

**FIRST REPORT OF *Telenomus remus* Nixon (Hymenoptera:Scelionidae)
PARASITIZING *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) EGGS,
ITS FIELD AND LABORATORY PARASITISM AND SOME BIOLOGICAL
PARAMETERS IN THE PHILIPPINES**

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ABSTRACT

Spodoptera frugiperda (J.E. Smith) is an invasive species that has recently emerged as a serious pest of corn in the Philippines. To address this issue, efforts are underway to develop biological control methods for managing this pest. This investigation led to the pioneering discovery of *Telenomus remus* Nixon, a potential parasitoid of *S. frugiperda* eggs, in cornfields located in Pangasinan, Laguna, and Quezon from January 2021 to June 2023. The findings revealed varying rates of parasitism by *T. remus* on *S. frugiperda*, ranging from 80% egg-mass parasitism from Tayabas, Quezon, 10% from Bocboc, San Carlos City in Pangasinan, and 45.7% for egg-masses from the Central Experiment station (CES), University of the Philippines Los Baños, College, Laguna. The progenies exhibited parasitism rates, under laboratory conditions, reaching as high as 87.5% for egg-masses and 83.1 % for eggs by *T. remus* collected from Barangay Bocboc, San Carlos City (Pangasinan). In the CES population, a 100% egg-mass and 90.3% eggs parasitism of FAW was recorded. A DNA barcode of 633 bp was successfully generated (GenBank Accession No. OR619425), providing valuable molecular identity information for future comparative studies. *T. remus* undergoes complete metamorphosis, with the development of various stages from egg to adult, and it typically takes 9-10 days for the Bocboc population and 11-12 days for the CES-UPLB population. These results highlight the potential of *T. remus* as a natural enemy capable of exerting effective control over *S. frugiperda* populations. This is the first report of *T. remus* parasitizing *S. frugiperda* eggs in the Philippines. The information gathered from this study contributes to the growing body of knowledge on biology and potential utilization of *T. remus* as a biocontrol agent against *S. frugiperda*. Further research is necessary to investigate on some aspects of its biology and mass rearing, its effectiveness in the field and practicality of deploying *T. remus* as a viable pest management strategy in corn cultivation in the Philippines.

Key words: biological control, egg parasitoid, fall armyworm, integrated pest management, parasitism

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is a highly destructive pest with a wide range of host plants, including economically important crops like corn, rice, sorghum, and sugarcane (Montezano et al. 2018; Navasero et al. 2019). Originating from the Americas, it has become a significant threat in Africa (Georgen et al. 2016; Prassana et al. 2018) and Asia (Sharanabasapa et al. 2018). In recent years, the fall armyworm has been detected in the Philippines (Navasero et al. 2019; IPPC 2019) and has rapidly spread throughout the country.

The economic impact of this pest is staggering, with estimated crop losses reaching up to 13 billion US dollars annually in Africa (Day et al. 2017). In the Philippines, the government projected economic losses of around P20 billion in 8,000 to 12,000 hectares of conventional corn varieties from June to October 2020 (DA Communications Group 2020). The fall armyworm is predicted to affect corn production in the country, reducing it from 2.5 million metric tons to 1.6 million metric tons (Miraflor 2020). This infestation poses a significant threat to feed millers, food processors, livestock and poultry raisers, traders and consolidators, as well as consumers of corn and its by-products.

