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First report of *Telenomus remus* Nixon (Hymenoptera: Scelionidae) on *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) in Egypt

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Abstract

Background The polyphagous alien invasive pest, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), invaded Africa and has so far spread across the continent, causing devastating impacts on maize production. In Egypt, the management of the pest by maize growers has been characterized by the application of broad-spectrum synthetic chemical insecticides, a strategy which is associated with various detrimental effects on the environment and human health. To mitigate the overreliance and excessive use of those synthetic chemicals, biological control using egg parasitoids species provides an ecologically friendly and sustainable management strategy. In that regard, this study had the objective of identifying the natural existing egg parasitoids, which could be effectively used in augmentative biocontrol of the pest in Egypt.

Results For the first time in Egypt, natural occurrence and parasitism of *Telenomus remus* Nixon, 1937 (Hymenoptera: Scelionidae) on *S. frugiperda* egg masses was recorded. Infested maize farms in Qena Governorate, Egypt, were surveyed, and *S. frugiperda* egg masses were collected and incubated in the laboratory. Emerging parasitoids' wasps were grouped based on their morphological similarities. Natural parasitism of the egg parasitoids (*Telenomus* sp.) was computed from the parasitized field collected egg masses. In addition, to confirm the laboratory parasitism and suitability of the parasitoid, *Telenomus* sp. to develop on *S. frugiperda,* the parasitoid was reared on *S. frugiperda* egg masses for three generations under laboratory conditions. Moreover, both morphological and molecular identifications were conducted. The recovered parasitoid samples from the field *S. frugiperda* egg masses were *Telenomus remus* (Nixon) (Hymenoptera: Scelionidae). Moreover, the average field parasitism level by *T. remus* on *S. frugiperda* was 15.9%, while under laboratory conditions, the parasitism was 63.5%.

Conclusions The natural occurrence of *T. remus* in Egypt and its association with *S. frugiperda* is an important finding upon which augmentative biocontrol strategy can be leveraged on to sustainably manage the pest populations.

Keywords *Telenomus remus*, *Spodoptera frugiperda*, Maize, Egypt

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Background

The invasion of the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) into Africa (Goergen et al. 2016) and its rapid spread across the continent has had devastating impacts on continent's food security. The establishment of the pest in Africa has not only contributed to increase in crop yield losses, but also rise in the cost of production, especially with the use of wide-spectrum synthetic chemical insecticides (De Groote et al. 2020). In order to contain the devastating impacts of the pest, concerted efforts have been geared towards the development of eco-friendly and sustainable management practices, which aim to reduce the pest populations in the field to a sound economic threshold level. Among the strategies that have been deployed include Push–Pull technology, biopesticides, biorationals and natural enemies (i.e., parasitoids) (Kenis et al. 2022).

Biological control of invasive pests using parasitoids and/or predators in either classical or augmentative releases is an important component of the integrated pest management (IPM) strategies for the smallholder farmers. Importantly, since the establishment of *S. frugiperda* in Africa, several parasitoids have been reported to have formed new association or originally associated with the pest. They include: (1) newly associated larval parasitoids such as *Cotesia icipe* Fernandez-Triana and Fiaboe (Hymenoptera: Braconidae) (Mohamed et al. 2021); and (2) old-association with egg-larval parasitoid, *Chelonus bifoveolatus* Szépligeti (Braconidae: Hymenoptera) (Agboyi et al. 2020), and egg parasitoids such as *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) and *Telenomus remus* Nixon (Hymenoptera: Platygastridae) (Kenis et al. 2022). All these parasitoids have only been recorded in other regions within Africa and have so far been considered for utilization in augmentative biocontrol of the pest. In Egypt, several other parasitoids have been reported to be associated with *S. frugiperda* such as *Exorista sorbillans* (Wiedemann), *Pseudogonia rufifrons* (Wiedemann) (Diptera; Tachinidae) and *Microplitis* sp. (Hymenoptera: Braconidae) (Abd Elmageed et al. 2021), *Cotesia ruficrus* (Haliday) (Hymenoptera: Braconidae), *Dinarmus basalis* Rondani (Hymenoptera: Pteromalidae), and *Microplitis rufiventris* Kokujev (Hymenoptera: Braconidae) (Youssef 2021). Even though the natural occurrence of egg and larval parasitoids has been reported in Egypt, it was important to conduct a survey to establish the presence of the widely reported and effective egg parasitoid, *T. remus*. In that regard, maize farms in Qena Governorate, Egypt, were surveyed for the presence *T. remus* on *S. frugiperda*. In addition, this study also aimed at identifying other naturally co-existing egg parasitoids with *T. remus* and their parasitism field level on *S. frugiperda*.

