the maize fields infested with *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in Karnataka, Tamil Nadu, Rajasthan and Meghalaya revealed that *Cotesia ruficrus* (Haliday) (Hymenoptera: Braconidae) was the common gregarious larval parasitoid in the maize fields parasitizing *S. frugiperda*. This is the first report of *C. ruficrus* parasitizing *S. frugiperda* in India, earlier reports being from Trinidad and Tobago. The present study (using integrated approach) provides morphological and molecular identification details along with host data, cocoon characters and geographical distribution of *C. ruficrus*.

KEYWORDS: Fall army worm, larval parasitoid

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INTRODUCTION

Cotesia ruficrus (Haliday, 1834) (Hymenoptera: Braconidae) also known from various synonyms – *Microgaster ruficrus* Haliday, 1834, *Apanteles antipoda* (Ashmead, 1900), *Apanteles anila* (Ashmead, 1904), *Apanteles sydneyensis* (Cameron, 1911), and *Apanteles narangae* (Viereck, 1913) has a wide range of geographical distribution namely Afrotropical, Australasian, Neotropical, Oriental, and Palaearctic (Yu *et al*., 2016). Wilkinson (1928) mentioned many hosts of this parasitoid – *Agrotis* sp. as the host of *A. antipoda*; *Naranga diffusa* Walker as the host of *A. narangae*; some other hosts like *Hypsipyla robusta* Moore and *Perigea capensis* Guen. Wilkinson (1928) examined a series of *A. antipoda* wasps bred from the larvae of *Spodoptera mauritia* Boisd., which is interesting to note in the present context, as *C. ruficrus* was recorded from the same host genus. More specifically, *C. ruficrus* is also recorded parasitizing the pestiferous host *S. frugiperda* from Trinidad and Tobago (CABI, 2019). Gupta & Fernández-Triana (2014) reported *C. ruficrus* from *Spodoptera* sp. in Karnataka and from an indeterminate larva feeding on maize in the Andamans and Nicobar Islands in 2012. The present study not only reports and confirms the identity of *C. ruficrus* (combining morphology, host data and Cytochrome Oxidase I – COI gene) additionally also highlights the affinity of natural parasitism by the native parasitoids towards the notorious invasive pest *S. frugiperda* in India which indeed is a good indication of its active and expanding parasitoid complex. A brief diagnosis of the wasp is also provided to aid researchers in quick identification.

MATERIALS AND METHODS

Larvae of *Spodoptera frugiperda* were collected from the maize fields (multiple locations) in southern India (Karnataka and Tamil Nadu) in 2018-19 and during February to April, 2019 in Banswara (north-western part of India) which is located in the humid southern plain zone of Rajasthan. The field collected larvae were reared in the laboratory at 25+2^{0}C and 60-70% humidity on the maize leaves (provided *ad libitum*). Parasitoids were collected from the infested larvae

of *S. frugiperda* and were preserved in 95% ethanol at -20^{O}C until further use.

Morphological studies were conducted at the ICAR- National Bureau of Agricultural Insect Resources, Bengaluru. The specimens reared from *S. frugiperda* were compared with the types and voucher specimens present in the Natural History Museum, London (BMNH) by the first author. DNA extraction, amplification and sequencing were done at Banswara by the second author for the specimens collected from Rajasthan. A portion of the tissue was dissected, air-dried for few minutes and rinsed with molecular grade water to remove the excess ethanol in the sample. Total genomic DNA was extracted using DNA Sure Tissue mini kit (Nucleo-pore, Genetix Brand, India), following the manufacturer's instructions. The intact genomic DNA was visualized in 1.2% agarose gel (Pure Gene, Genetix Biotech India PVT. Ltd., New Delhi). The concentration of DNA sample was adjusted to 50 ng/µl and stored at -20°C for further use. The PCR reaction was carried out for the amplification of Cytochrome Oxidase subunit I (COI) gene which is of ~700 by using universal primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAAT CA3') (Folmer *et al*., 1994). PCR (C1000Touch™ Thermal cycler of Bio-Rad, U) was performed with initial denaturation for 4 min at 94°C, followed by 35 cycles of 30 sec denaturation at 94°C, 45 sec primer annealing at 47°C, 45 sec initial extension at 72°C and a final extension of 20 min at 72°C (Rama Subramanian *et al*., 2016). The PCR amplification was performed for 50µL containing 25 µL DreamTaq PCR Master Mix (2X) (Thermo Fisher, Scientific, UK), 2µL of template DNA, 10 pmol of each forward and reverse primer and final volume was made by using nuclease free water. The amplified PCR products were separated by electrophoresis in a 1.2% agarose gel containing ethidium bromide (0.5µg/µL) for 60 min at 80 V (BIO-RAD, USA) and visualized in gel documentation system (Gel Doc™ EZ Imager, BIO-RAD, USA). The PCR products were purified by using GeneJET PCR purification Kit (Thermo Fisher, Scientific, UK) and sequenced by using ABI PRISM 3730xl Genetic Analyzer develop by Applied Biosystems, USA (Agile Life science Technologies India Pvt. Ltd, Pune).

