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First report of *Telenomus remus* parasitizing *Spodoptera frugiperda* and its field parasitism in southern China

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Abstract

The fall armyworm, *Spodoptera frugiperda*, is a lepidopteran pest that feeds on many economically important cereal crops such as corn, rice, sorghum, and sugarcane. Native to the Americas, it has become a serious invasive pest in Africa and Asia. Recently, this pest was found in China and has spread quickly across the country. As *S. frugiperda* will most likely become a major pest in China, Integrated Pest Management strategies, including biological control methods, should be developed to manage its populations. Here, we report the detection of *Telenomus remus* parasitizing *S. frugiperda* eggs in cornfields in southern China based on morphological and molecular evidence. Our preliminary surveys indicated that the parasitism rates of *T. remus* on *S. frugiperda* could reach 30% and 50% for egg masses and per egg mass, respectively. Further application of *T. remus* against *S. frugiperda* in biological control programs are discussed.

Keywords

Scelionidae, egg parasitoid, parasitism rates, biological control

* These two authors contributed equally to this work.

Introduction

The fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), which originates from tropical and subtropical areas of North, Central, and South America, has become an invasive pest of cereals in Africa, India, Myanmar, Thailand, etc, where it has caused serious damage (Kenis et al. 2019). In China, this species was first detected in the southeast province of Yunnan in January, 2019, and it has quickly spread northward to 17 other provinces (Cui et al. 2019, Jiang et al. 2019). Geographic distribution models have indicated that large parts of China are potentially suitable for the survival of this devastating pest (Lin et al. 2019).

In its native and introduced ranges, *S. frugiperda* feeds on a wide range of crops. Over 350 different host plants in numerous families have been recorded, and almost 40% of them are economically important (Montezano et al. 2018). It is estimated that, just for corn, rice, sorghum and sugarcane, this pest could cause up to 13 billion USD per annum in crop losses in Africa (Day et al. 2017). Given that corn, sugarcane, and rice are widely grew in southern China, *S. frugiperda* will most likely establish as a major pest in this region (Wang et al. 2019). Currently, chemical control is still the main strategy against this pest in China, although some biological control experiments using predators (*Picromerus lewisi* Scott (Hemiptera: Pentatomidae)) have been conducted in the laboratory (Tang et al. 2019). However, in the long run, more biological control strategies should be adopted to against *S. frugiperda* under the perspectives of Integrated Pest Management (IPM).

Among the ~150 parasitoid species that attack *S. frugiperda*, the egg parasitoid species *Telenomus remus* Nixon (Hymenoptera: Scelionidae) appears to be a promising biological control candidate (Kenis et al. 2019) and is reported to attack eggs of various *Spodoptera* species that are known from China (Chou 1987, Tang et al. 2010). In this study, we report the detection of *T. remus* parasitizing *S. frugiperda* eggs and its parasitism rates in cornfields in southern China.

Materials and methods

Field sampling

Eggs of *S. frugiperda* were collected from cornfields of three sites in Guangzhou and Foshan, China, in May and June, 2019 (Table 1). Egg masses were brought to the laboratory and individual egg masses were placed in a 10 cm glass tube and kept in a growth chamber set at 26 °C±1 °C, 40–60% humidity and a 12L:12D light cycle and checked daily for emergence of *S. frugiperda* or parasitoids. Larvae of *S. frugiperda* from the non-parasitized eggs in the same egg mass usually emerged first and were transferred to artificial diet (originally designed for rearing the tobacco cutworm, *Spodoptera litura* (Fabricius)) and reared to adulthood to confirm the identification of the hosts. Any parasitoids that emerged were placed in 100% ethanol for morphological and molecular analyses. The number of eggs in each egg mass, emerged larvae, parasitism and sex ratio of the parasitoids were recorded.

Table 1. Details of the sampling, numbers, and accession numbers of parasitoids sequenced.