Methods

Study area

The surveys for *S. frugiperda* egg masses were carried out between August and October 2021 in maize growing areas of Naqada, Naga Hammadi and Dashna Districts, in Qena Governorate, Upper Egypt. In the selected study sites, local maize varieties (Indigo Corn™ seeds) were planted in small fields of about half to two (½–2) Feddan (Feddan = 4200 m^2). In the surveyed areas, maize was grown under irrigation, with the average climatic conditions of 23% relative humidity and 24.6 °C as minimum temperature and 39 °C as maximum temperature (El-Marsafawy et al. 2019). Moreover, pesticide application was stopped at least 1 month post-planting in all the surveyed fields, a strategy which ensured that the population of the natural existing egg parasitoids build up in the fields and the egg laying of *S. frugiperda* and parasitism by natural enemies were not affected.

Field sampling of *S. frugiperda* eggs masses and larvae

Idiobiont parasitoids such as *T. remus* can only be recovered on *S. frugiperda* egg masses, while the koinobiont egg-larval parasitoids such as *C. bifoveolatus* can be recovered at both egg and larval stages of the host. In that regard, different developmental stages (egg masses and all larval instars) of *S. frugiperda* were collected from the selected farms. In each district and in every farm, 25 infested maize plants were selected using the "W" scouting and sampling technique recommended by the FAO (2018). Egg masses and all the larval instars were collected from each of the selected maize plants. To collect the egg masses, sections of maize leaves, with the egg masses, were cut using pair of scissors, and the egg masses individually placed in plastic vials. Moreover, from each maize plant, different larval instars were collected using a pair of soft forceps and placed in plastic buckets holding pieces of maize leaves, with similar larval instars placed in separate buckets. The larvae were continuously provided with clean maize leaves for feeding, and to minimize cannibalism. All field samples were labelled according to location and date of collection, as well as the development stages. The field samples were then transported to the laboratory at El Marashda Research Station, Qena for sorting, where each larva or egg mass was placed in individual plastic vials before they were transferred to the BioLab at Shandaweel, Sohag Governorate for processing.

Laboratory sorting and incubation of field-collected samples

At the BioLab Shandaweel, all samples were incubated at 25 ± 2 °C; 12HL: 12HD; 70 ± 5%RH, as the ambient rearing conditions. The egg masses were regularly observed

for hatched larvae, or emergence of the idiobionts. The developing larvae from the egg masses were maintained on maize leaves until pupation formation from the parasitized individuals as recommended in the method developed by Mohamed et al. (2021). Moreover, in cases whereby on a single egg mass, there are both parasitized eggs and unparasitized ones, dark colouration of the potentially parasitized ones and their failure to hatch 5 days after the initial day of incubation were the strategy used to separate them. Thus, to prevent cannibalism by the hatched larvae on the parasitized eggs, the neonates were separated from the vials and maintained on plastic buckets until population, as described above. From the suspectedly parasitized egg masses, where parasitized failed to emerge, at least 20 days post-incubation date, were all dissected under stereomicroscope (Noval/NS2-405) to confirm the cause of mortality, either by the presence of a parasitoid or otherwise.

