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New Record of the Egg-larval Parasitoid, *Chelonus formosanus* Sonan of Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) in the Philippines

Orlando Adona Calcetas[1)], Ravindra Chandra Joshi[2)*], Ankita Gupta[3)], Avinjikkattu Parambil Ranjith[4)], Mary Ann Madrid[5)], Joash Fameronag[5)]

[1)]Department of Agriculture, Regional Field Office-IVA-CALABARZON, Regional Crop Protection Center, Marawoy, Lipa City, Batangas, Philippines.
[2)]Philippine Rice Research Institute, Maligaya, Science City Muñoz, Nueva Ecija, Philippines
[3)]ICAR-National Bureau of Agricultural Insect Resources, P.B. No. 2491, H.A. Farm Post, Bellary Road, Hebbal, Bengaluru, Karnataka, India.
[4)]Insect Biosystematics and Conservation Laboratory, Ashoka Trust for Research in Ecology and the Environment, Bengaluru, Karnataka, India.
[5)]Batangas State University- The National Engineering University, Pablo Borbon Campus, 12 Rizal Avenue, Poblacion, Batangas City, Batangas, Philippines.

*E-mail: rcjoshi4@gmail.com

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Abstract

The egg-larval parasitoid on fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) have not reported in the Philippines. This paper summarizes the global biological information on two species of *Chelonus* and their role in the regulation of FAW. The FAW egg masses and larvae were collected in the corn plantations inside the Lipa Agricultural Research Station, Marawoy, Lipa City, Batangas. The collected egg masses and larvae were reared at the Entomology Laboratory of the Department of Agriculture-Regional Crop Protection Center IVA. The larvae of FAW were observed for the emergence of parasitoids. Two cocoons of the parasitoid from the rearing cages were obtained for species determination. Three adults from the emerged parasitoids, each male and female, were initially preserved and labeled. Color images of habitus and other morphological characters of the parasitoid were taken using a DSLR camera attached to an Olympus SZ61-60 microscope. This research found two species that emerged from FAW: *Chelonus formosanus* and the new record of *C. semihyalinus*. We also highlight the need for more research in the Philippines on exploring the diversity of indigenous natural enemies, the development of mass-rearing techniques, and their utilization to manage fall armyworms.

Keywords: *Chelonus semihyalinus,* corn, natural enemy, rice, parasitoid

Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), a transboundary invasive pest, is threatening global food, nutrition, and income security of millions of farming households because it feeds and develops on over 350 plant species belonging to 76 plant families, and causing economic damage to key food crops such as corn, sorghum, rice, soybean (Casmuz et al., 2010; Montezano et al., 2018; Overton et al., 2021; Perier et al., 2022).

It is native to the tropical and subtropical regions of North, Central, and South America (Kenis et al., 2023) and was first detected on the African continent in early 2016 in Nigeria (Goergen et al., 2016). Since then, FAW has invaded many territories worldwide, including almost sub-Saharan Africa and most tropical and subtropical Asian countries, including southern Australia, New Zealand, and some Pacific islands (Hruska, 2019; CABI, 2023). FAW is also regularly intercepted on imported plant material in Europe (EPPO, 2020). Regular updates on countries affected by FAW and the global distribution are available on CABI's FAW portal (www.cabi.org/ ISC/fall armyworm).

In almost all the countries FAW invaded, corn is the most susceptible crop (Rwomushana et al., 2018; FAO, 2022). Also, in the Philippines, its first reported damage on corn was in June 2019 in Piat, Cagayan (Navasero et al., 2019), and in rice only in May 2021 in Pateng, Gonzaga, Cagayan and subsequently reported in 13 other municipalities in the Cagayan Valley Region (Valdez et al., 2021; Valdez et al., 2023a). However, there are recurrent invasions of FAW in the Philippines in rice nurseries and direct-seeded rice fields in the earlier invaded areas, as well as in the new rice growing areas with the expansion of FAW distribution (Valdez et al., in press). DNA Barcoding studies revealed the existence of two FAW strains, viz, the Corn strain (C-strain) and the Rice strain (R-strain) (Pashley et al., 1985). Recent molecular analysis of the field-collected FAW larvae in the Philippines from corn and rice fields suggested that both strains can damage corn and rice (Valdez et al., 2023a).