Locality (City)	Coordinates	Collection date	Host plant	No. egg mass	No. barcoded	Code & GenBank accession number
Huadu District, Guangzhou	23°29'6.11"N, 113°16'11.99"E	8.vi.2019	corn	28	2	Huadu_F (MN123241) Huadu_M (MN123242)
Panyu District, Guangzhou	23°3'21"N, 113°24'41"E	7.vi.2019	corn	5	2	Panyu_F (MN123243) Panyu_M (MN123244)
Gaoming, Foshan	22°48'22.28"N, 112°34'19.83"E	18.vi.2019	corn	3	2	Gaoming_F (MN123239) Gaoming_M (MN123240)

Species identification

Species of *Telenomus* were determined using the characters of Johnson (1984). Considering the similarity of females between *Telenomus* species, genitalia of males collected from each locality were examined. To confirm morphological identifications, genomic DNA was extracted from a female and male collected from each locality using a nondestructive DNA extraction protocol as described in Taekul et al. (2014). Voucher specimens for all molecular data are deposited in the Museum of Biology at Sun Yat-sen University, Guangzhou, China. Following extraction, the "barcode" region of the mitochondrial cytochrome oxidase subunit 1 (*COI*) was amplified using the LCO1490/HCO2198 primer pair (Folmer et al. 1994). Polymerase chain reactions (PCRs) were performed using Tks Gflex DNA Polymerase (Takara) and conducted in a T100 Thermal Cycler (Bio-Rad). Thermocycling conditions were: an initial denaturing step at 94 °C for 1 min, followed by 5 cycles of 98 °C for 10s, 45 °C for 15s, 68 °C for 30s; 35 cycles of 98 °C for 10s, 52 °C for 15s, 68 °C for 30s and an additional extension at 68 °C for 5 min. Amplicons were directly sequenced in both directions with forward and reverse primers on an Applied Biosysttems (ABI) 3730XL by Sangon Biotech (Shanghai, China). Chromatograms were assembled with Sequencing Analysis 6 (ThermoFisher Scientific, Gloucester, UK). All the amplified sequences were deposited into GenBank (accession numbers in Table 1).

Sequences obtained in this study were compared with those analyzed by Kenis et al. (2019). Sequences were aligned by codons using MUSCLE implemented in MEGA6 (Tamura et al. 2013). The alignment was then analyzed using RAxML as implemented in Geneious 11.0.3 with *Gryon cultratum* Masner and *Gryon largi* (Ashmead) (Hymenoptera: Scelionidae) used as outgroups to root the tree.

Photography

Images of live specimens were captured using a Keyence VHX-6000 digital microscope. Images of mounted specimens were produced with Combine ZP and Auto-Montage extended-focus software, using a JVC KY-F75U digital camera, Leica Z16 APOA microscope, and 1X objective lens.

Results

Only one parasitoid species, *Telenomus remus* (Figure 1D), emerged from the 36 egg masses collected from the three sites. The specimens (Figure 1B, 1C) collected from the three sites show no morphological variation and match well with the holotype (Figure 1A) of *T. remus* (deposited in British Museum of Natural History, **BMNH**) as well as the description of this species developed by Nixon (1937) and Chou (1987). The *COI* sequences were identical among females and males sampled from the three collecting sites, and over 99% of the pairs of bases were identical to a series of sequences labeled as *T. remus* available from the Barcoding of Life Data system and the GenBank database. Phylogenetic analysis based on *COI* sequences generated from this study and those used by Kenis et al. (2019) showed that the six specimens collected from the three sites of southern China were grouped well within the clade of *T. remus* specimens collected from Asia, Africa, and the Americas (Figure 2).

Twenty-eight, five, and three egg masses of *S. frugiperda* were collected from Huadu, Panyu, and Gaoming, respectively (Table 1). Of the 36 egg masses collected, 11 egg masses (30.6%) were parasitized by *T. remus*. For the 28 egg masses collected from Huadu, we counted the number of layers of each egg mass, parasitism per egg