Laboratory rearing of parasitoid progenies

As a confirmatory test that indeed the parasitoid(s) that emerged from *S. frugiperda* eggs can actually parasitize and develop in the host, the progenies were reared on this host for three filial generations using the method developed by Tefara et al. (2019). The regeneration of the parasitoids was done using *S. frugiperda* and *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) as an alternative host for optimization of the parasitoids rearing.

In the laboratory rearing, 150 wasps (2♀:1♂) of *T. remus* were placed in plastic jars and provided with honey streaks on Manila paper for feeding. Fall armyworm egg masses of different ages (1, 2 and 3 days old) were glued on Manila cards and introduced into the jars, and the parasitoids were allowed to parasitize them for 8 h, after which, the parasitized egg masses (on the cards) were transferred into clean jars. The number of larvae that hatched was recorded, and per cent parasitism by *T. remus* was also calculated.

Morphological identification of the egg parasitoid(s)

All parasitoids that emerged from the field collected samples, from either egg masses or larvae, were sorted based on the egg/larval stage sampled, location and date of collection. Initially, based on sexual dimorphism on the external morphology (differences in genitalia and antennae), male and female parasitoids were separated under the stereomicroscope and individually placed in 70% ethanol for detailed morphological identification at the Department of Survey Research and Classification at the Plant Protection Research Institute, ARC, Egypt. All the recovered egg parasitoids were carefully examined for possible occurrence of two or more closely related or morphologically similar species that could emerge from a single egg mass. From all collected samples, only one species of egg parasitoid was recorded, and tentatively identified as *Telenomus* species. Consequently, using the guidelines provided by Polaszek and Kimani (1990), morphological identification of *Telenomus* species was performed. Thus, for the systematic morphological identification, specimens were prepared following the procedure of slide mounting of sample wasps, dissection and separation of various organs from each sample, male and female samples separately. Moreover, male genitalia were adjusted and illustrated using Adobe illustrator program version CS6. The morphological identification of the parasitoids was done at Biological Control laboratory (BioLab), Shandaweel and in the Collection of Survey and Classification Department at the Plant Protection Research Institute, ARC, Egypt.

Molecular identification of the parasitoid

In Egypt, there exists a closely related species, *Telenomus nawai* Ashmead, 1904; thus, to clearly and authoritatively confirm the identity of *T. remus*, molecular identification was conducted. The genomic DNA was extracted using Gene JET genomic DNA purification Kit Thermo Scientific (cat No. K0702) from *T. remus* specimen according to the manufacturer's instructions. One percent of agarose gel electrophoresis was utilized for DNA integrity determination. Extracted DNA was treated with RNase A solution (cat # A7973) to get rid of RNA residues. Polymerase chain reaction (PCR) amplification was performed using universal primers that amplify mitochondrial cytochrome c oxidase subunit I (COI) and ITS2 region. The COI fragment was amplified using the primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTG G-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCA AAAAATCA-3′) (Folmer et al. 1994); while ITS2 region was amplified using forward primer, ITS2F (5′-TGTGAA CTGCAGGACACATG-3′) and Reverse primer, ITS2R (5′-GTCTTGCCTGCTCTGAG-3′) (Stouthamer et al. 1999). The PCR was carried out in a 50-µL reaction containing 25 µL of One PCR™ master mix (cat # MB203-0100), 1 µL of the DNA extract (40 ng of total DNA), 1 µL of 10 µM of each primer, and the reaction was completed to 50 µL with Nuclease-free water. The amplification conditions were 95 °C for 5 min for initial denaturation, followed by 35 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 2 min, with a final extension at 72 °C for 10 min. The amplicons were visualized on 1.5% agarose gel (Fig. 1).

The amplified PCR product corresponding to the cytochrome c oxidase subunit I (COI) and internal transcribed spacer (ITS) gene underwent sequencing analysis employing the Big TriDye sequencing kit (ABI Applied Biosystems), conducted at the LGC facility in Germany.

