

Patterns of abundance in the beneficial insects associated with the fall armyworm *Spodoptera frugiperda* (J.E. Smith) egg masses in selected Philippines cornfields

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Abstract

In February 2022, preliminary field surveys were conducted to characterize patterns of abundance in the beneficial insect populations associated with fall armyworm (FAW) egg masses in selected Philippines corn fields. It was found that approximately 47.64% of the field-collected FAW egg masses were naturally parasitized by hymenopteran egg parasitoids. The most abundant, and possibly a habitual egg parasitoid of FAW, was *Telenomus remus* Nixon. The other egg parasitoids, *Te. nawai* Ashmead and *Trichogramma chilonis*, were likely only incidental parasitoids. This was based on the data gathered for population density, daily emergence patterns, local occurrence, and parasitism rates of these parasitoids. On the other hand, the remaining 52.36% of these egg masses successfully hatched and survived into the first instars. Further, the generalist predator *Euborellia annulata* was collected from soil samples in corn fields but with low patterns of abundance, and a possible explanation for this observation is provided. Overall, *Te. remus* and *E. annulata* are potential biocontrol agents and can be further evaluated for their ability to cause significant mortality on the first-generation FAW egg masses and the 2nd instars in corn plants. Future studies are recommended to establish their host and prey relationships in local corn agricultural landscapes. They are discussed in the context of developing conservation bio-control (CoBC) tactics for preventing the establishment of FAW populations early in the cropping season.

Keywords: egg parasitoids, *Euborellia annulata*, predators, *Telenomus remus*, *Telenomus nawai*, *Trichogramma chilonis*

Introduction

Native to the Americas, the fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) is a corn pest recently found in Africa and Asia, including the Philippines (Navasero *et al.* 2019a; Kenis *et al.* 2023). It is an insect that undergoes a holometabolous type of development, hence its life stages consist of the egg, larva, pupa, and adult. Among these stages, only the larval

stage is economically destructive, as it damages both the vegetative and reproductive parts of corn. Mature larvae then pupate in the soil, and adult moths emerge from the ground to locate mates and then lay eggs (Luginbill 1928; Goergen *et al.* 2016). Eggs are oviposited by mated female moths on the surface of leaves and these hatch into the first instars or neonates

(Kasige *et al.* 2022). To manage FAW moth populations, sex pheromones are usually used to trap males and prevent mating with females (Meagher *et al.* 2019; FAO 2021). However, this tactic may not be effective against already mated, migrant female moths, which can initiate oviposition upon arrival (Ge *et al.* 2021; He *et al.* 2023). Mated female FAW moths are the ones physiologically attracted to corn plants to lay eggs (Wu *et al.* 2023). If corn farmers fail to prevent the colonization of early-season, mated females in their fields, then the next life stages that can be targeted for control are, namely the first-generation FAW egg masses and early larval instars. These life stages are usually managed using biological control approaches, which involve the use of living organisms like egg parasitoids and predators. Many species of these biocontrol agents are found in the Americas, Africa and recently, Asia (Kenis *et al.* 2023; Wyckhuys *et al.* 2024). Biological control, being non-chemical and environmentally safe, is preferred as an alternative to chemical control, which utilizes insecticides that can cause environmental and health problems when misused by farmers (Tambo *et al.* 2019). However, issues with biological control approaches are also known, which include biocontrol agents being slower-acting than insecticides to control insect pests as well as dispersing away from crop fields after augmentative release (Collier and Steenwyck 2004; Hajek and Eilenberg 2018). An exception to this, however, is seen in tropical rice fields in Indonesia, where highly abundant and locally occurring beneficial organisms early in the cropping season effectively controlled late-season insect pest populations (Settle *et al.* 1996).

In the Philippines, biocontrol began in the 1850s with the importation or introduction of exotic beneficial organisms into the country (Baltazar 1980). About 42 exotic species of insect predators and parasitoids were then introduced into the Philippines from the 1850s to 1960s to solve pest problems, an approach known as classical biocontrol (CBC) (Baltazar 1963; Vänninen 2005). In the 1980s, the concept of CBC was combined with augmentative biocontrol (ABC) (Hoy 2008), leading to the repeated mass release of laboratory-reared biocontrol agents into the fields. An example of CBC-ABC in the Philippines is the mass-rearing and release of an exotic species of *Trichogramma* to control the Asian corn borer *Ostrinia furnacalis* G., a major insect pest of corn (Tran and Hassan 1986). However, ABC in general also has some issues, such as the dispersal of biocontrol agents away from the release sites. It can target insect hosts or prey which are not amenable to insectary mass-production. It also has high over-all production and labor costs that raise questions in terms of sustainability (Collier and Steenwyck 2004). Hence, aside from ABC programs, alternative biocontrol approaches are also