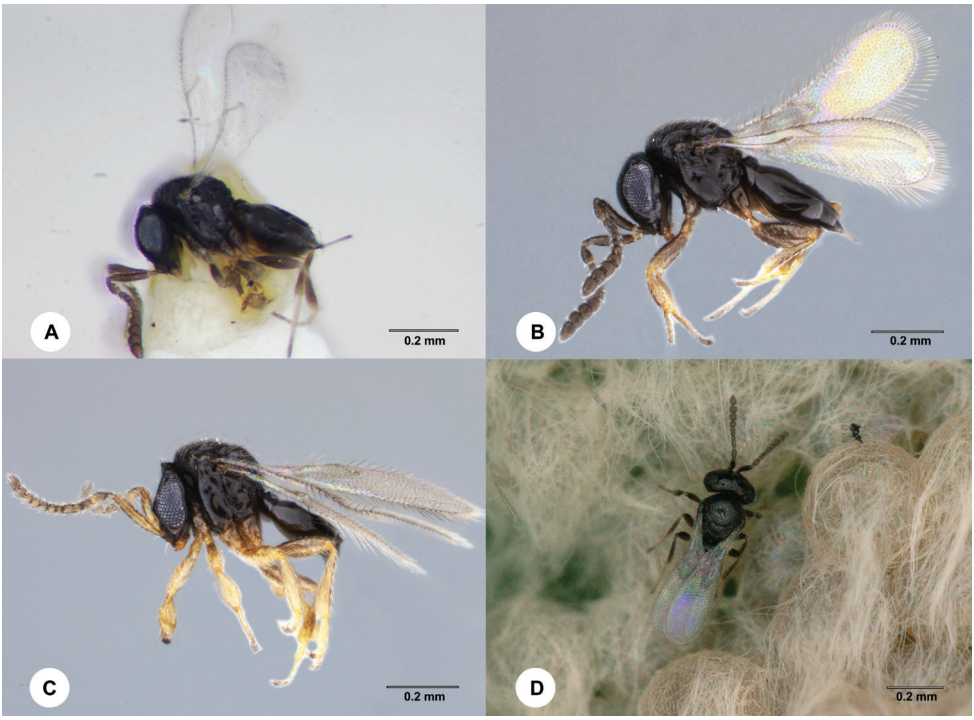

Figure 1. *Telenomus remus* Nixon. **A** Holotype (NHMUK010576395), female, lateral habitus **B** female (SCAU 3040967), lateral habitus **C** male (SCAU 3040968), lateral habitus **D** a female on egg mass of *Spodoptera frugiperda*.

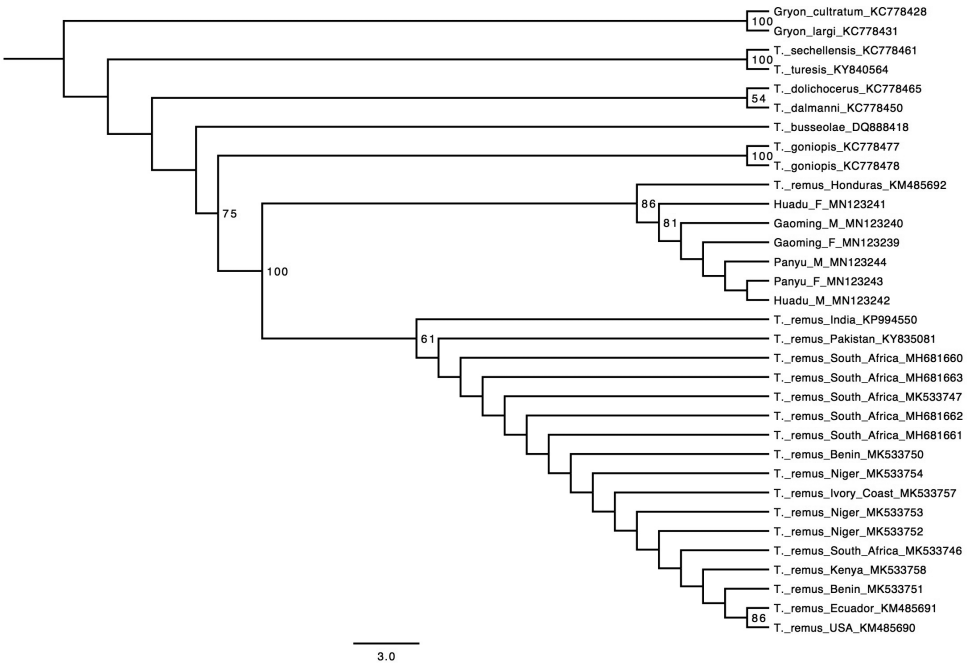

Figure 2. Phylogenetic analysis of *Telenomus remus* and related species by maximum likelihood method based on *COI* sequences. The six sequences generated from this study are indicated with codes and GenBank accession numbers (see Table 1). Boostrap values above 50 indicated on branches.

mass and parasitoid sex ratio in detail (Figure 3 and Table 2). Of the 28 egg masses, one-layer and two-layer egg masses seem to be dominant (13 of each), followed by three-layer egg masses (3). Of the seven parasitized egg masses, six were two-layer and one was one-layer. The number of eggs of each parasitized egg masses ranges from 64 to 163. The number of emerged *T. remus* adults from these parasitized egg masses ranges from 29 to 87, with 79.2%±2.14 females, resulting in an approximate 50.86%±2.24 parasitism rate per egg mass.