Currently, chemical control remains the primary strategy for managing the fall armyworm, although some experiments on entomopathogenic nematodes (Felicitas et al. 2022) and bacteria (Latina and Caoili 2023), entomopathogenic fungi, and viruses (Montecalvo and Navasero 2021 a & b) have been conducted in laboratory settings. However, there is a lack of research on parasitoids. In the Americas and the Caribbean Basin, approximately 172 species of parasitoids and parasites have been reported (Molina-Ochoa et al. 2003). Among these, the egg parasitoid *T. remus* shows promise as a potential biological control candidate (Kenis et al. 2019; Dong et al. 2021); it is aggressive and efficient ability to parasitize the large egg masses with multiple, superimposed layers covered with scales that limit the attack from other parasitoids; it can penetrate all the layers of the egg mass resulting in 80-100% parasitization in laboratory studies (reviewed by Hay-roe et al. 2015). *T. remus* has been observed to attack eggs of various *Spodoptera* spp. as well as other species belonging to the families Noctuidae, Pyralidae, and Arctiidae of the order Lepidoptera (Wojcik et al. 1976; Cave 2000; Tang et al. 2003). Parasitism of FAW by *T. remus* has been reported in Africa, India, and China (Li et al. 2019; Ning et al. 2019; Wang et al. 2020). However, *T. remus* is not included in the earlier listing of associated natural enemies of FAW in the Philippines (Navasero et al. 2019; Navasero and Navasero 2020; Valdez et al. 2023).

Telenomus remus is widely utilized in numerous countries as a biological control agent against *S. frugiperda* and currently being mass reared and released in several Central and South American countries (Cave 2000; Hay-roe et al. 2015). This egg parasitoid is naturally found in peninsular Malaysia and Papua New Guinea (Wengrat et al. 2021). It has been introduced into the Western Hemisphere in multiple locations and times with successful establishment in the Caribbean, Venezuela, and Honduras (reviewed by Cave 2000; Hay-roe et al. 2015) and with evidence for permanent populations in Ecuador (Hay-roe et al. 2015). It has been used as a biological control agent against various *Spodoptera* pest species. Previous studies have examined the biology and ecology of this parasitoid (reviewed by Cave 2000). *T. remus* is known for its high fecundity and its ability to effectively parasitize eggs of *Spodoptera* spp. even when they are located deep within an egg-mass. It also possesses strong dispersal capabilities and efficient host searching behavior, making it suitable for augmentative biological control programs. Notably, *T. remus* was released on a large scale (several thousands of hectares) in Venezuela during the 1990s as part of an integrated pest management (IPM) program targeting fall armyworm (FAW) in maize (Colmenarez et al. 2022). These releases resulted in a significant reduction in insecticide use against FAW, ranging from 49% to 80%. *T. remus* achieved impressive rates of FAW egg parasitism, reaching up to 90% following the releases (Colmenarez et al. 2022). Early reports in Brazil, indicated parasitism of *S. frugiperda* egg-masses by *T. remus* reaching 54-99% in maize, cotton, and soybean fields (Pomari et al. 2013). Other research studies have demonstrated the effectiveness of *T. remus* as a biological control agent against various lepidopteran pests, particularly in agricultural systems (Agboyi et al. 2021). Its use offers an environmentally friendly and sustainable approach to pest management, reducing the reliance on synthetic pesticides and promoting the conservation of natural enemies in agroecosystems.

Molecular identification plays a crucial role in accurately identifying species within the genus *Telenomus*, primarily due to the challenges posed by the close morphological similarity among species (Wengrat et al. 2021). This is particularly important in biological control programs, where the correct

identification of biological control agents is essential for their success (Wengrat et al. 2021). DNA-based approaches have demonstrated their utility in characterizing closely related or cryptic species, thereby improving the accuracy of species identification in biological control work (Cengiz et al. 2016). Molecular identification techniques have been used to confirm the presence of *T. remus* in various regions (Agboyi et al. 2020; Wengrat et al. 2021). Identifying *T. remus* molecularly is critical for species resolution as a biological control agent.

In this paper, the first-time occurrence of *T. remus* in corn fields parasitizing eggs of *S. frugiperda* is reported in the Philippines. Morphological and molecular profiles and some biological parameters such as life stages, egg to larval periods and sex ratio of *T. remus* are presented.

