

Mapping the potential of parasitoid species for biological control of *Spodoptera frugiperda* in Bali, Indonesia

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Abstract. *Ustriyana ING, Supartha IW, Susila IW, Yuliadhi KA, Sudiarta IP, Mokhtar ASB, Jamian SB, Mutiah B, Yasa IWS, Utama IWEK, Yudha IKW. 2024. Mapping the potential of parasitoid species for biological control of Spodoptera frugiperda in Bali, Indonesia. Biodiversitas 25: 3664-3671.* Parasitoids are natural enemies that can play a role in controlling the *Spodoptera frugiperda* (J. E Smith) (Lepidoptera: Noctuidae) pest population. This research aims to determine the morphological and molecular characteristics of these pests as well as distribution patterns in the field. Samples of *S. frugiperda* eggs and larvae were taken from corn (*Zea mays* L.) plants from several locations in Bali, Indonesia using the purposive sampling method. Identification of the *S. frugiperda* parasitoid was carried out using morphological and molecular methods. Based on the morphological and molecular characteristics, the types of *S. frugiperda* parasitoids in Bali are *Telenomus remus* as an egg parasitoid and *Microplitis prodeniae* as a larval parasitoid. The results of molecular analysis of the two types of parasitoids show that *T. remus* Bali has the same genetic sequence as *T. remus* from China and the larval parasitoid *M. prodeniae* Bali has the closest genetic sequence to *M. prodeniae* China, Hong Kong and India. According to the results of haplotype analysis, it is known that the *T. remus* parasitoid has one haplotype group with the Chinese *T. remus* sequence, while *M. prodeniae* has its own haplotype group when compared with sequences from the same species from several countries. Every sampling location in the Bali cornfield had the distribution of *T. remus*. Based on the percent parasitism value at each location point, *T. remus* is a very promising and effective parasitoid as a field mortality factor for *S. frugiperda* eggs.

Keywords: Biocontrol, corn plants, distribution, identification, percent parasitism

INTRODUCTION

Corn (*Zea mays* L.) is the second most important food crop after rice and an alternative staple food and a source of renewable bioenergy raw materials in Indonesia (Adiaha 2017). Therefore, the national demand for corn continues to increase but is not balanced by the rate of production (Varina 2018). The rate of corn production fluctuates from year to year, which is caused by the main limiting factor in the cultivation system, namely plant pests, the number of which varies from season to season (Sari et al. 2023). In early 2019, a new pest was discovered in Indonesia, which is an invasive pest of corn in the world, which was first found in West Sumatra, precisely in West Pasaman District, namely the corn armyworm *Spodoptera frugiperda* JE. Smith (Lepidoptera: Noctuidae) (Nonci 2019). This pest attacks the growing points of plants, which causes the failure of the formation of shoots or young leaves of the plant (Supartha et al. 2022b; Yudha et al. 2024). *S. frugiperda* originates from Central America and South America, then spreads to Africa and Asia. In 2020, this pest was found in Bali attacking corn plants owned by farmers, especially in Tabanan District, then spread to other districts in Bali

(Supartha et al. 2021). *S. frugiperda* spreads very quickly to several regions because the moth's flying ability reaches 100 km in one night (Nonci 2019). The adult pest is a strong flier and has a high cruising range (CABI 2019; FAO 2019), as well as good adaptability to new environments (Westbrook et al. 2015; Faulkner et al. 2017).

The attack of *S. frugiperda* in the Lampung area was reported to have a total damage intensity of 100% in corn crops 2 weeks after planting (Trisyono et al. 2019). *S. frugiperda* was first identified in Bali in 2019 (Supartha et al. 2021). There are two key factors for the strong spread of *S. frugiperda* throughout the world, namely its strong migration ability and its high proliferation potential (Sharanabasappa et al. 2018). Due to the support of these factors, *S. frugiperda* attacks on corn plants can cause significant losses if not protected (Nonci et al. 2019; Yudha et al. 2024). The significance of these losses is not only caused by decreased yields but also by crop failures. The decrease in sweet corn (*Zea mays saccharate* Sturt) yields in Bali due to *S. frugiperda* attacks can reach 28.08%, while glutinous corn reaches 25.04% (Sunari et al. 2022). The phenomenon of decreased corn production has a direct impact on decreasing farmers income (Supartha et al. 2021;

Sunari et al. 2022; Supartha et al. 2022b). In such conditions, farmers tend to take quick action by using synthetic pesticides. The use of synthetic pesticides has the potential to cause adverse effects such as environmental pollution, death of natural enemies, the emergence of resistance to target pests, death of non-target organisms, the emergence of secondary pest explosions, and the danger of poisoning to humans (Khan and Ahmad 2019). The potential for this incident is even greater if farmers' knowledge and recognition of the types, formulations, and methods of pesticide application in the field are still minimal.

