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Research Note

First report of *Exorista xanthaspis* (Wiedemann, 1830) (Diptera: Tachinidae), a larval-pupal parasitoid on invasive pest, *Spodoptera frugiperda* (J. E. Smith) in maize from India

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ABSTRACT: For the first time, the tachinid fly, *Exorista xanthaspis* (Wiedemann, 1830) (Diptera: Tachinidae), was found to parasitize the larvae of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) collected from maize fields in Karnataka, India. The field survey conducted during 2018-19 revealed the occurrence of *E. xanthaspis* on the larvae of *S. frugiperda* with the parasitism rate of 1.85 to 4.55% in maize fields. The identity of tachinid parasitoid was confirmed by amplifying Cytochrome Oxidase I gene (CO1-658 bp) and DNA

KEY WORDS: Parasitoid, *Spodoptera frugiperda*, Tachinid fly

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Tachinid flies are the important parasitoids of major pests attacking the crops (Crosskey, 1984). Tachinidae is one of the most diverse and species-rich families of Diptera with almost 10,000 described species worldwide (O'Hara, *et al.,* 2019). All tachinids are obligate parasitoids, and their larvae develop as endoparasitoids on various stages of insect and other arthropods (Stireman *et al.,* 2006; Tschorsnig, 2017). They are usually polyphagous, yet some are species-specific and preferably parasitize the lepidopteran larvae (Inclán *et al.,* 2014; O'Hara, 2019). Tachinids are considered as crucial agents for the biological control of pests because of its parasitic nature and the ability to parasitize insect pests belonging to larger insect orders (Grenier, 1988; Stireman *et al.,* 2006).

Tachinid fly, *Exorista xanthaspis* (Wiedemann) is a parasitic fly of subfamily Exoristinae distributed in Asia, Africa and Europe and is parasitic on larvae and pupae of many crop pests (Kara and Tschorsnig, 2003; CABI, 2019). In India, the presence of *E. xanthaspis* has been recorded on crop pests, *Amsacta moorei* Butler (Verma, 1983) and *Helicoverpa armigera* (Hübner) (Bhatnagar, 1982; Patel and Talati, 1987). It has also been recorded on the larvae of *Spodoptera* spp. *viz*., *S. exempta* (Walker), *S. exigua* (Hübner) and *S. mauritia acronyctoides* Guenee in different parts of the world (CABI, 2019).

Spodoptera frugiperda is one of the most destructive pests in the genus *Spodoptera* and recognized as a major pest of maize in North and South Americas, Africa and now in the Asian countries (Qiu-Lin, 2019). At present, *S. frugiperda* has become a key pest of maize across the country after its invasion in 2018. With the establishment of the pest, the affinity of local parasitoids has increased over the period towards *S. frugiperda* on maize. Further, many native parasitoids were found to have expanded their host range and were able to parasitize it successfully in the native environment (Shylesha *et al.,* 2018; Visalakshi *et al*., 2019). In the present study, we have found a tachinid fly parasitizing the larvae of *S. frugiperda* on maize in Karnataka during the field surveys and the identity of the parasitoid was further confirmed through DNA bar-coding.

Field surveys were conducted in major maize growing areas in 2018-19 from July to November 2019 in Chikkaballapur, Karnataka. The different stages of *S. frugiperda* larvae were collected from the infested maize fields based on the feeding injury on maize leaf and presence of frass (larval excreta) on maize leaf whorl. The larvae were pulled out from the infested maize leaf whorl and placed in the plastic bags along with maize leaf. However, younger larvae were collected along with a portion of maize leaf and

kept in sealed plastic bags. *Spodoptera frugiperda* larvae were identified based on inverted 'Y' shaped marking on head and four dot square on upper surface of last abdominal segment (EPPO, 2015; Prasanna *et al.,* 2018). On reaching laboratory, the larvae were segregated instar wise and later reared individually on the artificial diet as as per methodology developed by Ballal *et al.* (1995) in growth chamber (27 ± 2°C; RH 65 ± 5%; 12L: 12D h photoperiod) till parasitoid emergence or adults of *S. frugiperda*. The emerged parasitoids from infested larvae were collected and preserved in 95% ethanol for molecular identification.