MATERIALS AND METHODS

Field collection. Egg-masses of *S. frugiperda* were collected from corn fields in Luzon Island Philippines (Table 1). These were brought to the laboratory and individual egg-masses were placed in 10 cm diameter plastic plates and kept at 27°C, 50-60% RH and 12L:12D light cycle and checked daily for neonates of *S. frugiperda* or *T. remus* adult parasitoids. Emerging FAW larvae were promptly removed to prevent egg damage, transferred onto fresh leaves of 10-15 days after sowing (DAS) IPB var 6, reared to adulthood, and allowed to mate and produce eggs for parasitization. This was done for the population of *T. remus* collected from Barangay Bocboc, San Carlos City in Pangasinan and those from the Central Experiment Station, UPLB, Los Baños, Laguna. Those from Barangay Isabang, Tayabas, and Quezon were retrieved from preserved collections of FAW and associated organisms.

Table 1. Site of collection, date, host plant and number of egg-masses of *Spodoptera frugiperda* (J.E. Smith) with emerged adults of *Telenomus remus* Nixon parasitoids.

Locality	Collection Date	Host Plant	No. Egg Mass
Barangay Isabang, Tayabas, Quezon 13.96077 N 121.5584227 E	January 21, 2021	Corn	15
Barangay Bocboc, San Carlos City, Pangasinan 15.8934842 N 120.2664141 E	February 4, 2023	Corn	50
Central Experiment Station, Pili Drive, UPLB, College Laguna 14.09589 N 121.15117 E	June 5, 2023 June 9, 2023 June 14, 2023	Corn Corn Corn	49 22 23

Specimen processing and slide-mounting. The wings, head, thorax, gaster, and/or male genitalia of the specimens were dissected, processed, and mounted in synthetic Canada balsam separately but on the same microscope slide following the protocol of Polaszek and Kimani (1990). In some cases, whole insects were mounted. Voucher specimens were deposited in the insect repository of the National Crop Protection Center, University of the Philippines Los Baños (UPLB), UPLB Museum of Natural History, and the National Museum of the Philippines.

Species identification. The specimens were identified to species level by consulting the keys and species descriptions of Nixon (1937) and Polaszek and Kimani (1990). However, the morphology of the male genitalia of *T. remus* in the original description was not described but an illustration, excluding the basal ring, was provided (Nixon 1937). This line drawing, along with that of the other species

described therein and those illustrated by Polaszek and Kimani (1990), as well as the scanning electron micrographs of Masner (1980), were visually compared with the series of successfully slide-mounted male genitalia that may or may not have the basal ring attached.

To complement morphological identification, DNA barcoding was performed on a subset of the laboratory-reared population from Bocboc, San Carlos City (Pangasinan). The genomic DNA of adult *T. remus* was extracted using the GF-1 nucleic acid extraction kit (Vivantis Technologies) according to the manufacturer's protocol, with the addition of 8 ul of RNase per sample and an increase in centrifugation time from 1 minute to 5 minutes during the DNA precipitation step. Following DNA extraction, the mitochondrial cytochrome oxidase subunit 1 (*COI*), corresponding to the "barcode" region, was amplified using Taq DNA Polymerase (New England Biolabs, Inc) using primer pairs LCO1490 and HCO2198. The polymerase chain reactions (PCR) were conducted in Q-Cycler 96 Thermal Cycler (Hain Lifescience). The PCR conditions include an initial denaturation at 94 °C for 1 minute, followed by five cycles of 98 °C for 10s, 45 °C for 15s, 68 °C for 30s, and 35 cycles of 98 °C for 10s, 52 °C for 15s, 68 °C for 30s, as well as an extension at 68 °C for 5 minutes. The amplified DNA barcode was resolved in 1.2% agarose gel (Vivantis Technologies) and stained with Gel Red (Biotium) before viewing under UV light. Samples with positive amplification were sent to Macrogen (Macrogen Inc., South Korea) via Kinovett Scientific Solutions Co. (Quezon City, Philippines) for sequencing. The sequence chromatograms of each individual were checked, edited, and aligned to produce the consensus sequence using BioEdit Sequence Alignment Editor (Hall 1999). The nucleotide sequences were then compared with those deposited in GenBank using BLASTn (Altschul et al. 1990). This integrative taxonomy of combining a taxonomic key based on male genitalia with DNA barcoding using *COI* mitochondrial gene fragment is recommended for *Telenomus* species with their close morphological similarity (Wengrat et al. 2021).