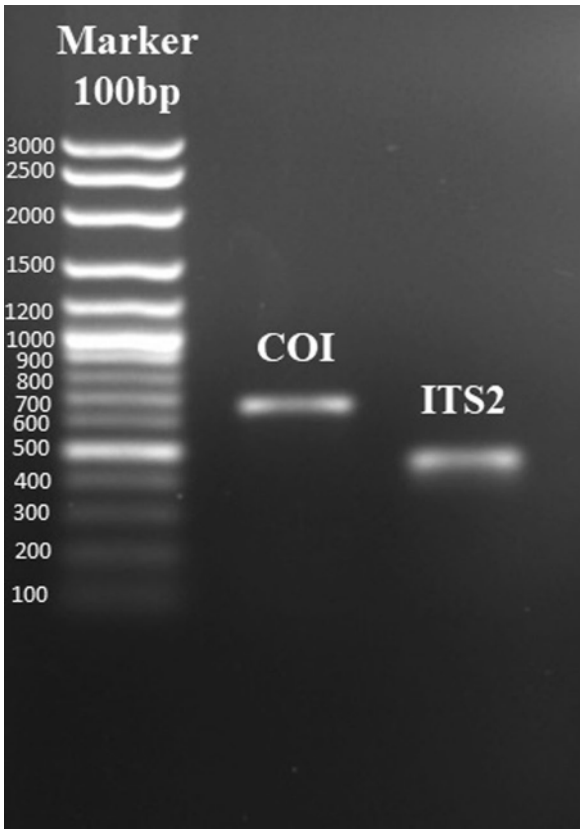

Fig. 1 VC 100 bp plus DNA ladder (Vivantis, Malaysia, Cat. #NL1407), PCR amplification product of COI and ITS2 region of *Telenomus* sp.

Subsequently, a homology search was executed for the COI and ITS region DNA sequence utilizing the Basic Local Alignment Search Tool (BLAST) against the National Center for Biotechnology Information (NCBI) database located in the United States (http://www.ncbi.nlm.nih.gov). Retrieved sequences were juxtaposed with ITS sequences within the National Center for Biotechnology Information (NCBI) GeneBank database utilizing the BLASTN algorithm (Altschup et al. 1990). Sequences closely related to the obtained data were retrieved and manually imported for alignment within the Molecular Evolutionary Genetics Analysis software, version 7.0 (Kumar et al. 2018), employing the Clustal W tool.

Relative abundance of the egg parasitoids in the study areas

The relative abundance of the egg parasitoids was determined using the formula adopted by Caniço et al. (2020), where the number of individuals of a specific parasitoid species was divided by the total number of individuals of all the parasitoid species collected, and the quotient converted into per cent values.

Parasitism rate of egg parasitoids

The parasitism rates of the only recovered egg parasitoid, *T. remus*, were computed, and compared between old and fresh egg masses, as well as parasitism per each study site. Rate of parasitism by the parasitoid species was determined by dividing the total number of parasitized egg masses by the number of collected egg masses and the quotient converted to percent values. The parasitism rates of larval parasitoids were not calculated since no parasitoid species was recovered from the field samples.

Regeneration of *T. remus* and its parasitism under laboratory conditions

The initial colony of *T. remus* that emerged from the field samples was reared on both *S. frugiperda* and *S. cerealella* eggs for three filial generations. For the *T. remus* rearing on *S. frugiperda*, host egg masses which were collected on castor oil leaves as the oviposition surface and stuck on the Manila paper (also referred to as *Tele* cards) measuring 5×2 cm-size paper cuttings, were introduced to 100 parasitoid wasps for oviposition over 24-h period. The estimated rates of parasitism on *S. frugiperda* and hatchability of the unparasitized eggs are shown in Table 1. For the regeneration of the parasitoid on *S. cerealella,* eggs of this host were collected and glued on the Manila paper, and then exposed to the parasitoid using the method described above for *S. frugiperda*. Importantly, for each generation, *S. frugiperda* eggs were exposed on a 3-day strategy, albeit with differential number of egg masses.