Therefore, an environmentally friendly control method is needed, namely biological control, by utilizing natural enemies as biological agents, such as parasitoids, predators, and insect pathogens. Biological control techniques have a history of success in various parts of the world. The use of natural enemies in pest control is a potential control method to suppress the *S. frugiperda* pest population permanently and sustainably (Paredes-Sánchez et al. 2021). Several types of natural enemies that have been introduced to control *S. frugiperda* in several countries in the Americas, Africa, and Asia, especially from egg parasitoid species include *Telonomus remus*, *Trissolcus japonicus* (Peverieri et al. 2018), *Trichogramma* sp., *Gonatocerus* sp., *Nasonia giraulti*, *Philotrypesis pilosa*, *Megapharagma amalphantanum*, *Trichogramma dendrolimi*, *Trissolcus basalidis*, *Ceratobaeus* sp., *Leptopilina bouvardi*, *Ibalia leucospoides*, *Vanhornia eucnemidarum*, *Mesocomys trabalae*, *Pelecinius polyurator*, *Aprostocetus brevipedicellus*, *Monomachus antipodalis* (Chen et al. 2022). Meanwhile, the types of larval-pupal parasitoids include *Chelonus curvimaculatus*, *Charops ater*, *Coccygidium luteum*, and *Palexorista zonata* (Abbas et al. 2022). The focus of our research is to conduct an inventory and identify potential egg and larval parasitoid species as candidates for biological control agents of *S. frugiperda* and their distribution areas on corn in Bali.

MATERIALS AND METHODS

Sampling and rearing of eggs and larvae of *S. frugiperda*

In the field research, sampling was carried out using the purposive sampling method on groups of *S. frugiperda* eggs and larvae in corn fields in each district in Bali, Indonesia, i.e. Denpasar City, Gianyar District, Badung District, Klungkung District, Karangasem District, Bangli District, Tabanan District, Jembrana District and Buleleng District. Sampling of *S. frugiperda* eggs and larvae was done as much as possible depending on the area of the field and the number of *S. frugiperda* eggs and larvae in the field. Then morphological and molecular identification was done in the laboratory by observing the *Spodoptera* eggs that emerged. Samples of eggs and larvae found at the research location were then maintained at the Integrated Pest Management Faculty of Agriculture, Universitas Udayana, by placing them in transparent plastic cups and covering them with gauze. Each cup was labeled with the date of collection. One to two groups of *S. frugiperda* eggs were placed in one maintenance cup, then grouped according to the location of the sampling. Furthermore,

daily observations were made on the development of the eggs, whether they became imago or parasitoids emerged. Meanwhile, for the larvae, observations were made on the types of parasitoids that appeared.

Mapping analysis of geographic distribution of *S. frugiperda* parasitoid

Mapping of the *S. frugiperda* parasitoid was carried out in corn planting centers spread across Bali, Indonesia. The research location was carried out by recording each coordinate point of the predetermined sampling location (Supartha et al. 2021; Yudha et al. 2024). Environmental parameters including temperature, humidity, and altitude are also documented using the Open Camera application. Environmental parameters (extrinsic factors) including temperature, humidity, and rainfall are documented. Scattered data were visualized using Arc-GIS tools and combined with MaxEnt software.

Morphometric analysis parasitoid species of *S. frugiperda*

Morphometric identification of parasitoid species of *S. frugiperda* was carried out by observing parasitoid adults and larvae of *S. frugiperda* emerge from eggs using the identification guide according to Otim et al. (2021). Several species of parasitoid groups that had just emerged from the rearing were put into a 1.5 mL tube, and then put in the refrigerator at -40°C for 10 minutes. The parasitoids were then immersed in 5% potassium hydroxide in a plastic cup (diameter: 2.0 cm) for 24 hours and then washed 3-4 times with distilled water. Next, the specimens were examined with a Nikon SMZ 1500 binocular microscope, and photographs were made with a Keyence digital microscope (VHX-2000). The photos were lightly processed (especially cutting and background modification) in Adobe Photoshop CC 2018.