Rearing of the host and parasitoid. The existing population of FAW at the Biocontrol Laboratory of the National Crop Protection Center, UPLB, originally collected from Gonzaga, Cagayan, was the source of egg-masses as host of the parasitoid. The larvae of FAW were reared on young leaves of corn until pupation. Pupae were pooled in Petri plates and when about to emerge, usually after seven days, the pupae were placed in an oviposition cage with 20% sugar solution dispensed in cotton balls inside a plastic plate and a few seedlings of corn in a bottle filled with water to maintain freshness served as oviposition substrates of female adults. Newly laid egg-masses of FAW on corn leaves were fastened onto a strip of paper in groups of 5-10 using a stapler and offered to ovipositing females of *T. remus* inside 500 ml Erlenmeyer flasks. The flasks were covered with two-ply paper towel kept in place using a rubber band. A cotton ball moistened with 20% sugar solution embedded inside a 1 ml Eppendorf plastic tube served as food to the adult parasitoids.

The strips of paper with stapled egg-masses of FAW were exposed for oviposition for 24 h to newly mated parasitoids. After 24 h exposure, the egg strips were removed and carefully cleaned with any parasitoids, placed into another Erlenmeyer flask for holding, and examined daily for emergence of parasitoids or host larvae. Neonates were trapped and removed from the flask to prevent cannibalizing the remaining parasitized eggs of *S. frugiperda*.

Strips of FAW egg-masses were exposed daily to adult parasitoids for oviposition up to four days after which the adult *T. remus* were preserved, coded, and kept as reference material. The cycle is repeated to maintain the parasitoids. Biological parameters observed were: number of parasitized egg-masses and eggs, duration of egg-adult period (in days), and sex ratio. The duration of egg-adult period was determined through daily observations from emergence to the adult stage of the parasitoids. All rearing was done at about 27 °C, 50-65% humidity, and photoperiod of 12:12 (L: D) h.

Documentation. Images of live and preserved specimens were captured using a Carl Zeiss Stemi 305 stereomicroscope (Zeiss Research Microscopy Solutions, Germany) equipped with a microscope

camera (AxioCam ERc5s, Zeiss Research Microscopy Solutions, Germany) wirelessly connected to an iPad. The images were processed and annotated using an imaging software (Labscope version 2.8.1, Zeiss Research Microscopy Solutions, Germany).

For the slide-mounted specimens, several partially focused images were captured at intervals of 2 mm by using a phase-contrast compound microscope with an integrated high-definition camera (Primostar, Carl Zeiss; Jena, Germany). These images were stacked into a single focused image by using Zerene Stacker (professional edition, version 1.99, Zerene Systems LLC; Washington, USA) and the free software Combine ZP. Images of better resolution were saved and annotated.

Statistical analysis. Percentage parasitism between field and laboratory reared *T. remus* were analyzed statistically using t-test. Male to female sex ratio was computed based on emerged males and females.

RESULTS AND DISCUSSION

Field and laboratory parasitism. Field parasitism of egg-masses of *S. frugiperda* by *T. remus* was highest (80%) from Barangay Isabang, Tayabas, Quezon, lowest (10%) from Barangay Bocboc, San Carlos City, Pangasinan, and moderate (45.7 %) from Central Experiment Station, UPLB on corn (Table 2). Recently, in China, natural parasitism rates of *T. remus* in corn fields could reach up to 30% and 50% for egg-masses and eggs, respectively (Liao et al. 2021). Other studies showed that the parasitic capacity of *T. remus* on *S. frugiperda* egg-masses in the field was high (Ferrer 2001; Bueno et al. 2010; Pomari et al. 2013). Earlier, report of parasitism rates of *T. remus* on *S. frugiperda* reached 90% through inundative releases in corn fields in Venezuela (Ferrer 2001).