RESULTS AND DISCUSSION

Detailed morphological and molecular characterization of the gregarious larval parasitoid *Cotesia ruficrus* was done.

***Cotesia ruficrus* (Haliday, 1834)**

Microgaster ruficrus Haliday, 1834.
Apanteles antipoda (Ashmead, 1900).
Apanteles manilae (Ashmead, 1904).
Apanteles sydneyensis (Cameron, 1911).
Apanteles narangae (Viereck, 1913).

Diagnosis of *Cotesia ruficrus*

(Figs 1 A-C)

Female: Body length 2 mm; general body colour black; legs except hind coxae, tegulae yellowish brown; apices of hind femora and hind tibiae black.

Mesosoma: Mesonotum coarsely and closely punctate posteriorly, scutellum strongly punctate, punctures well separated from each other. Mesopleuron posteriorly and above mostly smooth and shining. Fore wing with first abscissa of radial vein almost equal in length to transverse cubital vein. Hind coxa mostly rugulose. Propodeum coarsely rugose.

Metasoma: Metasoma with first and second tergites rugulose, remaining tergites smooth. Ovipositor sheaths well exserted but not longer than hind tibial spurs.

Host: Larvae of *S. frugiperda* (Fig. 2).

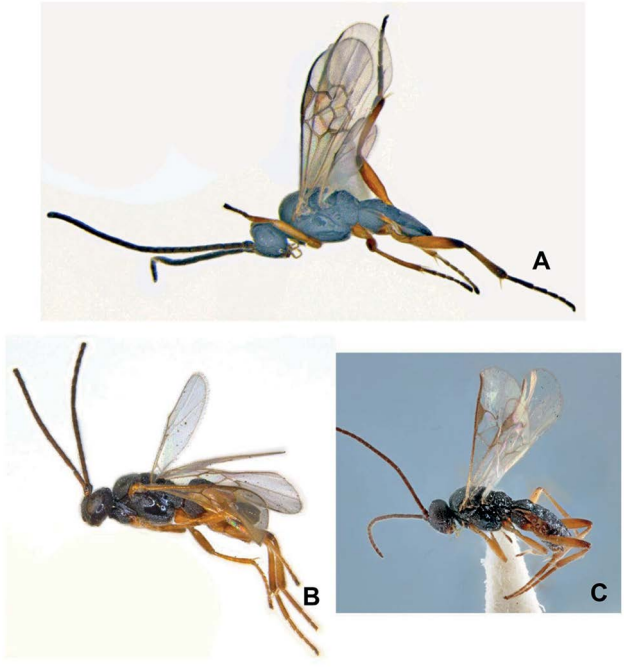

Fig. 1. (A−C) *Cotesia ruficrus*. A- Karnataka specimen ICAR/ NBAIR/Brac/Microg/Cot/9818, female in habitus; B - Paratype no. 4912, USNM, habitus; C- BMNH specimen, NHMUK010635854_ *Cotesia ruficrus*_nontype_ 3c 653, habitus.

Cocoons: Gregarious in nature; all the cocoons observed from India were white in colour and arranged in two rows (ranging from 11−29 cocoons per larva) (Fig. 3) contrary to

Fig. 2. ***Spodoptera frugiperda*** **larva with** ***Cotesia ruficrus*** **cocoons in laboratory (Banswara).**

Fig. 3. ***Cotesia ruficrus*** **cocoons collected from** ***Spodoptera frugiperda*** **larva in maize field (Karnataka).**

the yellowish brown cocoons of *A. narangae* mentioned in Wilkinson (1928). However Wilkinson (1928) did mention that cocoons of *A. sydneyensis* were white in colour and cocoons of the Indian specimens were not observed.