Molecular analysis parasitoid species of *S. frugiperda*

Molecular analysis was carried out on parasitoid species from parasitoids using COI and ITS primers. The stages of molecular identification include (i) Total DNA isolation, (ii) Polymerase Chain Reaction (PCR) amplification, (iii) DNA visualization, and (iv) Nucleotide sequencing and alignment (Alignment).

The sequencing process produces a nucleotide sequence in the form of an ABI file, then editing is carried out in the BioEdit version 7.2.5 program. The results of good editing are then entered into NCBI BLAST (Basic Local Alignment Search Tool) to determine the sample sequence homology with the closest species from the GenBank (www.ncbi.nlm.nih.gov) collection. The results of the percentage of base content were aligned using Cluster W, then a phylogenetic tree reconstruction was carried out using MEGA software version 11. The method used was a Maximum Likelihood Tree using bootstrap 1000x repetitions. Bootstrap values of 100 to 1000 replications are used to estimate the confidence level of a phylogenetic tree. The confidence in the rebuilt tree topology, which is dependent on the character distribution in the data, increases with the bootstrap value employed. The Larger the bootstrap value used, the higher the confidence of the reconstructed tree

topology, which is based on the distribution of characters in the data which is strongly influenced by random effects.

Haplotype analysis

Sequences were aligned and cropped using Clustal W (Kumar et al. 2018). A haplotype network from DNA sequences was constructed by minimum spanning network using PopART (Population Analysis with Reticulate Trees) software (Leigh and Bryant 2015).

Percent parasitism

Groups of parasitized *S. frugiperda* eggs and larvae were taken from the field and then collected in the laboratory, then the percent parasitism was calculated by observing the number of parasitoids that came out and the total number of *S. frugiperda* eggs or larvae observed using the formula (Wahyuni et al. 2017; Yuliadhi et al. 2022):

$$\text{Percent parasitism} = \frac{\text{Number of parasitized of eggs or larvae}}{\text{Number of eggs or larvae observed}} \times 100 \%$$

RESULTS AND DISCUSSION

Morphological identification of the *S. frugiperda* parasitoid

The findings of the research team after conducting observations in the field, egg and larval maintenance in the laboratory, that several species of parasitoids of *S. frugiperda* were found, especially the parasitoid species that attack *S. frugiperda* eggs, namely *Telenomus remus* (Figure 1). The parasitoid species that attack *S. frugiperda* eggs are commonly found in Bali. In addition to egg parasitoids, parasitoids that attack *S. frugiperda* larvae were also found, which are known to be the species *Microplitis prodeniae* (Figure 2). These parasitoids were found in almost all sampling locations.

The morphological characteristics of the *S. frugiperda* egg parasitoid specimen can be distinguished from the body being more or less cylindrical in shape and having a black and shiny color with a body measuring 0.6 mm and the body parts of the parasitoid specimen consisting of the head, wings, antennae, thorax, abdomen, and limbs (Figure 3.F). The antenna type in females is clavate, brownish

black with 10 flagellum segments and the base segment of the antenna scape is dark (Figure 3.A). The type of antenna in males is monoliform, which is shaped like a string of prayer beads and is round attached to the head, the color of the head is black and shiny, in males the flagellum segments have 11-12 antenna segments (Figure 3.B). The legs of this egg parasitoid have 3 pairs of legs, in females the front coxa and the middle femur, the back are dark black. The trochanter of the female adult is slightly brownish in color. The front, middle, and back of the tibia are dark brownish, and the tarsus is light colored with the metatarsus (apical) dark colored (Figure 3.C). Meanwhile, in males, almost all parts are pale, the coxa is dark brown and the trochanter is light brown. The front, middle, and back of the femur are darker in color, while the tibia and tarsus are yellowish brown and the metatarsus is dark (Figure 3.D). The wings consist of two pairs where the front wings are wider than the hind wings, with a transparent color, fine spines on the edges are clearly visible and brownish black on the costal edge of each wing, the tips of the front and hind wings are attached to the interior edge, there are hamuli which useful for connecting the two wings so that they stick together perfectly (Figure 3.E). Have a slightly convex thorax and is higher than the abdomen. The tip of the abdomen contains the genitalia in male and female ovipositors. Males have longer reproductive organs than females. Based on the characteristics above, according to Wengrat et al. (2021) the parasitoid specimens in *S. frugiperda* eggs are the same as *Telenomus remus* Nixon.