R-strain has been reported from corn plants in Gonzaga, Cagayan (Navasero et al., 2019) and was also reported in Pakistan (Gilal et al., 2020; Yousaf et al., 2022) and Australia (Piggott et al., 2021). The possibility of the presence of FAW hybrids of the two strains in the monitoring areas needs to be verified for better management of FAW in relation to its host preference. Rice, a key food security crop for the Philippines and in Asia, has been reported elsewhere and is amongst the key hosts of the FAW (Pantoja et al., 1986; Wu et al., 2019). Based on the field study Pantoja et al. (1986) conducted, the increased larval density of FAW resulted in rice yield reductions due to the observed increased defoliation and decreased plant stand and panicle density. In rice production, the main concern with FAW specifically is to protect the two upper canopy leaves of the rice crop from being clipped or damaged during the reproductive stage and mostly during grain filling (Hardke and Lorenz, 2016). In the Philippines, the Department of Agriculture – Philippine Rice Research Institute (DA-PhilRice), together with the Centre for Agriculture and Bioscience International (CABI), and other agencies attached to the Philippines' Department of Agriculture is conducting intensive training, seminars and also disseminating knowledge-based FAW pest alert information materials to increase the rice farmer's awareness of the possible FAW invasion in rice and ways to manage it with nature-based solutions.

The use of parasitoids provides comparatively long-term efficacy at low cost, without inducing significant resistance, and is environment-friendly (Molina-Ochoa et al., 2003). Currently, more than 50 species of insect natural enemies are recorded for FAW; the majority, or 90%, are parasitic wasps (Hymenoptera). There are 19 recorded species of Trichogrammatidae followed by 18 species of braconids (Hymenoptera: Braconidae), wherein there are ten species of *Chelonus* Panzer, four species of *Coccygidium*, three species of *Cotesia* (3), and one species of *Campoletis sonorensis* (Cameron) (Amadou et al., 2018; Lopez et al., 2018; Li et al., 2019; Sisay at al., 2019; Tendeng et al., 2019; Firake and Behere 2020a, 2020b; Garcia-Gonzales et al., 2020; Gupta et al., 2020; Jaraleno-Teniente et al., 2020; Tang et al., 2020; Youssef, 2021; Sagar et al., 2022; Li et al., 2023).

Other natural enemies are the two species of tachinid flies *Drino quadrizonula* Thomson and an undetermined tachinid species (Diptera: Tachinidae), two species of minute pirate bug *Orius insidiosus* (Say) and *O. similis* (Zheng) (Hemiptera: Anthocoridae) and one pentatomid bug *Eocanthecona furcellata* (Wolff) (Hemiptera: Pentatomidae). Valdez et al. (2023b) reported two parasitoids on FAW namely, a larval-pupal parasitoid *Brachymeria lasus* (Walker) (Hymenoptera: Chalcididae) and a larval parasitoid *Copidosoma floridanum* (Ashmead) (Hymenoptera: Encyrtidae). These two parasitoids are new records on FAW in the Philippines and elsewhere.

Thus, adequate early preparedness and mitigation measures must be in place in the Philippines to counter any level of FAW incursions in rice by understanding the naturally occurring biocontrol agents in the Philippines, which are vital for developing ecologically-based integrated FAW management systems in rice and rice-based farming systems. This paper summarizes the global biology of *Chelonus formosanus* and its role in the regulation of fall armyworms.

Methods

The FAW egg masses and larvae that were collected in the corn plantations inside the Lipa Agricultural Research Station (LARES), Marawoy, Lipa City, Batangas (13° 57' 31.0 "N; 121° 09' 44.2" E), and also during the regular monitoring and surveillance activities.

The collected egg masses and larvae were reared individually in plastic cups at the Entomology Laboratory of the Department of Agriculture-Regional Crop Protection Center IVA (DA-RCPC-IVA). Fresh corn leaves were used to feed larvae and were replaced every two days. The larvae of FAW were observed for the emergence of parasitoids. Three adults from the emerged parasitoids, each male and female, were initially preserved and labeled correctly in glass vials containing 85% ethanol and later pinned using minuten. In addition, two cocoons of the parasitoid from the rearing cages were obtained for species determination.

Color images of habitus and other morphological characters of the parasitoid were taken using a DSLR camera attached to an Olympus SZ61-60 microscope. Digital photographs were tethered using Helicon Remote® (ver. 3.9.12 M) and combined using Helicon Focus® (ver. 7.7.4) stacking software. The parasitoids were identified by using available relevant taxonomic keys. The voucher specimens were deposited in the insect collections at the University of the Philippines Los Baños - Museum of the Natural History, Philippine Rice Research Institute, and DA-RCPC-IVA.

The parasitoid specimens were compared with the digital photos of the holotype female of *Chelonus semihyalinus* Ashmead, a voucher specimen received from the National Museum of Natural History (NMNH), Smithsonian, USA., since *C. semihyalinus* is

endemic to the Philippines. Preliminary biological information on parasitoid feeding habits, emergence from the host body, and parasitism in the Philippines were collected during the laboratory rearing of the parasitoid.