needed for managing insect pest outbreaks. Improving crop production practices and promoting sustainable agriculture is one of the 17 important sustainable development goals (particularly SDG 2, zero hunger) mentioned by the United Nations to promote prosperity while protecting the planet (United Nations 2015). A potential alternative biocontrol approach is conservation biocontrol (CoBC) (Eilenberg *et al.* 2001; Khan *et al.* 2008). CoBC aims to enhance biological control by modifying the environment of locally-occurring beneficial organisms; for example, the release of scents or volatiles to attract and retain a high number of beneficial insect populations at specific time periods, addressing issues in the timing of pest and natural enemy colonization patterns (Lewis and Norlund 1984; Avelo *et al.* 2021; Kansman *et al.* 2023). In the Philippines, no study has yet explored using scents or volatiles for FAW biological control, which initially requires a good understanding of the foraging behavior of the most important biocontrol agent (Mills and Wajnberg 2008; Mills and Heimpel 2018). Moreover, the question of which beneficial insect will have the most potential for FAW CoBC has not yet been investigated in the country. Hence, preliminary field surveys were conducted to characterize abundance patterns of beneficial insect populations associated with FAW egg masses in corn-fields, with the assumption that abundant beneficial insects are the most effective biocontrol agents; thus, they might also have the most potential for developing a CoBC tactic for preventing FAW establishment early in the corn cropping season.

Materials and Methods

Survey sites

The Municipal Agriculture Offices (M.A.O.s) in Tanauan, Batangas; Tiaong, Quezon; and Antipolo City, Rizal, Philippines, were examined in order to identify corn-producing areas affected by recent infestations of fall armyworm (FAW), *Spodoptera frugiperda*. This led to the identification of 10 farm sites with varying crop development stages, varieties planted, pest control practices, and farm sizes. Field surveys were carried out on the 8th, 14th and 22nd of February 2022. Farm attributes and pest control practices were documented during five-minute informal interviews with farmers, covering field size, corn variety, plant age, other crops within 50 meters, and pest control methods (Table 1). In this research, farm sites were referred to by municipality and province initials (TB means Tanauan, Batangas; TQ means Tiaong, Quezon; AR means Antipolo City, Rizal), followed by the site number (S1 to S4 for sites one to four), e.g., TBSI refers to site 1 in Tanauan, Batangas.

Table 1. Smallholder cornfield sites last surveyed in February 2022

Sampling date	Site code	Location	Soil pH	Farm size [ha]	Days after planting	Purpose	Non corn crops	Pest control
Feb. 08, 2022	TBS1	Montania, Tanuan, Batangas	5.0	0.50	47	food	string beans	*
	TBS2	Montania, Tanuan, Batangas	5.0	0.25	33	food	string beans, bottle gourd, cassava	*
	TBS3	Santol, Tanauan, Batangas	5.4	0.40	46	feed	eggplant, string beans	*
	TBS4	Santol, Tanauan, Batangas	5.8	0.50	48	feed	squash	*
Feb. 14, 2022	TQS5	Lagalag, Tiaong, Quezon	5.4	10.00	48	feed	eggplant, bitter gourd, string beans, chili, papaya	*
	TQS6	San Juan, Tiaong, Quezon	5.8	1.00	27	feed	eggplant, bitter gourd, banana	*
	TQS7	San Juan, Tiaong, Quezon	5.4	1.00	12	feed	bottle gourd	**
	TQS8	Ayusan 1, Tiaong, Quezon	5.4	3.00	45	food	papaya	*
Feb. 22, 2022	ARS9	Manggahan, San Jose, Antipolo City, Rizal	5.0	0.50	66	food	rice	*
	ARS10	Kaysakat, San Jose, Antipolo City, Rizal	6.0	3.50	59	food	eggplant, okra, tomato	*

* chemical control,

** no control

Insect and soil samples collection

First, the presence of FAW larvae in corn plants was confirmed using larval characteristics reported by Navasero *et al.* (2019a). Second, FAW egg masses were collected by a three-person team over one hour per corn field. The team followed a zigzag route over the field, locating egg masses on leaves or stalks. Egg masses' physical characteristics were referenced from Luginbil (1928) and prior experience of the team in rearing an initial FAW population collected in 2019. Third, leaf sections with egg masses were cut, placed in glass vials, sealed with cotton plugs, and then labeled accordingly. Only one egg mass was placed per glass vial.

To collect soil-dwelling, nocturnal natural enemies associated with FAW egg masses, soil samples were taken from each field. A 20 m × 20 m plot from the cropping area was chosen, and four strata were identified: inside, border, 1 meter from border, and random. The inside stratum covered the selected farm plot; the border stratum was the border of the field nearest to the selected plot; the 1 meter from border stratum was the area which was 1 meter from the selected border containing non-crop plants like grasses and shrubs; and the random stratum was within the corn field but outside the selected plot. Ten soil samples (500 g per sample, collected 3 to 5 inches deep using a hand shovel) were taken from each of the four strata per field. All insect and soil samples were brought to the Insect Ecology Laboratory (Room 106), National Crop Protection Center, University of the Philippines Los Baños for immediate processing and data gathering.

FAW egg mass density and parasitism

In the laboratory, the total number of field-collected FAW egg masses was recorded per corn field, and each egg mass in the vial was labeled again for data gathering (24–26°C; 50–70% RH; photoperiod of 12:12 L:D hour). The numbers of healthy vs. parasitized egg masses were determined, with healthy egg masses having all their eggs hatched into first instars and parasitized ones having at least one emerging parasitoid during the 11-day observation period. Representative larvae from each type of egg mass were collected and reared in separate containers using clean corn leaves (IPB Var. 6) to confirm the presence of key morphological characters of FAW (Navasero *et al.* 2019a). All egg masses collected were then confirmed to be FAW using this reference.