The parasitoid *Telenomus remus* (Hymenoptera: Scelionidae) originates from Peninsular Malaysia and Papua New Guinea (Wengrat et al. 2021). *T. remus* in biological control programs in several countries such as India, Pakistan, Australia, New Zealand and Venezuela has been successful in controlling pests, especially pests of the *Spodoptera* genus (Ferrer 2001). *T. remus* is described as an aggressive parasitoid due to its larger and stronger size (compared to *Trichogrammatoidea*), which allows it to penetrate all layers of the fall armyworm egg mass and parasitize more eggs (Cave 2000). *T. remus* females lay their eggs in the *S. frugiperda* egg group, then the parasitoid larvae will parasitize the *S. frugiperda* eggs (Nonci 2019).



Figure 1. *Telenomus remus* as egg parasitoid *Spodoptera frugiperda*



Figure 2. *Microplitis prodeniae* as parasitoid larvae *S. frugiperda*

The morphological characteristics of the larval parasitoid found in Bali are larger than the *S. frugiperda* egg parasitoid specimens with a body length of approximately 2 mm. The body of a larval parasitoid specimen consists of head, antennae, thorax, abdomen, wings, legs (Figure 4.A). The head, thorax and abdomen have a shiny black color (Figure 4.C). The antennae with 16 black segments, the antenna type on the parasitoid specimen *S. frugiperda* Bali is filiform which looks like a thread, the segments are the same size with a cylindrical shape (Figure 4.B). A pair of transparent wings with clear venation, the wing type has similarities to a wasp, the wings are attached to the abdomen (Figure 4.D). The femur is reddish brown, the tibia is clear to brownish below, the tarsus is clearly black. At the end of the abdomen there are reproductive organs. The characteristics above are similar to the morphology of *M. prodeniae* according to Yang et al. (2018). In previous research, two types of *S. frugiperda* egg parasitoids were reported, consisting of *Telenomus remus* (Nixon) and *Trichogramma* sp., as well as twelve types of larval parasitoids, namely *Chelonus Formosanus* Sonan, *Chelonus Oculator* F., *Chelonus Annulipes* Wesm., *Chelonus cautus* (Cresson), *Microplitis Manilae* Ashmead, *Microplitis Marshallii* Kokujev, *Euplectrus corriemoreuae* Hansson, *Compsilura concinnata* (Meigen), *Sarcophaga* sp., *Macrocentrus* sp., *Exorista* sp., and *Megaselia* sp. (Herlinda et al. 2023). *M. prodeniae* has never been reported in Indonesia, thus *M. prodeniae* is the first larval parasitoid recorded in Bali-Indonesia. *M. prodeniae* Rao & Kurian (Hymenoptera: Braconidae) is a larval parasitoid that parasitizes its host by consuming the host's body tissue and hemolymph, so that the parasitized host does not pupate and eventually dies (Yang et al. 2018).

Molecular identification of the *S. frugiperda* parasitoid

Exploration of the Cytochrome Oxidase I (COI) gene sequence in parasitoid specimens on *S. frugiperda* Bali eggs. From the results of PCR amplification, the COI fragment of parasitoid specimens in *S. frugiperda* Bali eggs can be amplified well using the forward primer HCO-2198 and the reverse primer LCO-1490. Factors that cause the success of PCR amplification are the purity of the extracted DNA, the composition of the reagent material, and the conditions Correct PCR, especially in the annealing process (primer attachment). Based on the attachment location of the forward primer HCO-2198 and the reverse primer LCO-1490, it can amplify the Cytochrome Oxidase I gene of 642 bp. Good PCR amplification results are continued to the DNA sequencing stage.