Specimens examined: 10 (females and males), INDIA: Karnataka: Bommenahalli, 12.5601° N, 76.3724° E, 09.viii.2018, ex larva of *Spodoptera frugiperda* (J. E. Smith), coll. M. Sampath Kumar, specimen code: ICAR/ NBAIR/ Brac/Microg/Cot/9818; 40 (females and males), INDIA: Rajasthan: Agricultural Research Station: Borwat Farm: Banswara, located between 73°2' to 75°E' longitude and 23°11' to 24°23' N latitude, 660 mt above msl, 05.vi.2019, coll. S. Ramesh Babu, specimen code: ICAR/ NBAIR/Brac/ Microg/Cot/5719;9 (females and males), INDIA: Andamans: Neil island, ex indet. larva feeding on maize, 27.ii.2012, coll. NBAIR team, specimen code: ICAR/ NBAIR/Brac/Microg/ Cot/27212AN.

Type specimens and vouchers examined at BMNH London: *Apanteles antipoda* ASHM. paratype; 14; paratype no. 4912; USNM. One female, NHMUK010635854_*Cotesia ruficrus*_nontype_ 3c 653.

Distribution: Afrotropical, Australasian, Neotropical, Oriental, and Palaearctic (Yu *et al*., 2016). India- present in mainland as well as in Andaman & Nicobar Islands (Gupta & Fernández-Triana, 2014).

Molecular characterization of *Cotesia ruficrus*

Gupta *et al.,* (2016) have emphasized on the use of integrated approach (combining morphology, host data, and COI gene) as a dependable method of species delimitation for the identification of microgastrine wasps (Braconidae) as they are super diverse and highly speciose. Hence molecular characterization was performed for *C. ruficrus.*

The obtained sequences were aligned using BioEdit sequence alignment editor (version 7.0.5.3) and homology was confirmed using NCBI-BLAST (BLASTn, http:// www. ncbLn1m.nih.gov). The sequences were deposited in the Genbank of National Center for Biotechnology Information (NCBI), USA and accession number obtained (MN337571). The below mentioned submitted sequence shows 95% query cover with *C. ruficrus* (from Pakistan) with GenBank accession: ARS77476.1 and 96% query cover with *Cotesia* sp. SMH-2016 submitted from Meghalaya, India.

Sequence of *Cotesia* ruficrus (NCBI Accession number - M N337571)

TTAGGAATACCTGGAAGATTAATTGGTAAT
GATCAAATTTATAATAGAGTTGTAACTTCTCAT
GCATTTATTATAATTTTTTTTATAGTTATACCAG
TAATAATTGGTGGATTTGGAAATTGATTAATTC
CTTTAATATTAGGTTCTCCAGATATATCTTTCC
CTCGAATAAATAATATAAGTTTTTGATTATTAATTC
CTTCTTTATTATTATTAATTTTAAGAATATTTAT
TAATGTTGGTGTTGGAACAGGATGAACAGTATATC
CACCATTATCATTAATTTTAGGTCATGGAGGTA
TATCAGTTGATTTAGGAATTTTTTCTTTACATTTG
GCTGGTGCTTCATCAATTATAGGAGCTGTTAATTT
TATTACTACAATTATTAATATACGTTCTAATTTATT
TAATATAGATAAAATATCTTTATTTTCTTGATCAGT
GTTTATTACTGCAATTTTATTATTATTATCTTTAC
CTGTTTTAGCAGGTGCAATTACTATATTATTAACT
GATCGAAATATAAATACTAGATTTTTTGATCCATCAG
GTGGTGGTGATCCAATTCTTTATCAACATTTATTTT
GATTTTTTGGTCACCTTGGAAGTTT

Shylesha *et al.* (2018) have documented natural parasitism by egg parasitoids *viz*., *Telenomus* sp. (Platygastridae) and *Trichogramma* sp. (Trichogrammatidae), gregarious larval parasitoid *Glyptapanteles creatonoti* (Viereck) (Braconidae), solitary larval parasitoid *Campoletis chlorideae* Uchida (Ichneumonidae), and a solitary

indeterminate larval-pupal ichneumonid parasitoid. *Cotesia ruficrus* is a new addition to the existing parasitoid species complex. On an average, *C. ruficrus* emergence ranged from 11-29 wasps/larva. Perhaps with cosmopolitan distribution in addition to being a well established gregarious parasitoid in maize ecosystem, *C. ruficrus* is emerging as a natural biological control agent of *S. frugiperda* in the maize fields across India. Conservation of this wasp species and relocation of the unhatched wasp cocoons to the infested sites can help in the natural control of the pest.

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