Under laboratory conditions, it was observed that *T. remus* adults readily parasitized freshly laid egg-masses (but not thawed frozen eggs) resulting in parasitism rates up to 87.5% for egg-masses and 83.1% for eggs of *S. frugiperda* originally collected from Barangay Bocboc, San Carlos City in Pangasinan. Reports show that *S. frugiperda* usually laid eggs in masses in one to three layers on leaf surfaces, usually covered with a layer of scales from female abdomen serving as physical barriers to parasitoids (Dong et al. 2021). However, *T. remus* can penetrate all the layers of the egg-mass resulting in 80-100% parasitization in laboratory studies (reviewed by Hay-roe et al. 2015), as was observed in this study (Table 2). Further, *T. remus* females could overcome the scale covering on egg-masses of *S. frugiperda*, are more aggressive, with higher searching capacity and proportion of parasitism when compared to *Trichogramma dendrolimi* and *T. pretiosum* (Dong et al. 2021). This is attributed to the co-evolution between *T. remus* and *Spodoptera* spp. The parasitoid females are attracted to the kairomone (z)-9-dodecene-1-ol acetate component of the sex pheromone of *S. frugiperda*, secreted by the accessory glands located at the abdominal tip of female moths and attached to the scale-hair cover of eggs during oviposition (Dong et al. 2021).

Sex ratio in *T. remus*. Based on observations, the male and female sex ratio of *T. remus* in the laboratory is 0.99: 1.01 for the Bocboc population (Table 2). On the average, for every one male individual, there is a female individual. This was translated to 87.5% egg-mass and 83.3% egg parasitism of FAW.

In the CES population, the sex ratio of the progenies is similar to Bocboc (0.97:1.03) with 100% egg-mass and 90.3% eggs parasitism of FAW. The sex ratio is an important biological parameter in assessing performance of *T. remus* for use in biological control program for *S. frugiperda* and should be in favor of the females since the females are involved in host selection and oviposition. In instances where there is preponderance of males, as in field collected parasitized egg-masses from Isabang (Quezon) (2:1) and Bocboc (1.3:0.70) (Table 2), this can be explained either by competition between *T. remus* females which causes imbalance in favor of the males.

Table 2. Some biological parameters of *Telenomus remus* Nixon collected from Luzon Island, Philippines.

Locality	Field Collected				Progeny				
	Total number of Egg- Masses	No. of Egg- Masses parasitized	% Parasitism Egg- Mass	% Parasitism Eggs	Sex ratio (M:F)	Egg- adult period (days)	% Parasitism Egg- Mass	% Parasitism Eggs	Sex ratio (M:F)
Barangay Isabang, Tayabas, Quezon	15	12	80	ND	2:1	ND	ND	ND	ND
Barangay Bocboc, San Carlos City, Pangasinan	50	5	10	47.8	1.3:0.7	9-10	87.5	83.1	0.99:1.01
Central Experiment Station, Pili Drive, UPLB College Laguna	94	43	45.7	85.7	0.7:1.3	11-12	100	90.3	0.97:1.03

Sex ratio between males and females from and Bocboc and CES (field collected and progeny) are not significantly different by t-test.

Species verification of *T. remus*. Morphological examination revealed that the species of egg parasitoid from the three collection sites is *T. remus* (Fig. 1 A-E). This identification is confirmed using molecular identification. The DNA barcode of the egg parasitoid, consisting of 633 bp, was successfully generated (GenBank Accession No. OR619425) (Fig. 2). This barcode sequence provides invaluable insights into the molecular identity of the parasitoid and can serve as a reference for future comparative studies. The sequence alignment revealed a significant percentage identity of 99.3% to 99.8% with *T. remus* (accession number MT906647.1), suggesting that the samples in Bocboc, San Carlos, Pangasinan is *T. remus* (Table 3). The generated DNA barcodes were uploaded in the GenBank and assigned accession numbers OR619417 to OR619426.