Based on the COI gene, a homology search was carried out in GenBank using BLAST software, and several nucleic acid sequence data of the *Telenomus remus* species were found in GenBank data which were similar to parasitoid specimens in *S. frugiperda* Bali eggs. From the results of the sequence alignment that has been carried out, the COI gene nucleic acid in *T. remus* that shows the highest homology is *T. remus* (MW419148.1), *T. remus* (MW243584.1), and *T. remus* (MN123239.1) with levels identical, namely 99.69%. The genetic characters of parasitoid specimens in *S. frugiperda* Bali eggs are close to the genetic characteristics

of *T. remus* in GenBank based on the level of homologous DNA sequences of the COI gene and phylogenetic analysis. Based on the Maximum Likelihood Tree with 1000x bootstrap replication, a phylogenetic tree construction was obtained from parasitoid samples from parasitoid specimens on *S. frugiperda* Bali eggs.

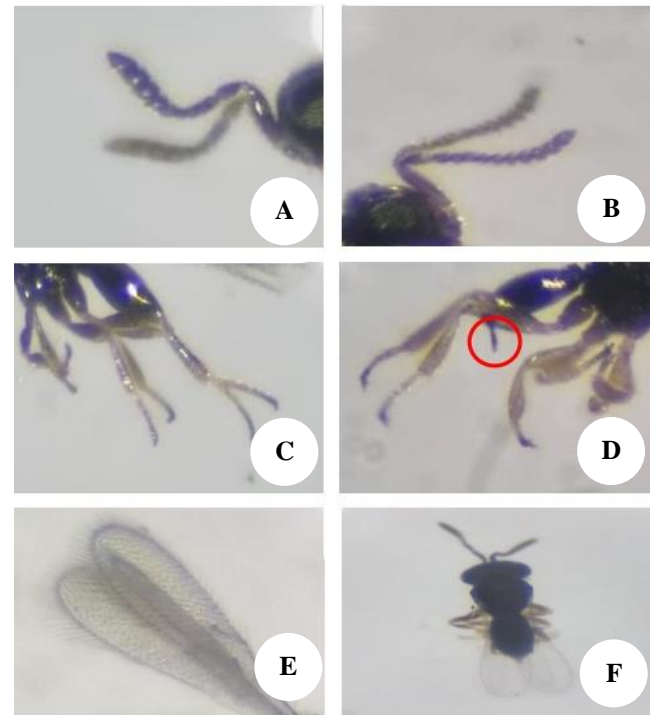


Figure 3. Morphological characteristics of the parasitoid insect *T. remus*. A. Male adult antennae; B. Female adult antennae; C. Female limbs; D. Male limbs, E. Parasitoid adult wings; F. Dorsalis adult parasitoid specimens

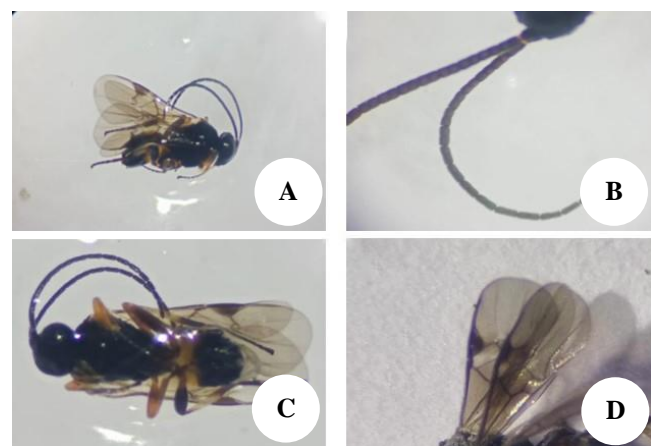


Figure 4. Morphological characteristics of the *Microplitis prodeniae* larval parasitoid insect: A. Side view of the body; B. Shape of the parasitoid specimen's antennae; C. Dorsal view of the insect's body; D. Parasitoid specimen's wings

Each phylogeographic region has a unique nucleotide sequence due to geographic isolation known as local populations (native populations). The results of the analysis presented in the phylogenetic tree (Figure 5) show that the egg parasitoid (*T. remus*) sample found in Bali (PP860918.1) is genetically close to the *T. remus* sequence from China (MW419148.1), this is a new finding in this study because no one has reported the parasitoid species of the egg parasitoid *S. frugiperda* from the Bali-Indonesia sequence in GenBank. It can also be seen in the phylogenetic tree, sequences from Bali and China have different groups between several countries in Asia, Africa, and America. Outgroup groups are very necessary to provide polarization of characteristics or traits, namely apomorphic and plesiomorphic characters. Apomorphic characters are characters that change and are inherited from the ingroup, while plesiomorphic characters are primitive characters found in the outgroup.

