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

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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

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°C and 60-70% humidity on the maize leaves (provided ad libitum). Parasitoids were collected from the infested larvae.
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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 bases by using universal primers LCO1490 (5’-GGTCAACAAATCATAAAGATATTGG-3’) and HCO2198 (5’-TAAACTTCAGGGTGACCAAAAAATCA3’) (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).

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
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.ncbi.nlm.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 - MN337571)

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TTAGGAAATACCTGGAAGATTAATTGGTAAT
GATCAAATTTATAATAGAGTTGTAACTTCTCAT
GCATTTATTATAATTTTTTTATAGTTATACCAG
TAAATTGGGTGATTTGGAATTGATTAATTC
CTTTAATTATAGGTTCCTCCAGATATATCTTTCC
CTCGAATAAAATAATATAAGTTTTTGATTATATCC
CTTCCTTTATATTTAATTTAATGAGATATTTAT
TAATGTTGGTGTTGGAAACAGGAAGATAGAAGTATATC
CACCATTATCAATTATTTTAGTCAATGGAGGTA
TATCAGTTGATTTAGGAAATTTTCTTTCATTCTG
GCTGGTGCTTCATCATATTAGGAGGCTTATATTT
TATTACTCAAATTATAATACGTCTCAAATTTATT
TAATAGATAAAATATTTATTTCTTGTACGT
GTTTTACTGCAATTTATTATATATCTCTAC
CTGTTTTTAGCAGTTGCAATCTATATATTAATAC
GATCGAATTAAATACGATTTTTGATCCATCG
GTGGGTGTTGGATCAAATTTTATCAACATTATTT
GATTATTTGTCACCTTTAAGGT
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Shylesha et al. (2018) have documented natural parasitism by egg parasitoids viz., *Telenomus* sp. (Platygastridae) and *Trichogramma* sp. (Trichogrammatidae), gregarious larval parasitoid *Glyptaenantes creatonoti* (Viereck) (Braconidae), solitary larval parasitoid *Campoletis chloridae* Uchida (Ichneumonidae), and a solitary...
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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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