Egg parasitoid identity and density

Emerging egg parasitoids from field-collected FAW egg masses were initially assessed based on key morphological characteristics and then classified into insect families Trichogrammatidae, Hymenoptera (designated as parasitoid A or P.A.), and Scelionidae, Hymenoptera (designated as parasitoid B or P.B.) (Goulet and Hubner 1993). Based on key characters, trichogrammatids and scelionids can be distinguished from each other easily, and so the numbers of P.A. and P.B. parasitoids emerging from each egg mass per collection site were recorded daily under a dissecting microscope for 11 days. Voucher specimens, which included six to nine male and female individuals, were preserved in 85% ethanol and sent to the Corn Insect Pest Laboratory, Institute

of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China for molecular identification using internal transcribed spacer 2 (ITS-2) gene, and for genitalia dissection of P.B., the most abundant egg parasitoid of FAW in all field surveys. For molecular identification, whole-worm genomic DNA (gDNA) of the parasitoid was extracted by the traditional salting-out method for subsequent PCR. The specific operation was to fully mix 625 µl of 2 M NaOH with 20 µl of 0.5 M EDTA and dilute it to 50 mL as a lysis buffer 1 (R1), and 1 M Tris-HCl of 2 ml was diluted to 50 ml as an extract 2 (R2). To fully lyse the whole parasitoid 100 µl R1 was added and heated at 95°C for 30 minutes. After cooling, 100 µl R2 was added and centrifuged for 5 minutes, and the supernatant was the whole insect gDNA. The sequencing comparison after PCR was completed by Sangon Bioengineering (Shanghai) Co., Ltd. Genitalia of male P.B. specimens were dissected, described, and then photographed using a Zeiss microscope.

In sites with few P.A., parasitoids were allowed to parasitize first *Corcyra cephalonica* Stainton, 1886 eggs to maintain a laboratory culture before preservation. However, attempts to rear different populations of P.B. using *C. cephalonica* eggs were unsuccessful. Finally, the percentage parasitism by P.A., P.B. or both on FAW egg masses was determined using Equations 1 and 2.

$$\% \text{ Parasitism by both P.A. or P.B.} = \frac{\text{Total number of FAW egg masses}}{\text{Total number of parasitized FAW egg masses}} \quad (1)$$

$$= \frac{\text{with at least 1 emerging egg parasitoid}}{\text{Total number of parasitized FAW egg masses}} \quad (1)$$

$$\% \text{ Parasitism by both P.A. and P.B.} = \frac{\text{Total number of FAW egg masses}}{\text{Total number of parasitized FAW egg masses}} \quad (2)$$

$$= \frac{\text{with at least 1 emerging egg parasitoid of P.A. and P.B.}}{\text{Total number of parasitized FAW egg masses}} \quad (2)$$

Predatory earwig identity and density

Each soil sample was placed on a clean plastic tray and observed under a dissecting microscope to identify predatory earwigs or other potential invertebrate natural enemies. The number and developmental stage of predatory earwigs in each soil sample were recorded and collected. Immature stages were carefully removed from the soil, transferred to properly-aerated plastic sauce cups with sterilized garden soil (50.15 g), and fed fish meal (Fry Mash 9, 1 g) for laboratory rearing until the adult stage. Adults were then sexed and preserved in 85% ethanol for species identification using available taxonomic keys (Srivastava 1976; Steinmann 1989).

Statistical analysis

To compare the mean numbers of FAW egg masses, parasitized egg masses and healthy egg masses between

provinces, Quade's test was used because the assumptions for ANCOVA were not satisfied. The Mann-Whitney test compared the numbers of P.A. and P.B. egg parasitoids emerging per corn field, as the t-test assumptions were also not met. To test differences in earwig *E. annulata* numbers between farm strata and sites, a two-way ANOVA was initially used. However, the Kruskal-Wallis test had to be performed due to unmet assumptions of normality and homogeneity. Dunn's test was used for pairwise comparisons between farm sites. All statistical tests were performed using the R software.

Results

Numbers of parasitized vs. healthy FAW egg masses

The average numbers of FAW egg masses, healthy egg masses and parasitized egg masses collected from the three provinces are presented (Table 2). Approximately 57.100 egg masses were collected, with 52.36% classified as healthy and 47.64% parasitized. In the provinces, the highest number of egg masses was recorded in Antipolo City, Rizal, which was not significantly different from Tiaong, Quezon and Tanauan, Batangas ($p = 0.123$, $F = 2.874$). Further, the highest number of parasitized egg masses was found in Tiaong, Quezon, but it was not significantly different from Antipolo City, Rizal and Tanauan, Batangas ($p = 0.856$, $F = 0.159$). In terms of healthy egg masses, the highest number was recorded in Antipolo City, Rizal, which again, was not significantly different from Tiaong, Quezon and Tanauan, Batangas ($p = 0.270$, $F = 1.585$).