Parasitoid DNA sequencing results of *S. frugiperda* larval parasitoid specimens analyzed in the COI gene were searched for homologies in GenBank using BLAST. Several nucleic acid sequence data of the *Microplitis prodeniae* species were found in GenBank data which had similarities to specimens of the Balinese *S. frugiperda* larval parasitoid. According to the results of the sequence alignment that has been carried out, it shows that the nucleic acid of the COI gene of the larval parasitoid specimen of *S. frugiperda* Bali shows the highest homology to *M. prodeniae*. In this study, an exploration of the COI gene sequence in *M. prodeniae* was carried out. In the PCR amplification process, the forward primer HCO-2198 and the reverse primer LCO-1490 can be amplified well. This is due to the purity of the extracted DNA, the composition of the reagents, and the correct PCR conditions, especially in the annealing process (primer attachment). As a result of the PCR amplification process, the COI gene produces 665 bp. Next, the DNA sequencing process is carried out.

Based on phylogenetic tree analysis using the Maximum Likelihood Tree method with Bootstrap 1000x repetitions, it shows that analysis using COI gene fragments will

produce geographic kinship groupings (phylogeography). In the phylogenetic tree (Figure 6), it is known that the larval parasitoid specimens are in the same clade as the Chinese samples (KX096874.1), (MW739950.1), (MW250775.1), and Hong Kong (ON368856.1).

The haplotype appearance was analyzed based on egg and larva parasitoid sequences found in corn planting centers in Bali, then compared based on the same species taken from sequences at NCBI showing the results that the *T. remus* sequence had five haplotypes (Figure 7.A), where the sequence *T. remus* found in Bali shows one haplotype group with the *T. remus* sequence from China with one base difference to the haplotype group from African countries, Uganda, Kenya and Nigeria, then the haplotype that is furthest from the *T. remus* Bali sequence is the *T. remus* sequence found in Brazil.

The *M. prodeniae* sequence found in Bali shows three haplotypes (Figure 7.B). The Bali *M. prodeniae* sequence shows closeness to the Chinese and Hong Kong *M. prodeniae* haplotype groups, then the India *M. prodeniae* sequence has its own haplotype with the furthest genetic distance to the *M. prodeniae* sequence found in Bali.

Distribution of the *S. frugiperda* parasitoid in Bali

Based on observations of several locations in Bali, almost all of the affected locations as in Figure 8 were found at various altitudes, namely >0 meters above sea level to <500 meters above sea level in several areas in Bali. *T. remus* parasitoids have various categories in their distribution patterns in the field, *T. remus* parasitoids regularly spread in Denpasar. The areas of Gianyar, Badung, Klungkung, Karangasem, Bangli, Tabanan, Jembrana and Buleleng have clustered distribution patterns. The *M. prodeniae* larvae parasitoid has a regular distribution pattern, which occurs in the Denpasar and Karangasem areas, in the Gianyar, Badung, Klungkung, Bangli, Tabanan, Jembrana areas, no parasitoids were found so there is no distribution pattern.

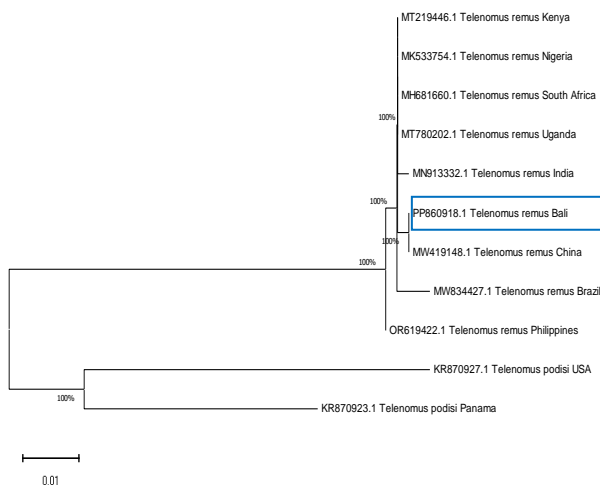


Figure 5. Phylogenetic tree *Telenomus remus* (The blue box indicates the *T. remus* sequence described in this study)