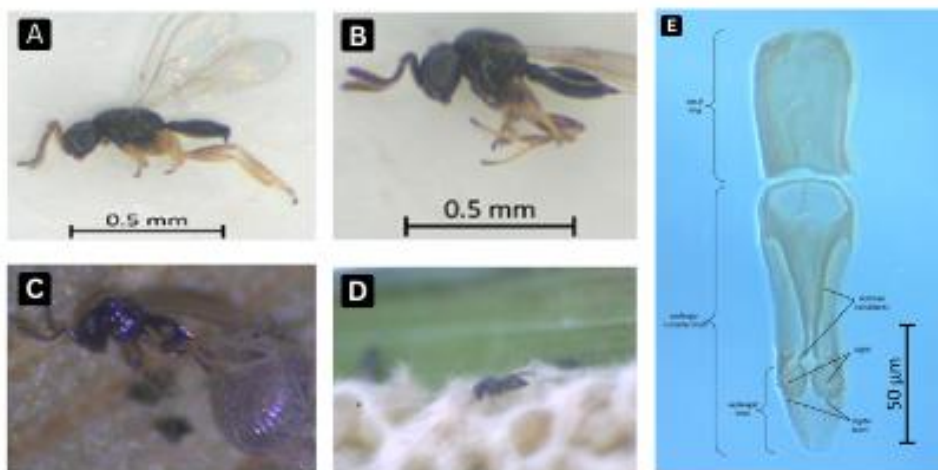


Fig. 1. *Telenomus remus* Nixon: (A) Male, (B) Female, (C) Newly emerged female, (D) Parasitized egg-mass with fully emerged adult, (E) Male aedeagus

Table 3. Summary of the most significant BLAST hit of the *cytochrome C oxidase I (COI)* barcode gene region of the *Telenomus remus* Nixon from laboratory-reared samples collected from Bocboc, San Carlos City, Pangasinan, Luzon, Philippines with the published reference sequence from GenBank.

Query Sequence	Query Length	Percentage Nucleotide Identity (%)	E-value	GenBank Accession Number	Description	Author/Country
OR619417	611	99.3	0	MT906647.1	<i>Telenomus remus</i> mitochondrion, complete genome (1792 to 3309 of the gene region)	Li et al. 2021/China
OR619418	611	99.3				
OR619419	611	99.3				
OR619420	611	99.3				
OR619421	611	99.3				
OR619422	633	99.8				
OR619423	633	99.8				
OR619424	633	99.8				
OR619425	633	99.8				
OR619426	633	99.8				

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1      10      20      30      40      50
GAGCAGGAATATTAGGTTTCAGCAATAAGAGCTTTAATTCGAATAGAACTTAGAGTTCC
60
AGGAATACTTATTGGAAATGACCAAATTTATAAATTCATTGTAACATTCACACGCATTT
120
ATTATAATTTTTTTTTATAGTTATACCAATTATACTTTGGAGGATTTGGAAATTTGACTTG
180
TACCATTAAATAAATGCTCCAGATATAGCATTCCCACGATTAAATATAAAGTTT
240
TTGATTATAATTCATCTTTAATTTTATTAATTTATAGAAATTTTTGGATCAGGA
300
ACAGGAACAGGATGAAGTGTATACCCACCTTTATCTTCACAATTAATCCATCAATTG
360
ATTTAACTATTTTTTCACTTCATATTGCAGGAATTTTCATCTATTCTTAGATCAATTA
420
TTTTTTATGTACAATTATTAATATATCAAATACCTCAATAAATAATTGAACATTTT
480
ACTTGATCTGTATTAATTAACAACAATTTTATTATTATTATCACTTCCAGTACTAGCAG
540
GAGCAATTACAATAATTTTAACTGATCGAACTTAAATACAGCATTTTTTAATCCAGC
600
AGGAGGAGGAGATCCAATTTTATACCAACATCTATTTTTGATTTTTTGGTCCAC
633
    
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Fig. 2. DNA barcode of *Telenomus remus* Nixon (GenBank Accession No. OR619425) from laboratory-reared samples collected from Bocboc, San Carlos City, Pangasinan.