Molecular Based Identification of Tachinid Parasitoid

DNA Extraction and Sequencing

DNA extraction was done using a hind leg of the tachinid parasitoid using Qiagen DNeasy® kit, following the manufacturer's protocols. The DNA extracts were subjected to Polymerase Chain Reaction (PCR) amplification of a 658bp region near the 5' terminus of the COX1 gene following the standard protocol (Hebert *et al.*, 2003). Primers used were: forward primer (LCO 1490: 5'- GGTCAACAAATCATAAAGATATTGG-3'), and reverse primer (HCO 2198: 5'- TAAACTTCAGGGT GACCAAAAAATCA-3'). PCR reactions were carried out in 96-well plates, 50µL reaction volume containing: 5 µL GeNeiTM Taq buffer, 1 µL GeNeiTM 10mM dNTP mix, 2.5 µL (20 pmol/µL) forward primer, 2.5 µL (20 pmol/ µL) reverse primer, 1 µL GeNeiTM Taq DNA polymerase (1 U/µL), 2µL DNA (50 ng/µL), and 36µL sterile water. Thermocycling consisted of an initial denaturation of 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 46°C for 1 min and extension at 72°C for 1 min. PCR was performed using a C1000™ Thermal Cycler. The amplified products were analyzed on a 1.5% agarose gel electrophoresis as described by Sambrook and Russell (2001). The amplified products were sequenced. The species was bidirectional sequenced and checked for homology, insertions and deletions, stop codons, and frame shifts by using NCBI BLAST and ORF finder. The sequence was uploaded to GenBank and the Barcode of Life Database (Ratnasingham and Hebert, 2007).

In total, 92 larvae of *S. frugiperda* were collected from the infested maize fields and the tachinid parasitoids emerged from the pupae of *S. frugiperda* and they were found to be larval-pupal parasitoids (Fig. 3). The extent of parasitism by the parasitoid was 1.85 to 4.55% on larvae during rainy season in maize habitat (Table 1).

Table 1. Collection locality and parasitism rate of *Exorista xanthaspis* on larvae of *Spodoptera frugiperda*

Species	Locality	Parasitism (%)
Exorista xanthaspis	Karnataka: Chikkaballapur, 06.vii.2019 (13°22' N 77°44' E)	1.85
Exorista xanthaspis	Karnataka: Chikkaballapur, 08.viii.2019 (13°30' N, 77°35' E)	4.55

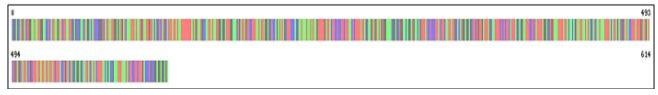

Fig. 1. DNA bar-code of *Exorista xanthaspis* (EX1).

Sequence Analysis of Tachinid Parasitoid

Molecular identification of the specimen revealed that the parasitoid was *Exorista xanthaspis* (Wiedemann, 1830) (Diptera: Tachinidae). The sequences showed 100% similarity to *Exorista xanthaspis* (AB700016) through BLAST sequence analysis and the sequences were submitted to NCBI and accession numbers were retrieved (GenBank Acc. No. MT007801 and MT007802) and DNA barcodes were obtained from BOLD systems (Fig. 1- 2) (BIN No. ACH9139).

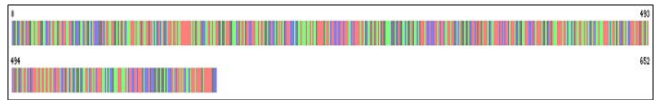

Figure 2. DNA bar-code of *Exorista xanthaspis* (EX2).

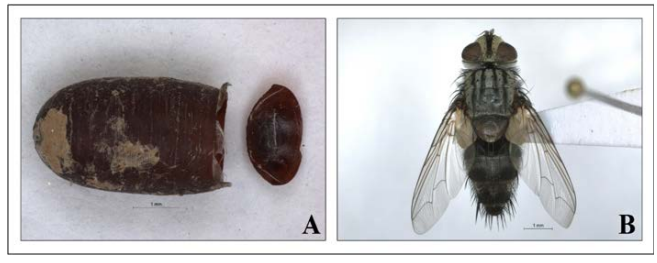

Fig. 3. Tachinid fly, *Exorista xanthaspis* emerged from the fall armyworm (A - puparium B- Adult).