Egg parasitoid identity and density

Molecular analysis using the ITS-2 gene indicated that P.A. from all 10 corn fields was *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), while P.B. collected in seven out of ten fields (TBS1, TBS4, TQS5, TQS6, TQS7, TQS8, and ARS10) was *Telenomus remus* Nixon (Hymenoptera: Scelionidae). Further, P.B. collected in three out of ten fields (TBS2, TBS3, and ARS9) was identified as *Te. nawai* Ashmead, 1904 (Hymenoptera: Scelionidae). Sequence alignment shows 93.44% for *Telenomus* and 95.77% identity for *Tr. chilonis* (Figs. 1, 2, respectively). From the gene sequence level of the species, it was possible to confirm that P.A. is *Tr. chilonis* and P.B. is *Telenomus*.

Dissection of the male genitalia of the P.B. samples also revealed that they belonged to the genus *Telenomus*, which is consistent with the study of Wengrat *et al.* (2021). Genitalia of the male parasitoid are rather short and broad, with three large digital teeth (dt)

Table 2. Mean numbers of fall armyworm (FAW) egg masses, parasitized and healthy egg masses collected during field surveys in three corn-producing regions: Batangas, Quezon and Rizal

FAW population	N of farm sites	Range	N of egg masses* Mean \pm STDEV	N of parasitized egg masses* Mean \pm STDEV	N of healthy egg masses* Mean \pm STDEV
Tanauan, Batangas	4	9–44	25,750 \pm 15,414	10,000 \pm 7,394	15,750 \pm 11,236
Tiaong, Quezon	4	36–88	66,250 \pm 23,415	45,500 \pm 25,040	20,750 \pm 25,040
Antipolo City, Rizal	2	73–130	101,500 \pm 40,305	25,000 \pm 29,698	76,500 \pm 10,607
		average	57,100 \pm 36,804	27,200 \pm 24,630	29,900 \pm 25,848
		<i>p</i> -value	0.123	0.856	0.270
		F	2.874	0.159	1.585

*not significantly different based on Quade's test

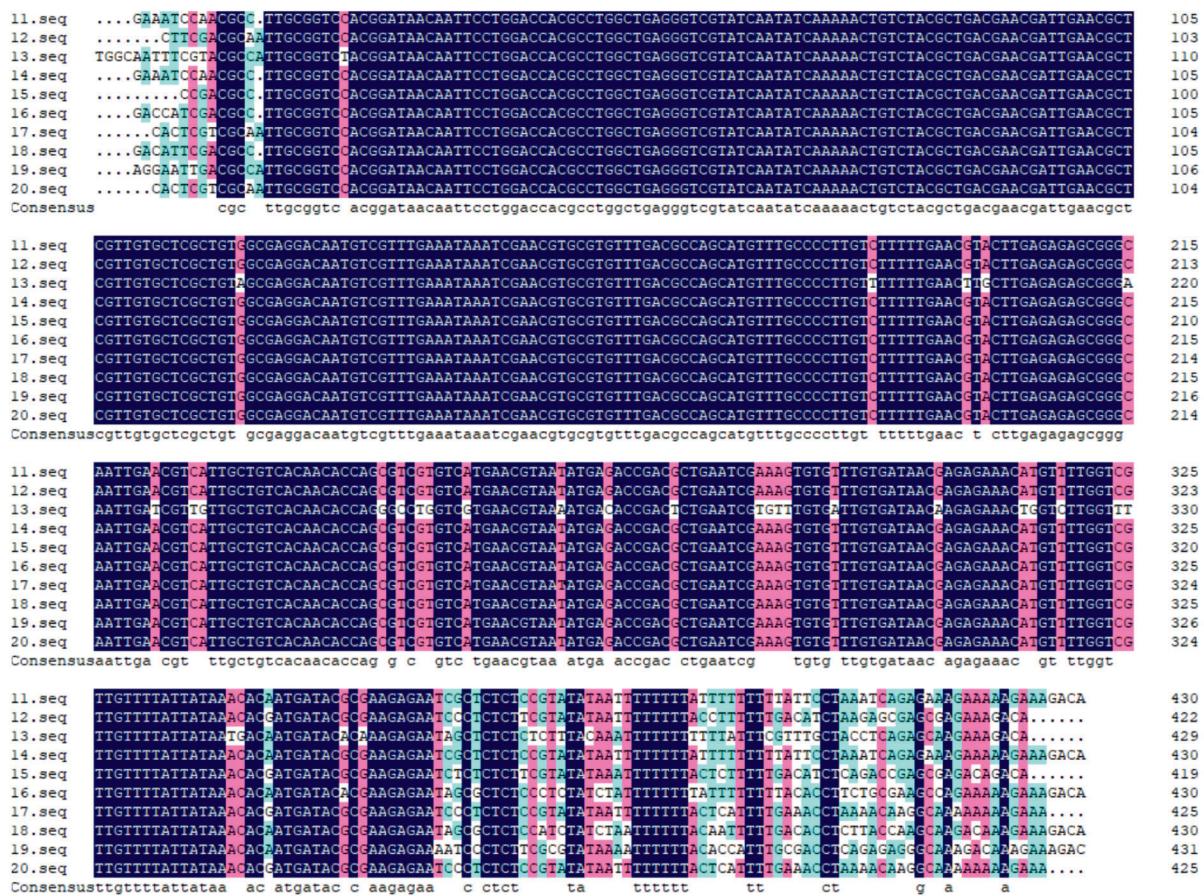


Fig. 1. Sequence alignment results of 10 *Telenomus* parasitoids from different sites and regions

(Fig. 3). Volsellar laminae are strongly pigmented. The aedeagus-volsellar shaft (av) appears to have two rods converging toward the digitii (di) before diverging for a short distance. On the aedeagus-volsellar shaft, in the latero-dorsal portion of digitii, there is a small projection that converges with the apex of the volsellar laminae, which has no central projection. However, the genitalia of *Te. remus* is indistinguishable from that of *Te. nawai*.