Results

Morphological identification of parasitoids

The only egg parasitoid that was recovered from field collected *S. frugiperda* egg masses was identified as *Telenomus remus* Nixon, (Hymenoptera: Scelionidae) (Fig. 2a, b). The male wasps (Fig. 2a) have 12-segmented moniliform antennae in which all the flagella segments are of approximately equal diameter. The female wasps (Fig. 2b) have 11-segmented clavate antennae, in which the last four or five segments are larger than the preceding ones. The limbs of male wasps were yellowish to brownish and were all lighter compared to that of the female limbs. Further, the genitalia of the males were used to confirm the identity of the parasitoid (Fig. 3) as described by Wengrat et al. (2021). The genitalia had basal ring of aedeagus which appeared to be pod-like. Laminae volsellares were formed apart from the base of aedeago volsellar shaft and gradually converge and diverge shortly before their end to meet each end of digiti, from which three digital teeth, and the end of digiti connects to a transparent membrane surrounding the aedeago volsellar shaft except, aedeagal

Table 1 Regeneration of *T. remus* on *S. frugiperda* and average parasitism by the parasitoid on different age of the egg masses under laboratory conditions

Age of *S. frugiperda* eggs exposed to *T. remus*	No. of egg masses	Number of hatched larvae (3 Days later)	Number of emerged wasps from parasitized eggs	Percent parasitism
1 day old	12	578	1222	67.89
	9	603	297	33.00
	10	317	183	36.60
2 days old	6	287	613	68.11
	7	309	391	55.86
	6	98	202	67.33
3 days old	3	209	241	53.56
	6	329	271	45.17
	4	62	138	69.00

Fig. 2 *Telenomus remus* Nixon 1937 male (**a**) and female (**b**)

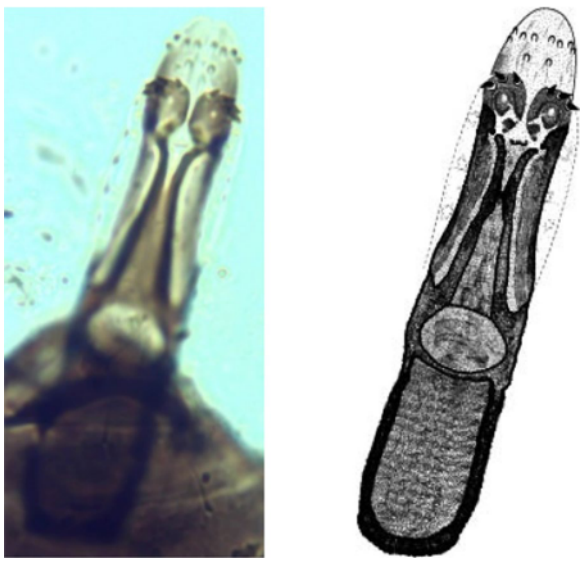

Fig. 3 Genitalia of male *T. remus*

lobe. Eight small nipples horizontally lined on both sides of aedeagal lobe (four on each side) and two slightly below (one on each side).

Molecular identification of the egg parasitoid

The result of BLASTn program, which shows the internally transcribed spacer 2 (ITS2), was deposited in NCBI database under accession number (PP430542). It exhibited a 98% homology with documented isolates such as *T. remus* (KM272554), while demonstrating a 97% homology with *T. nawai* (AF467102) and *T. remus* Korea isolate (ON721270). Furthermore, the DNA barcode sequence of COI of the samples were blasted and deposited in the GenBank with accession number OP932000. The sequence similarity was considered and dendrogram analysis was done as well (Fig. 4). Sequence was aligned by codons using MUSCLE implemented in MEGAX (Kumar et al. 2018). Our *T. remus* mtCOI sequence shared over 99.9% similarity with those of other