Occurrence of *E. xanthaspis* has been reported on many noctuid moths of crop pests in India. The tachinid fly was found to cause 3% parasitism on the larvae of *Amsacta moorei* Butler in Rajasthan (Verma, 1983). Parasitism of *E. xanthaspis* was recorded on *A. albistriga* (Walker) on groundnut from Karnataka (Veenakumari *et al*., 2008) and *H. armigera* infesting different field crops in India (Bhatnagar, 1982). However, *E. xanthaspis* was found to have expanded

its host range by parasitizing the invasive *S. frugiperda* in maize. *Exorista xanthaspis* is also known to attack abundantly the larvae of various species of genus *Spodoptera* with different parasitism rate worldwide (CABI, 2019). Verma (1983) reported 3% larval parasitism of *A. moorei* by *E. xanthaspis* in Rajasthan. The higher parasitism (8%) of this tachinid was observed on *S. exigua* larvae in cotton fields in Turkey (Efil and Kara, 2004). In Africa, another tachinid fly, *Palexorista zonata* appeared as a primary parasitoid with 12% larval parasitism in maize within two years of invasion of *S. frugiperda* (Sisay *et al.,* 2018). We recorded lower parasitism (4.55%) of *E. xanthaspis* in the early stage of pest invasion, as the pest is yet to establish in the native environment and parasitism level of local parasitoids may improve in future. Recently, the occurrence of tachinid, *E. sorbillans* was reported with negligible levels of parasitism of fall armyworm in India (Sharanabasappa *et al.,* 2019). Approximately 100 tachinid species have been incorporated in the biological control programme of crops or forest pests with partial or complete success (Grenier, 1988). Therefore, recruiting the local parasitoids or conserving them to suppress the population of invasive pest could be an alternative to the chemical approach. In the present study, we report the new association of *E. xanthaspis* with *S. frugiperda* for the first time and further a new addition to the parasitoid assemblages of fall armyworm in India. Conservation of the tachinid parasitoids may limit and regulate the pest population in structuring the natural control of pest in maize habitat.

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REFERENCES

Ballal CR, Kumar P, Ramani S. 1995. Laboratory evaluation, storability and economics of an artificial diet for rearing *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). *J Entomol Res.* **19**: 135-41.

Bhatnagar VS, Lateef SS, Sithanantham S, Pawar CS, Reed W. 1982. Research on *Heliothis* at ICRISAT; Proc. Int. Workshop on *Heliothis* Management, ICRISAT, Patancheru, Andhra Pradesh; p 385-96.

CABI. 2019. *Exorista xanthaspis*. In: Invasive Species Compendium. Wallingford, UK: CAB International. www.cabi.org/isc/datasheet/23770.

Crosskey RW. 1984. Annotated keys to the genera of Tachinidae (Diptera) found in tropical and southern Africa. *Ann Natal Mus.* **26**(1): 189-337.

Efil L, Kara K. 2004. Tachinid Parasitoids (Diptera: Tachinidae) of *Spodoptera exigua* in cotton fields in Diyarbakw, Turkey. *Phytoparasitica* **32**(4): 363-66. https://doi.org/10.1007/BF02979846.

EPPO. 2015. PM 7/124 (1) *Spodoptera littoralis, Spodoptera litura, Spodoptera frugiperda, Spodoptera eridania. EPPO Bull.* **45**: 410-44. https://doi.org/10.1111/epp.12258.

Grenier S. 1988. Applied biological control with tachinid flies (Diptera, Tachinidae): A review. *Anz Schadlingskde Pflanzenschutz Umweltschutz* **61**: 49-56. https://doi.org/10.1007/BF01906254.

Hebert PDN, Cywinska A, Ball SL, DeWaard JR. 2003. Biological identifications through DNA barcodes. *Proc R Soc Lond B Biol Sci*. **270**: 313-21. https://doi.org/10.1098/rspb.2002.2218. PMid: 12614582, PMCid: PMC1691236.