Average numbers of *Te. remus* were statistically higher than *Tr. chilonis* in six fields: TBS4 (17.522

vs. 0.795, respectively; $W = 780.5, p = 0.0301$), TQS5 (35.602 vs. 1.864, respectively; $W = 1246, p < 0.0001$), TQS6 (44.117 vs. 3.250, respectively; $W = 727, p < 0.0001$), TQS7 (11.028 vs. 1.806, respectively; $W = 497.5, p = 0.0145$), TQS8 (50.598 vs. 2.866, respectively; $W = 1446, p < 0.0001$), and ARS10 (12.908 vs. 0.315, respectively; $W = 5723, p < 0.0001$). Further, the number of *Te. nawai* was statistically higher than *Tr. chilonis* in only one field: TBS3 (18.594 vs. 0.437, respectively; $W = 305, p = 0.0012$). However, no statistical difference was found between the numbers of

1.seq	TCTCTAGGGCTCTTTCGAGAGTGTCGAGGCAAGCTGATAGCIGCCTAACAGAAAGCAGAAAATTTGATGATTGCTGTCTAGCTGGCGCGCGCTAACGGCTTGGAGATGAGCAGTACTTC	257
2.seq	TCTCTAGGGCTCTTTCGAGAGTGTCGAGGCAAGCTGATAGCIGCCTAACAGAAAGCAGAAAATTTGATGATTGCTGTCTAGCTGGCGCGCGCTAACGGCTTGGAGATGAGCAGTACTTC	249
3.seq	TCTCTAGGGCTCTTTCGAGAGTGTCGAGGCAAGCTGATAGCIGCCTAACAGAAAGCAGAAAATTTGATGATTGCTGTCTAGCTGGCGCGCGCTAACGGCTTGGAGATGAGCAGTACTTC	251
4.seq	TCTCTAGGGCTCTTTCGAGAGTGTCGAGGCAAGCTGATAGCIGCCTAACAGAAAGCAGAAAATTTGATGATTGCTGTCTAGCTGGCGCGCGCTAACGGCTTGGAGATGAGCAGTACTTC	259
5.seq	TCTCTAGGGCTCTTTCGAGAGTGTCGAGGCAAGCTGATAGCIGCCTAACAGAAAGCAGAAAATTTGATGATTGCTGTCTAGCTGGCGCGCGCTAACGGCTTGGAGATGAGCAGTACTTC	253
6.seq	TCTCTAGGGCTCTTTCGAGAGTGTCGAGGCAAGCTGATAGCIGCCTAACAGAAAGCAGAAAATTTGATGATTGCTGTCTAGCTGGCGCGCGCTAACGGCTTGGAGATGAGCAGTACTTC	249
7.seq	TCTCTAGGGCTCTTTCGAGAGTGTCGAGGCAAGCTGATAGCIGCCTAACAGAAAGCAGAAAATTTGATGATTGCTGTCTAGCTGGCGCGCGCTAACGGCTTGGAGATGAGCAGTACTTC	253
8.seq	TCTCTAGGGCTCTTTCGAGAGTGTCGAGGCAAGCTGATAGCIGCCTAACAGAAAGCAGAAAATTTGATGATTGCTGTCTAGCTGGCGCGCGCTAACGGCTTGGAGATGAGCAGTACTTC	259
9.seq	TCTCTAGGGCTCTTTCGAGAGTGTCGAGGCAAGCTGATAGCIGCCTAACAGAAAGCAGAAAATTTGATGATTGCTGTCTAGCTGGCGCGCGCTAACGGCTTGGAGATGAGCAGTACTTC	255
10.seq	TCTCTAGGGCTCTTTCGAGAGTGTCGAGGCAAGCTGATAGCIGCCTAACAGAAAGCAGAAAATTTGATGATTGCTGTCTAGCTGGCGCGCGCTAACGGCTTGGAGATGAGCAGTACTTC	249
Consensus	CTCTAGGGCTCTTTCGAGAGTGTCGAGGCAAGCTGATAGCIGCCTAACAGAAAGCAGAAAATTTGATGATTGCTGTCTAGCTGGCGCGCGCTAACGGCTTGGAGATGAGCAGTACTTC	
Consensus	CTCTAGGGCTCTTTCGAGAGTGTCGAGGCAAGCTGATAGCIGCCTAACAGAAAGCAGAAAATTTGATGATTGCTGTCTAGCTGGCGCGCGCTAACGGCTTGGAGATGAGCAGTACTTC	

Fig. 2. Sequence alignment results of 10 *Trichogramma chilonis* parasitoids from different sites and regions



Fig. 3. Genitalia of male *Telenomus* or P.B., the most dominant egg parasitoid of FAW egg masses in this study

Te. remus and *Tr. chilonis* collected at TBS1 (14.222 vs. 10.000, respectively; $W = 38, p = 0.8503$); as well as between *Te. nawai* and *Tr. chilonis* at TBS2 (10.389 vs. 0.278, respectively; $W = 134, p = 0.1480$) and ARS9 (3.698 vs. 0.0137, respectively; $W = 2553, p = 0.1679$) (Fig. 4).