Results and Discussion

Chelonus species Diversity

In the present study, the only species of the parasitoid wasp that emerged from the parasitized FAW larvae was an egg-larval parasitoid, *Chelonus formosanus* Sonan (Hymenoptera: Braconidae, Cheloninae). The images of *C. formosanus* (Philippines specimens) were compared with the specimens from India and Southeast Asia (deposited at NHM, London). Figures 1A, 1B & 2 Species of the subfamily Cheloninae are easily recognized by the shape of their abdomen or ventral side of the carapace, which looks like an inverted bathtub or a turtle shell, presence of postpectal carina (Figure 2) (Baltazar, 1962; van Achterberg 1993).

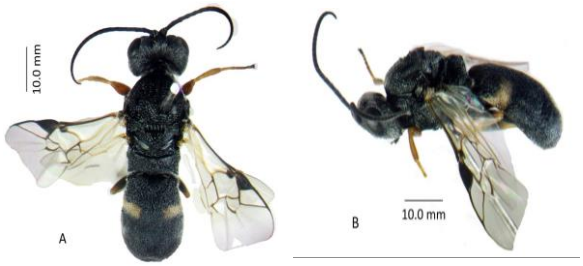

Figure 1**.** Female of *Chelonus formosanus* Sonan. A. dorsal, B. lateral

The parasitoid was originally described as one of the 18 braconids and ichneumonids identified by Sonan (1932) in Formosa (the old name of Taiwan), which is in the northern part of Taiwan. Currently, *C. formosanus* is distributed in China, Taiwan, and India (Firake and Behere, 2020a; Firake and Behere, 2020b; Gupta et al., 2020; Sagar et al., 2022; Shen et al., 2023). In India, Gupta et al. (2020) redescribed the species *C. formosanus* and compared it with all species of *Chelonus* in India and Southeast Asia. The front tibiae and tibial spurs of *C. formosanus* are all brownish (Figure 2). The parasitoid was originally described as one of the 18 braconids and ichneumonids identified by Sonan (1932) in Formosa (the old name of Taiwan). Currently, *C. formosanus* is distributed in China, Taiwan, and India (Firake and Behere, 2020a; Firake and Behere, 2020b; Gupta et al., 2020; Sagar et al., 2022; Shen et al., 2023). In India, Gupta et al. (2020) redescribed the species *C. formosanus* and compared it with all species of *Chelonus* in India and Southeast Asia.

Figure 2**.** *Chelonus formosanus* adult female, ventral

The antennal scape of *C. semihyalinus* and *C. formosanus* is black. The hind femur of *C. formosanus* is black or dark brown, but it is black in *C. semihyalinus.* The female of *C. formosanus* has 24–25 antennal segments (22–23 flagellomeres) (Figure 3A), while the male has 28 antennal segments (26 flagellomeres) (Figure 3B) (Gupta et al., 2020).

The female has a short tube-like ovipositor, occasionally concealed inside the carapace (Figure 4A). The fore wings of *the C. semihyalinus* SR1 vein are nearly straight (Figure 5A), while they are curved medially in *C. formosanus* (Figure 5B). However, the fore wings of both species are fuscous or dark and dull, while the hind wings are transparent or hyaline (Figures 5B, 5C, 6A, 6B). To our knowledge and based on available scientific databases, *C. formosanus* has never been reported from the Philippines, and therefore,

the Philippines is a new country record for *C. formosanus* (Yu et al., 2016).

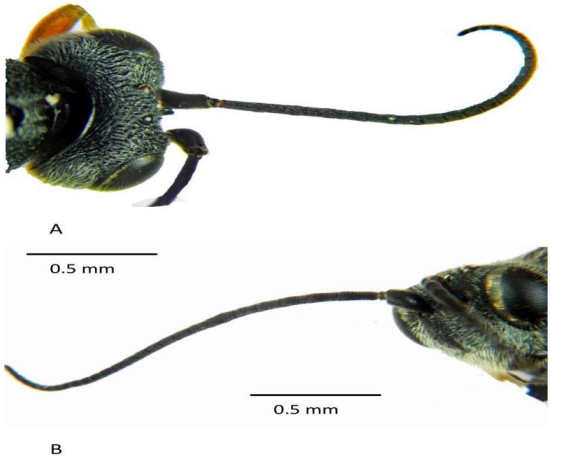

Figure 3. The antennae of *Chelonus formosanus,* adult: A. female, B. male

Figure 4. *Chelonus formosanus*; Adult female metasoma, showing the ovipositor (circled)

Figure 5. The adult female of *Chelonus semihyalinus* Ashmead. A. dorsal, B. lateral, and labels (Photograph of the holotype female courtesy of Dr. Floyd Shockley, NMNH, Smithsonian, USA).