Inclán DJ, Cerretti P, Marini L. 2014. Interactive effects of area and connectivity on the diversity of tachinid parasitoids in highly fragmented landscapes. *Landsc Ecol*. **29**(5): 879-89. https://doi.org/10.1007/s10980-014-0024-0.

Kara K, Tschorsnig HP. 2003. Host catalogue for the Turkish Tachinidae (Diptera). *J Appl Entomol*. **127**: 465-76. https://doi.org/10.1046/j.0931-2048.2003.00786.x.

O'Hara JE, Henderson SJ, Wood DM. 2019. Preliminary checklist of the Tachinidae of the world. Version 1.0. PDF document, p. 681. Accessed 24 December 2019. Available at: http://www.nadsdiptera.org/Tach/WorldTachs/Checklist/Worldchecklist.html.

Patel AJ, Talati GM. 1987. Biology of *Helicoverpa armigera* (Hb.) as a pest of sunflower. *Gujarat Agric Univ Res J*. **12**(2): 54.

Prasanna BM, Huesing JE, Eddy R, Peschke VM. 2018. Fall Armyworm in Africa: A Guide for Integrated Pest Management, First Edition. Mexico, CDMX: CIMMYT.

Qiu-Lin W, Li-Mei H, Xiu-Jing S, Yu-Ying J, Jie L, Gao H, Kong-Ming W. 2019. Estimation of the potential infestation area of newly-invaded fall armyworm *Spodoptera frugiperda* in the Yangtze River valley of China. *Insects* **10**(9): 298. https://doi.org/10.3390/

insects10090298. PMid: 31540256, PMCid: PMC6780859.

Ratnasingham, S., Hebert, PDN. 2007. "BOLD: The Barcode of Life Data System (http://www.barcodinglife.org)". *Mol. Ecol. Notes* 7 (3): 355–364.

Sambrook JF, Russell DW. 2001. Molecular Cloning: A Laboratory Manual, 3rd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

Sharanabasappa, Kalleshwaraswamy CM, Poorani J, Maruthi MS, Pavithra HB, Diraviam J. 2019. Natural enemies of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), a recent invasive pest on maize in South India. *Fla Entomol*. **102**(3):619-23. https://doi.org/10.1653/024.102.0335.

Shylesha AN, Jalali SK, Gupta A, Varshney R, Venkatesan T, Shetty P, Ojha R, Ganiger PC, Navik O, Subaharan K, Bakthavatsalam N, Ballal CR. 2018. Studies on new invasive pest *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and its natural enemies. *J Biol Control* **32**(3): 1−7.

Sisay B, Simiyu J, Malusi P, Likhayo P, Mendesil E, Elibariki N, Mulatu Wakgari M, Ayalew G, Tefera T. 2018. First report of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), natural enemies from Africa. *J Appl Entomol*. **142**: 800-04. https://doi.org/10.1111/jen.12534.

Stireman JO, O'Hara JE, Wood DM. 2006. Tachinidae: Evolution, behavior and ecology. *Annu Rev Entomol*. **51**(1): 525-55. https://doi.org/10.1146/annurev.ento.51.110104.151133. PMid: 16332222.

Tschorsnig H-P. 2017. Preliminary host catalogue of Palaearctic Tachinidae (Diptera). *Tschorsnig.* p. 480. http://www.nadsdiptera.org/Tach/WorldTachs/CatPalHosts/Cat_Pal_tach_hosts_Ver1.pdf.

Veenakumari K, Ramani S, Rabindra RJ. 2008. Additions to the natural enemy complex of the red hairy caterpillar, *Anlsacta albistriga* (Walker) (Lepidoptera: Arctiidae). *J. Biol Control* **22**(1): 203-04.

Verma SK. 1983. *Exorista xanthapis* (Wiedemann) (Tachinidae: Diptera), a new record on *Amsacta moorei* Butler. *Ann Arid Zone.* **22**: 41-42.

Visalakshi M, Ashika TR, Venkatesan T, Ballal CR, Laxman K, Nagarjuna D, Chittibabu G, Venkatarao P, Jamuna P. 2019. Report of the invasive fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) and its natural enemies on maize and other crops from Andhra Pradesh, India. *J Entomol Zool Stud*. **7**: 1348-52.