Metamorphosis in *T. remus*. The developmental stages of *T. remus* consist of the egg, two larval stages, pre-pupa, pupa, and adult male and female (Fig. 3). The durations of the egg to adult periods were 9-10 days for the Bocboc population and 11-12 days for the CES-UPLB (Table 2). The egg is a typical encyrtiform type (Fig. 3A) inserted within the FAW egg during oviposition, in which the egg stalk (slender portion, with an arrow) sticks out of the host chorion; light colored and viewed properly using phase contrast compound microscope. At 2 d after exposure to ovipositing female parasitoids, a minute first instar larva (Fig. 3B) appeared globular, white, and transformed into a bigger and cylindrical second instar larva at 3 d (Fig. 3C). At 4 d (Fig. 3D), the second instar larva had consumed the host egg contents and excreted the meconium and is called pre-pupa. At 5d, the pre-pupa transformed into a pupa (Fig. 3E), which is light brown initially. At 6d to 8d (Fig. 3F to H) the male pupa blackened, visibly exarate and a male adult emerged at 9d (Fig. 3I). The female adult emerged at 10d (Fig. 3J), a day after the male adult. It was observed that only one adult *T. remus* emerged from a parasitized egg of *S. frugiperda* indicating that it is a solitary parasitoid, although superparasitism was also observed. These observations are consistent with previous reports of others working on *T. remus* as reviewed by Cave (2000).

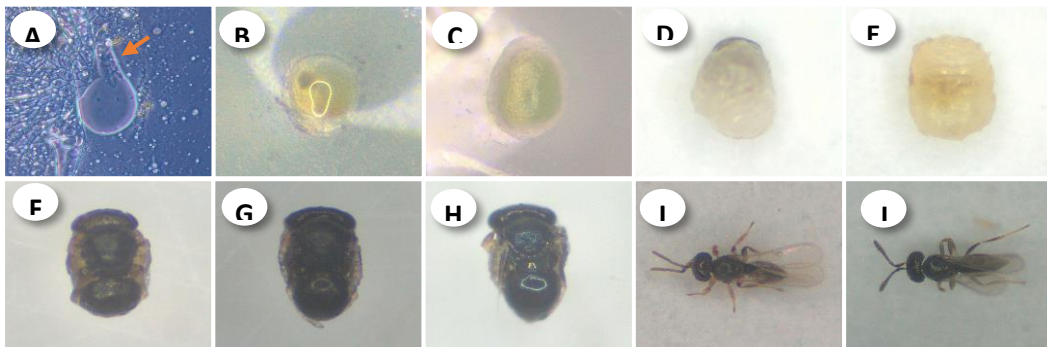


Fig. 3. Egg to adult periods of *Telenomus remus* Nixon from parasitized eggs of *Spodoptera frugiperda* (J.E. Smith): corresponding to the egg (A), first instar larva (B), second instar (C), late second instar with meconium, called pre-pupa (D), early pupal stage (E), advanced pupal stages (F,G,H), newly emerged adult male (I), and female (J). (Photo credits: M.D. Javier).

CONCLUSION

Integrative taxonomy and biological characterization in this study showed that a *Telenomus* species found parasitizing *S. frugiperda* eggs in the Philippines is *T. remus*. This first report lays the foundation for the inclusion of *T. remus* as an IPM tool against this pest in the country. Future studies should focus on the mass-rearing of *T. remus* on alternative hosts including field validation and assessment for cost effective mass production.

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