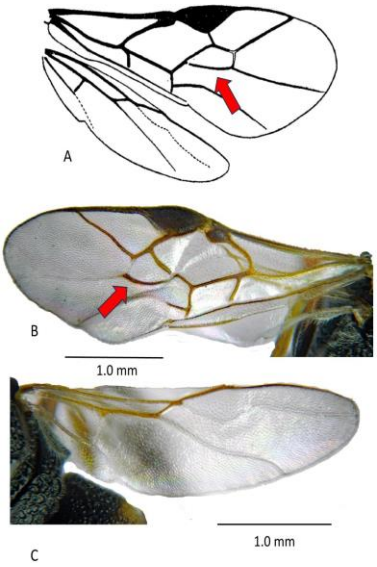

Figure 6. A. line drawings of fore and hind wings of *Chelonus semihyalinus*, by Baltazar (1962), *Chelonus formosanus*., B. digital photo of forewing, dorsal, C. digital photo of hindwing, dorsal

The only known endemic species, *Chelonus semihyalinus* Ashmead in the Philippines did not emerge from the parasitized FAW larvae. The three females of C. *formosanus* were compared with the digital photos of the holotype specimen of *C. semihyalinus*. Comparison showed that the dorsal metasoma of *C. formosanus* has two yellow spots near the base (Figure 1A) while in *C. semihyalinus* it has a transverse uninterrupted basal band (Figures 4A, 4B). Also, based on Ashmead (1904) original description of *C. semihyalinus* "the abdomen with transverse white band at base." In addition, all tibial spurs are white in *C. semihyalinus* (Figures 6A, 6B) (Ashmead, 1904) while the front tibiae and tibial spurs of *C. formosanus* are all brownish (Gupta et al., 2020) (Figure 2). Furthermore, after examining a range of specimens across India, it is observed that the two subbasal ivory spots of metasoma vary in size and the *C. formosanus* specimens from the Philippines fall within this range.

Alternate Hosts of Parasitoid

Chelonus has numerous host associations with many lepidopterous species, including some important agricultural pests in the world (Baltazar, 1962; Jones, 1985; Yu et al., 2016). Species of the genus have been successfully used to control other economically important agricultural pests such as common cutworm *S. litura* Fabricius, beet armyworm *S. exigua* (Hübner), and corn earworm or cotton bollworm *Helicoverpa armigera* (Hübner). However, *Chelonus* spp. reared in the laboratory using factitious host, have been used widely as biological control agents to control *Batrachedra arenosella* (Walker) (Lepidoptera: Batrachedridae), *Earias vittella* (Fabricius) (Lepidoptera: Noctuidae), *H. armigera* (Hübner) (Lepidoptera: Noctuidae), *Pectinophora gossy-piella* (Saunders) (Lepidoptera: Gelechiidae), *Prays oleae* (Bernard) (Lepidoptera: Praydidae) and *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) (Pawar et al., 1983; Baringbing, 1984; Hafez et al., 1980; Legner and Thomson, 1977; Arambourg et al., 1970).

Alternate hosts of *C. formosanus* are well known as it has been used successfully to control pests such as *H. armigera* (Liu, 2022), *Leucania loreyi* (Sonan, 1932; Sonan, 1944; Chou, 1981; Chen et al., 2009), *S. exigua* (Chou, 1981; Chen et al., 2009; Ji et al., 2013, Liu, 2022), *S. frugiperda* (Molina-Ochoa et al., 2003; Liu, 2022), and *S. litura* (Sonan, 1932; Sonan, 1937; Sonan, 1944; Rai, 1974; Rao and Patel, 1974; Chou, 1981; Chen et al., 2009; Liu, 2022). In India, *C. formosanus* was first reported during a survey of corn fields infested with *S. frugiperda* in 2018 in Karnataka and in 2019 in Andhra Pradesh (Gupta et al., 2019) and then in 2021 at Ludhiana, Punjab (Jindal, 2022). In ginger fields in Meghalaya State, Firake and Behere (2020b) recorded 5% larval mortality due to *C. formosanus*. A recent survey of FAW parasitoids in India showed that 80.46% larval mortality by the natural enemy complex (Sagar et al., 2022). The most predominant parasitoid is *Chelonus nr. blackburni* (Cameron) caused 49.24% larval mortality, followed by *C. formosanus*. However, in organic corn conditions in New Delhi, India, *C. formosanus* was the dominant parasitoid (12.55%), followed by *Chelonus nr. blackburni* (10.98%) and *Coccygidium* sp. (Hymenoptera: Braconidae) (4.85%) (Keerthi et al., 2023). Furthermore, *C. formosanus* was identified as FAW's most dominant egg-larval parasitoid in northern India (Sagar et al., 2022) and in India (Firake and Behere, 2020a; Gupta et al., 2020).