Discussion

Both morphological and molecular analyses in this study showed that the *Telenomus* species we found attacking *S. frugiperda* eggs in southern China is the promising biological control agent, *T. remus*. In China, this parasitoid species was reported to attack eggs of other *Spodoptera* species, including *S. litura* and *S. exigua* (Hübner) (Chou 1987, Tang et al. 2010), but its identity was not well established. Both our morphological and molecular data confirmed the presence of *T. remus* and its parasitism on *S. frugiperda* eggs in China. *Telenomus* species are generally small and morphologically simplified, rendering them difficult to distinguish and identify. However, in the case

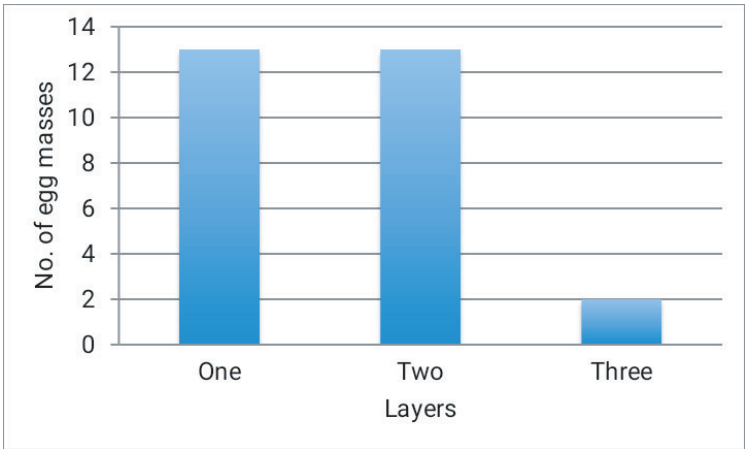

Figure 3. Number of layers in *Spodoptera frugiperda* egg masses.

Table 2. Status of parasitized *Spodoptera frugiperda* egg masses, field parasitism and sex ratio of *Telenomus remus* collected from Huadu, Guangzhou.

Layers per egg mass	No. eggs	No. parasitoids	% parasitism	No. female parasitoids	% female parasitoids
1	76	33	43.42105263	24	72.72727273
2	64	29	45.3125	22	75.86206897
2	103	62	60.19417476	48	77.41935484
2	99	48	48.48484848	38	79.16666667
2	163	87	53.37423313	66	75.86206897
2	79	44	55.69620253	37	84.09090909
2	113	56	49.55752212	50	89.28571429
Total	99.57±12.40	51.29±7.41	50.86±2.24	40.71±5.83	79.2±2.14

of using *T. remus* against *Spodoptera* pests, biological control practitioners should note that the color of the legs is sexually dimorphic (confirmed by *COI* sequences from both sexes) and not indicative of different species. We recommend that a strategy of integrated taxonomy should be applied to identify *Telenomus* species, especially in the case of introducing a parasitoid species into a new region for biological control of pests.

Eggs of *S. frugiperda* are usually laid in masses of approximately 100–200 eggs which are laid in one to three layers on the surface of a leaf (Guo et al. 2019) and the egg mass is usually covered with a layer of scales (setae) from the female abdomen. Our observations showed that *S. frugiperda* females usually lay one-layer and two-layer egg masses and rarely three-layers in cornfields (Figure 3). Although our current data do not allow us to analyze the preference of different egg masses attacked by *T. remus* due to small sample size (Table 2), this parasitoid species seems to be able to parasitize multiple-layer egg masses. We observed that even the bottom layer of a three-layer egg mass can be parasitized by *T. remus*. Determination of how architecture of *S. frugiperda* egg masses affects parasitism rates of *T. remus* requires further investigation and is crucial for mass rearing of *T. remus*.

Studies have shown that *T. remus* has great potential use in augmentative biological control against *S. frugiperda* in the field (Cave 2000). Our observations showed that natural parasitism rates of *T. remus* in corn fields in southern China can reach 30% and 50% for egg masses and per egg mass, respectively. In a study conducted in cornfields in Venezuela, parasitism rates of *T. remus* on *S. frugiperda* reached 90% through inundative release in corn cultivation areas (Ferrer 2001). Further research programs, such as long-term monitoring of natural parasitism and mass release in the filed, should be developed for *T. remus* to evaluate its impact on *S. frugiperda* populations in China.

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