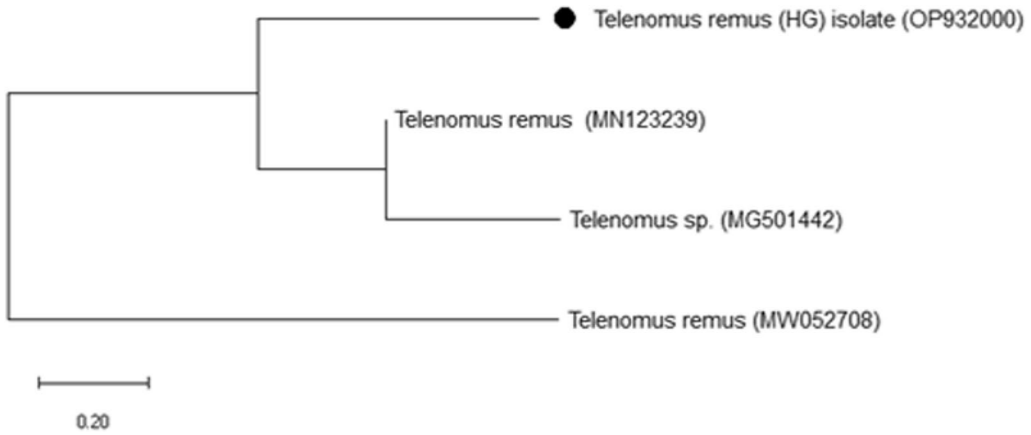

Fig. 4 Phylogenetic analysis of *Telenomus remus* and related species by maximum likelihood method based on COI sequences. The sequence generated from this study are indicated with codes and GenBank accession numbers (OP932000). Bootstrap values above 1000 indicated on branches

T. remus sequences that were deposited in the GenBank from East Africa MT780201.1 by Otim et al. (2021), India (MN879316.1) by Navik et al. (2021) and China (MT906647.1) by Li et al. (2021).

Relative abundance of the egg parasitoids in the study areas

From all the study areas, there was differential levels of *S. frugiperda* infestations, with the highest recorded infestation level of 100% in ElKhour Kebli, ElSarakhna, and ElAwsia, while the lowest infestation level of 36% was recorded in ElFars and ElHysha (Fig. 5). All the developmental stages of *S. frugiperda* were found, except the pupae. From the samples collected in the three study areas, *T. remus* was recovered in Nagaa Hammadi; therefore, the recommended formula by formula adopted by Caniço et al. (2020) was not adopted, and the only abundant egg parasitoid in Qena Governorate is *T. remus*.

Parasitism by *T. remus* on *S. frugiperda* under field conditions

It was only in Nagaa Hammadi where parasitized egg masses were obtained. Out of 2051 eggs sampled from freshly laid *S. frugiperda* eggs, only 2.3% parasitism rate was recorded, while from the old egg masses, an average of 34.7% parasitism rate from a total of 1474 eggs was obtained. Thus, overall parasitism rate of *T. remus*, which