Egg parasitoid parasitism and emergence

Overall, 47.64% (N = 27.200) of the field-collected egg masses were parasitized either solely or in combination with scelionid and trichogrammatid egg parasitoids. Particularly, *Telenomus* parasitoids contributed

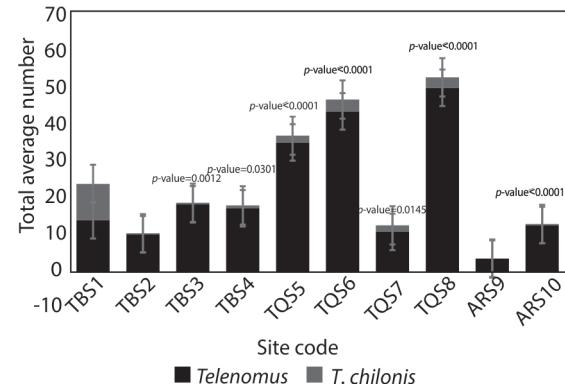


Fig. 4. Total average numbers of egg parasitoids *Telenomus* and *Tr. chilonis* adult individuals that emerged from FAW egg masses after 11 days observation period

an average of 56.976% parasitism on these parasitized egg masses (with the highest percentage recorded in ARS10, 82.609% by *Te. remus*); *Tr. chilonis* of 3.579% parasitism (with the highest percentage recorded in TBS1, 20%); and dual parasitism of 39.446%, either by the combination of *Te. remus* and *Tr. chilonis* or *Te. nawai* and *Tr. chilonis* (with the highest percentage recorded in TBS1, 80% by *Te. remus* and *Tr. chilonis*) (Fig.5).

Daily emergence of *Telenomus* and *Tr. chilonis* from FAW egg masses collected per corn field are also shown (Figs. 6, 7, respectively). *Telenomus* adults started emerging from egg masses as early as day 1

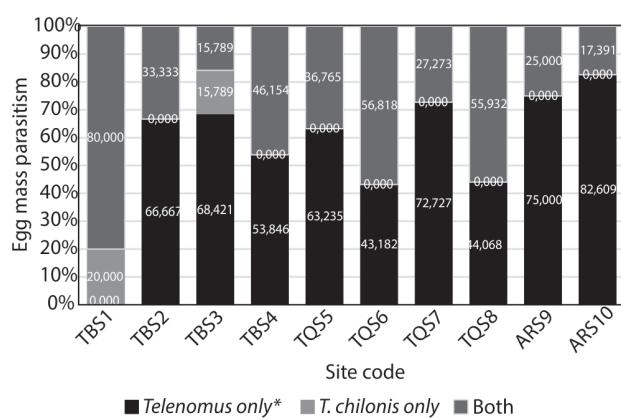


Fig. 5. Percentage distribution of egg mass parasitism by *Telenomus*, *Tr. chilonis*, or by both egg parasitoids in the total number of parasitized FAW egg masses per farm site

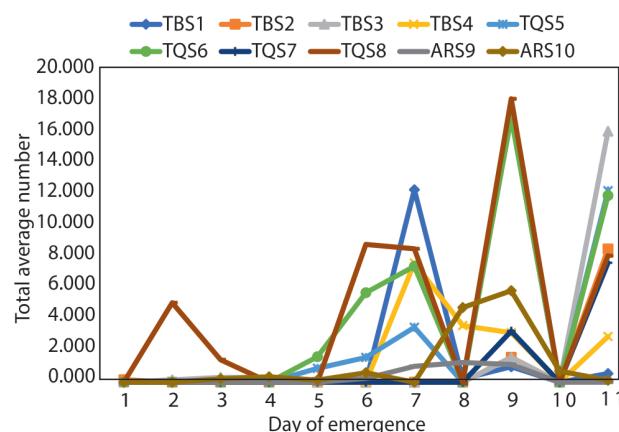


Fig. 6. Daily emergence of *Telenomus* parasitoids from field-collected FAW egg masses in the laboratory

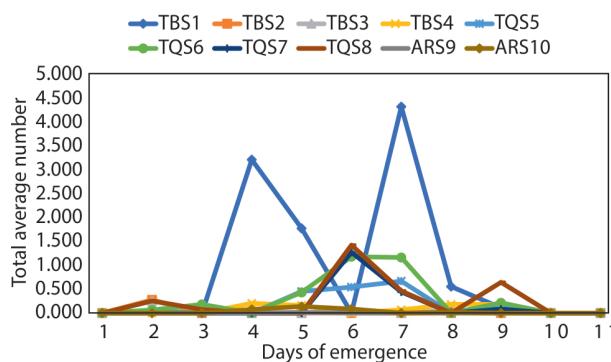


Fig. 7. Daily emergence of *Tr. chilonis* parasitoids from field-collected FAW egg masses in the laboratory

(TBS2, *Te. nawai*) and as late as day 9 (TQS7, *Te. remus*) of the observation period. The highest average number of adults emerging within a day was recorded in *Te. remus* in TQS8 on day 9 (18.317 individuals), with five emergence peaks observed over 11 days. On

the other hand, *Tr. chilonis* began emerging from days 2 to 6, with the highest average emergence at TQS8 on day 6 (1.427 individuals). About three peaks in adult emergence were observed in *Tr. chilonis* during the period.