In the Philippines, the species *C. semihyalinus* has no record of the host information, and this species is only reported from the Philippines (Ashmead, 1904; Baltazar, 1962). According to Shenefelt (1973), *C. semihyalinus* is known to be distributed in the Indo-Australian and Pacific regions aside from the Philippines. Further studies might confirm or refute this distribution.

Currently, ten Chelonus species are associated with FAW, five occurring in Central America and four in Africa and Asia (Li et al., 2023). Based on the *Chelonus* species global distribution as a parasitoid of FAW, our study concludes that *C. formosanus* is the new record from the Philippines, with FAW as its so far known host in the Philippines. *Chelonus formasanus* association with FAW in the Philippines can have an important role in enhancing the country's biological control program against FAW.

***C. formosanus* Sonan in the Philippines**

The parasitoid *C. formosanus* larvae emerged from the 3rd or 4th FAW larvae (Figure 7A). The FAW larval body sometimes splits open in the middle due to the large size of the emerging parasitoid larvae. Earlier studies also reported similar signs (Madrid

and Fameronag, 2023). This caused the instant death of the host larva. After emergence from its host larva, the *C. formosanus* continuously fed on the dead FAW larvae, sucking its juice with its mouth until the host became small and completely dried (Figure 7B).

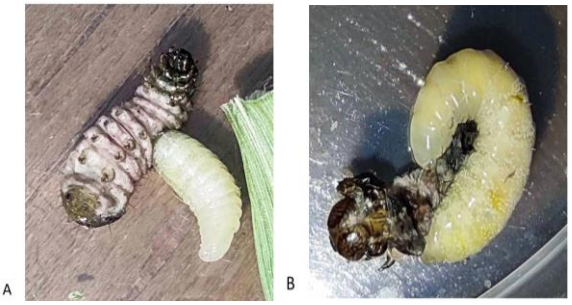

Figure 7. *Chelonus formosanus*: A. Larva emerging from the 3rd instar larva of fall armyworm, *S. frugiperda*, B. Larva feeding on the larva of fall armyworm, *S. frugiperda* after emergence

In contrast, *C. formosanus* grew larger (in length and width) outside the host's body after siphoning most of the larval body fluid. After this, it underwent pupation by creating a cigar-like whitish cocoon. *C. formosanus* is a predominantly solitary egg-larval and koinobiont endoparasitoid. Koinobionts allow the host to continue its development while feeding on it. Thus, the larval parasitoid feeds on the host while growing inside the host's body without causing it any damage until the moment larvae reach maturity, when they emerge from the body of the host, causing its death (Baltazar, 1962; Kalyanasundaram and Kamala, 2016). Furthermore, they lay their eggs into the host egg, but their larval development is delayed at the first instar until the host larva matures (Tang and Marsh, 1994).

Preliminary data from the FAW rearing showed that FAW eggs infested with *C. formosanus* in the field emerged from the host body after 12–14 days. However, the parasitized FAW larvae collected in the field emerged from the host body between 6–8 days. The randomly collected FAW egg masses on corn fields in the station during June 2023 were 8.38% (14 out of 167) were parasitized by *C. formosanus*. In contrast, the randomly collected FAW larvae also in the same month were 17.65% (6 out of 34) parasitized by *C. formosanus.*

Conclusion

In conclusion, the discovery of the *Chelonus formosanus* parasitoid associated with the FAW in the Philippines marks a notable advancement in developing nature-based solutions for agricultural challenges. The identification of this parasitoid holds significant promise for IPM strategies, aligning with the overarching goal of fostering agricultural sustainability. Moving forward, continuous research and the practical implementation of this newfound knowledge are imperative for addressing FAW-related challenges and promoting resilient and ecofriendly agricultural practices. It is recommended to foster collaboration in researching *C. formosanus* for FAW control, prioritize farmer education through extension services, and invest in sustainable practices to ensure the long-term food security of the Philippines.

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Declaration

Author contribution

Orlando A. Calcetas is the main contributor of this paper, Ravindra C. Joshi is the corresponding author, Ankita Gupta, A. P. Ranjith, Mary Ann Madrid, Joash Fameronag are co-author(s). All authors read and approved the final paper.

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Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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