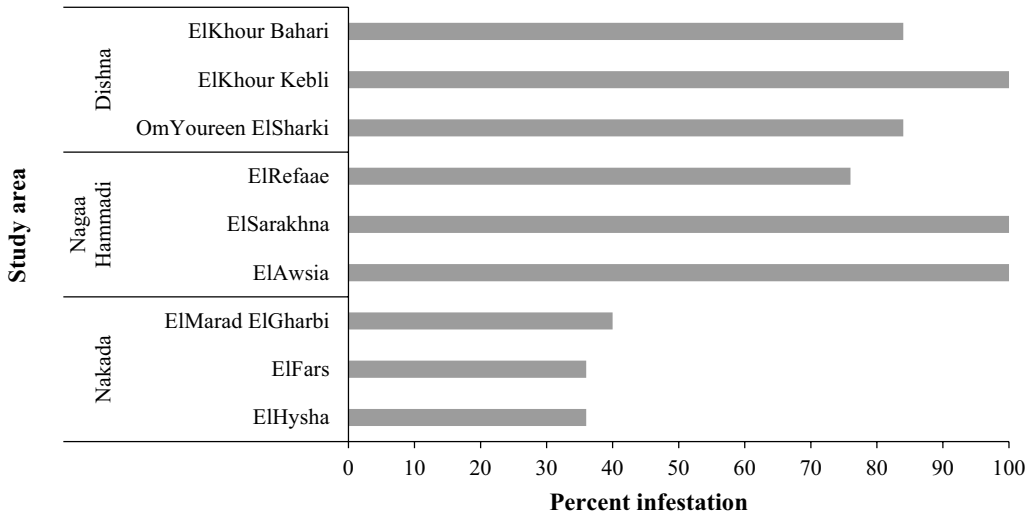

Fig. 5 Percent infestation level of *S. frugiperda* in the study areas

measured as the average parasitism rate from both newly and old egg masses, was estimated as 15.9%.

Regeneration of the parasitoids in the laboratory

For every exposure of *S. frugiperda*, different rates of parasitism were recorded, with the highest percent parasitism (63.5%) were obtained on the second exposure on *S. frugiperda*, while the third exposure resulted in the lowest rate of parasitism (52.0%) on the same host. There was no parasitism and regeneration success of *T. remus* on *S. cerealella.*

Discussion

The morphological and molecular identifications showed that the only egg parasitoid recovered from the *S. frugiperda* egg masses in Egypt was *T. remus.* This finding confirms the earlier work by Kenis et al. (2019) which provided the evidence that *T. remus* is already present in Africa even before the invasion of *S. frugiperda*. Moreover, the parasitoid has been recorded in other African regions such as East Africa (Sisay et al. 2018), and in southern Africa (Kenis et al. 2019). The record of this important biological control agent could also be beneficial in control of several other *Spodoptera* spp. that threaten crop production in in Egypt (Wojcik et al. 1976). Moreover, the natural parasitism by *T. remus* on *S. frugiperda* and the success in the regeneration of the parasitoid under laboratory conditions are indeed important milestones in the development of augmentative biocontrol of *S. frugiperda* in the Sahel region. Moreover, the recovery of the parasitoid in maize fields is an indication of its potential to be effectively employed in controlling the pest's populations to below economic threshold in maize farms across Egypt, as is the case in the Americas (Heinrichs et al. 2018).

The differential parasitism rates between the newly laid *S. frugiperda* egg masses and the old ones provided the indication of the comparative rates of parasitism over time, especially under field conditions. On the newly laid eggs, it was expected that only few eggs could be parasitized as opposed to the old egg masses, which could be attributed to the exposure time and consequently the parasitism by the potential egg parasitoid(s). Under field conditions, *T. remus* had more time to parasitize egg masses that were laid at least 1 day prior to our collection, which therefore confirms the low parasitism on the freshly laid egg masses. The laboratory results confirmed this presumption, where the highest parasitism rate was obtained from the old egg masses. Even though the recorded combined field parasitism rate by *T. remus* was as low as 15.9%, compared to other regions such as 69.3% in Kenya and 58.5% in Tanzania (Sisay et al. 2018), and 25.9% in Ghana (Agboyi et al. 2020), our finding highlights the much important aspect of the existence of the parasitoid in the country. Therefore, the low parasitism rate can be supplemented by argumentative releases of laboratory reared *T. remus* to boost on the natural population of the parasitoid in maize fields.

The successful regeneration of the parasitoid in the laboratory, using both *S. frugiperda* and *S. cerealella* eggs, provided the basis for mass production of *T. remus*. Given the success in the pilot releases of the parasitoid in Africa, with promising results of up to 33% parasitism rate in Ghana (Agboyi et al. 2021) and above 50% in Kenya (icipe 2021), mass rearing of this parasitoid in the laboratory is worth undertaking. Efforts are underway to undertake mass rearing of the parasitoid and thereafter launch a mass releases in the fields to help suppress the population of *S. frugiperda*.

Conclusions

In this study, the natural occurrence and parasitism with *T. remus* on *S. frugiperda* is reported for the first time in Egypt. Thus, the result of this study is an important indicator of the need to implement augmentative biological control using *T. remus* in the Sahel region.

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Author contributions

All phases of the research were prepared, conducted and formulated by the authors.

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Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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