Predatory earwig identity and density

Immature stages (the 1st to the 3rd instars) of the predatory earwigs *Euborellia annulata* (Dermaptera: Anisolabidae) were found in soil samples collected from TBS3, TQS5, TQS8, and ARS10 (4/10) (Fig. 8). Overall, the population density of *E. annulata* at the time of survey was low, averaging 0.035 individuals per soil sample. Among farm sites, the highest number was found at TBS3 with 0.225 individuals per soil sample, and with the median number of earwigs significantly different from other sites ($X_2 = 37.47$, $df = 9$, $p < .001$). Among farm strata, the average number of *E. annulata* was highest at the 1-meter farm stratum with 0.060 individuals per soil sample, but the median number was not significantly different ($X_2 = 4.775$, $df = 3$, $p = 0.1890$). Furthermore, no other predatory earwigs were collected in the corn fields surveyed except *E. annulata*.

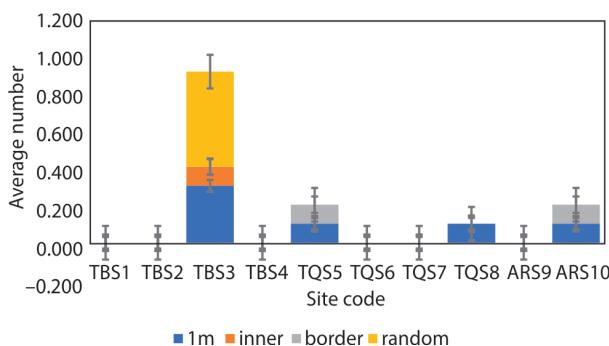


Fig. 8. Average number of *Euborellia annulata* per soil sample in surveyed corn farm sites

Discussion

It was found that 47.64% of the field-collected FAW egg masses were naturally parasitized by hymenopteran egg parasitoids, notably *Te. remus*, *Te. nawai* and *Tr. chilonis*. Among these parasitoids, the most abundant and potentially the most effective biocontrol agent was *Te. remus*, based on data on population density, daily emergence patterns, local occurrence, and parasitism rates. This observation aligns with studies in Asia and Africa, where *Te. remus* is also identified as the most dominant and widespread egg parasitoid attacking FAW eggs in corn plants, along with other

trichogrammatid parasitoids (Kenis *et al.* 2023). The widespread occurrence of *Te. remus* in Africa and Asia has often been attributed to local armyworm species present in these regions, for example, *S. litura* and *S. exigua* in China, and *S. tritumata* in Kenya (Liao *et al.* 2019; Kenis *et al.* 2019). This is not surprising, as *Te. remus* has been employed as a biocontrol agent against various species within the armyworm genus *Spodoptera*, in addition to FAW (Cave 2000). Further investigation is needed to determine if FAW eggs are regularly and consistently used by a large number of *Te. remus* individuals in local corn cropping areas. In the Philippines, the fact that other armyworm species such as *S. mauritia*, *S. litura*, *S. exempta*, or *S. exigua* may exist near corn or in non-cropping areas near cornfields (Gabriel 2000; Aguilon *et al.* 2015; Navasero *et al.* 2019b), also suggests the need to further investigate the host use pattern of this parasitoid under local conditions.

Conversely, *Te. nawai* possibly uses FAW egg masses only occasionally, resulting in low abundance on this host and minimal occurrence in the surveyed corn fields. This pattern may be due to the partial matching of cues it uses to locate its habitual host, possibly another species of *Spodoptera* less associated with corn farm sites (Fukuda *et al.* 2007; Arakaki *et al.* 2000; Walter 2003). In Africa and Asia, *Te. nawai* is rarely mentioned as an egg parasitoid of FAW, although unidentified *Telenomus* species emerging from FAW eggs have been reported in India and Indonesia (Shylesha *et al.* 2018; Wahyuningsih *et al.* 2022). In the Philippines, *Te. nawai* was one of the exotic egg parasitoids imported from Hawaii in 1928 to control lepidopteran pests (Baltazar 1963). Also, Cock (1985) documented *Te. nawai* in the Caribbean as a biocontrol agent for several *Spodoptera* species. *Te. remus* and *Te. nawai* may parasitize the same *Spodoptera* species but are biologically distinct (Cock 1985; Raveendranath 1987; Cave 2000). *Te. remus* was reported in the Philippines as an egg parasitoid of FAW in 2023 (Navasero *et al.* 2023), but a Philippines population of *Te. remus* emerging from *S. mauritia* eggs was documented as early as 1984 (Raveendranath 1987). Raveendranath (1987) also found that *Te. remus* develops faster and has higher fecundity on FAW eggs than *Te. nawai*, possibly explaining why *Te. remus* is more abundant than *Te. nawai* on FAW egg masses in the presented field surveys.

Further, daily emergence patterns of *Telenomus* parasitoids suggest that egg masses collected in the same corn field may vary significantly in the developmental stage. For instance, *Te. remus* at TQS8 started to emerge one day after the described survey, suggesting that some parasitized egg masses were old, and were oviposited by female FAW moths more than a week earlier. Other FAW egg masses might be newly-laid,

since *Te. remus* at TQS8 also had a peak of emergence on day 11. The same pattern might also apply to *Te. nawai*, based on their biology on FAW eggs (Raveendranath 1987). Moreover, since previous studies highlight the challenges in mass – producing *Te. remus* in insectaries, and therefore in developing cost-effective and sustainable ABC programs for FAW control (Colmenarez *et al.* 2022), it is recommended that other biocontrol approaches, particularly developing a CoBC tactic that can be incorporated into a pest management program be explored. Identifying the primary or preferred host of *Te. remus* populations in corn cropping systems in the Philippines could help develop attractants to manipulate its natural populations early in the cropping season. For instance, a study identifying kairomones from egg masses of a preferred host insect in Brazil was previously conducted to influence the behavior of a related scelionid egg parasitoid *Te. podisi* in soybean fields (Tognon *et al.* 2018, 2020).

Interestingly, *Tr. chilonis* was consistently found in FAW egg masses in all the corn fields surveyed in this study, but with very low patterns of abundance. In the Americas, similar patterns were seen with trichogrammatids *Tr. pretiosum* and *Tr. atopovirilia*, which seem to regularly parasitize FAW eggs but at low rates (Beserra *et al.* 2002). Nevertheless, the presented data indicates that *Tr. chilonis*' parasitism on FAW eggs is likely also incidental, and possibly associated with another host insect in corn cropping areas. In the Philippines, *Tr. chilonis* is commonly used as a biocontrol agent against *Helicoverpa armigera* Hübner, 1908, a polyphagous insect pest which is also found in corn fields (Javier *et al.* 2002, 2005). However, *Tr. chilonis*' host insect relationships in the Philippines are still not understood, as it has been initially documented to parasitize eggs of 10 different host insects across different agricultural environments (Alba 1988).

Furthermore, it was also observed that 52.36% of the field-collected FAW egg masses successfully hatched and reached the first instar stage. Under field conditions, newly-hatched first instars or neonates can engage in larval ballooning before feeding on corn leaves, increasing their exposure to other mortality factors (Sokame *et al.* 2020). This suggests that among young FAW larval instars, targeting the second instars for control, possibly by another biocontrol agent, would be more effective since the first instars would have naturally higher mortality rates. Lastly, the presence of *E. annulata* was confirmed in four out of the 10 corn fields surveyed, but its population density was also very low. In the Philippines, *E. annulata* populations in corn fields typically increase from tasseling to harvest, possibly explaining its low density in the surveyed fields (Situmorang and Gabriel 1988; Javier *et al.* 1993). Its absence in other fields may be due to unsuitable soil conditions in these areas characterized

as having dry, uncovered soil, as well as to the timing of the sampling of the presented research. According to Situmorang and Gabriel (1988), *E. annulata* prefers loose, moist soil, and is more active at night. Another explanation is that the population of *E. annulata* that might be present in corn plants at the time of the survey was not considered, although previous reports indicate that this beneficial insect prefers to stay in the soil during the day. Again, future studies are recommended on *E. annulata* in the context of the proposed CoBC tactic for preventing the establishment of the first generation FAW egg masses and the second instars. This includes studies on the role of corn pollen in *E. annulata*'s survival and reproduction in an attempt to develop attractants to manipulate its natural populations early in the cropping season, as well as studies on agricultural practices that will encourage the build-up of earwig populations in corn agricultural landscapes. In Brazil, adding corn pollen to the diet of *Doru luteipes* Scudder, a nocturnal predatory earwig associated with FAW in corn plants, enhanced the earwigs' survival and fecundity compared to those given only insect prey suggesting the important role of corn pollen in the diet of this biocontrol agent (Pasini *et al.* 2007; Marucci *et al.* 2017; Pacheco *et al.* 2023). Exploiting *D. luteipes*' omnivorous nature is a strategy to attract and maintain significant predator populations in maize fields (Pasini *et al.* 2007). Understanding the role of pollen in the survival and reproduction of *D. luteipes* is fundamental to its use and possible manipulation in the field.

Conclusions

The initial field surveys in February 2022 revealed that nearly half of the field-collected FAW egg masses were naturally parasitized by hymenopteran egg parasitoids, with *Te. remus* being the most abundant and likely a habitual egg parasitoid of FAW. In contrast, parasitism of *Te. nawai* and *Tr. chilonis* on this pest species appeared to be incidental, as suggested by their limited field occurrence and low density per egg mass in the cornfields surveyed. Likewise, *E. annulata* seemed to be less associated with cornfields, but this observation requires further validation as the sampling design for this predator may not fully capture the spatial range of its activity within the corn agricultural landscape. Although the data sets were preliminary and were collected from a limited number of sampling sites and cropping systems, it was still proposed and the potential of using *Te. remus* and *E. annulata* as biocontrol agents within the framework of conservation biological control (CoBC) for FAW IPM was discussed. This is an aspect for pest control that remains largely unexplored for small-scale corn farming in the Philippines.

It will complement the ongoing research on developing augmentative biological control (CBC) for FAW IPM to achieve long-term pest management, reduce farm input costs, and promote more sustainable practices tailored to the needs of smallholder farmers in the country.

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