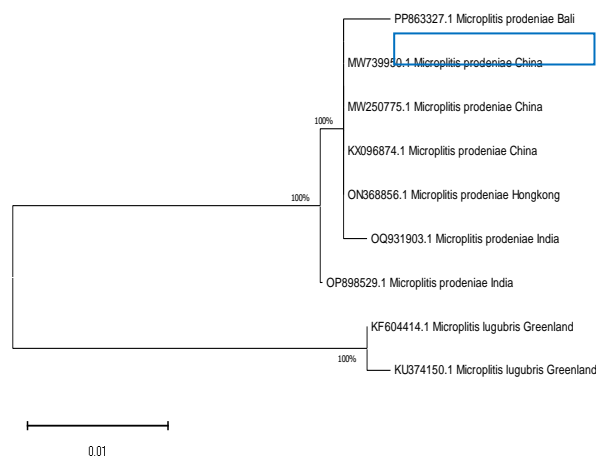


Figure 6. Phylogenetic tree *Microplitis prodeniae* (The blue box indicates the *M. prodeniae* sequence described in this study)

The ability of parasitoids to spread and evenly distribute natural enemy types can be due to environmental conditions, and food factors that are quite abundant. Apart from that, agricultural land management can be a factor in the ability of parasitoids to spread, the ratio factor between male and female parasitoids also influences the high level of parasitization of the host. The higher the ratio between female parasitoids and hosts, the higher the number of eggs laid in each host. Nonci et al. (2019) stated that control with less efficient insecticides can kill natural enemies, so the distribution of parasitoids is relatively low.

The presence of richness and abundance of individuals, as well as the diversity of parasitoids in agricultural ecosystems to regulate their host populations is influenced

by various factors such as altitude, cultivation practices carried out landscape, complexity of the agroecosystem, and planting patterns applied. A community is said to have high species diversity if the community is composed of many species. In general, the number of species will be influenced by temporal and spatial factors. The dominant ecosystem found in the field is a polyculture planting pattern with corn as the main crop and combined with other plants such as plants from the Fabaceae family. Sampling was also carried out at various altitudes ranging from <500 masl to >1000 masl so that the species diversity of this study describes the planting pattern and topography of the island of Bali.

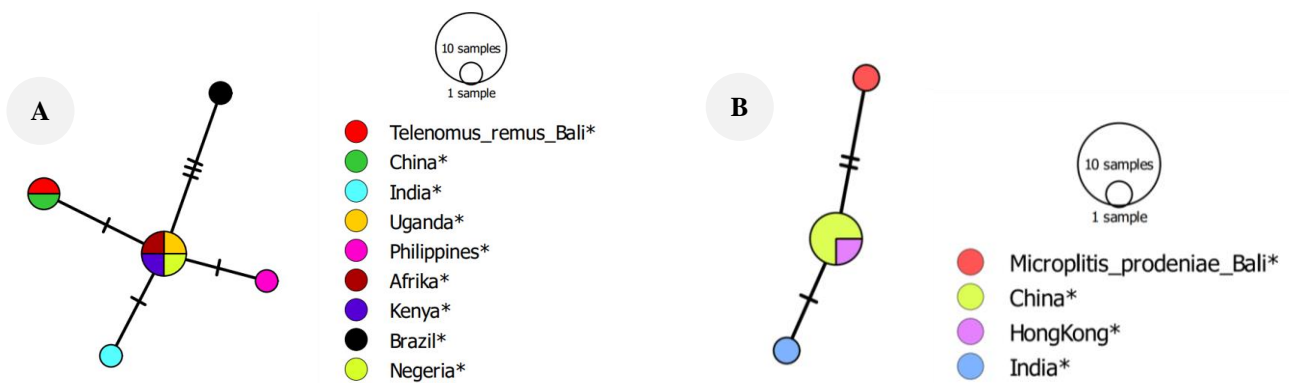


Figure 7. Haplotype eggs and larval parasitoid *Spodoptera frugiperda*: A. *Telenomus remus*; B. *Microplitis prodeniae*

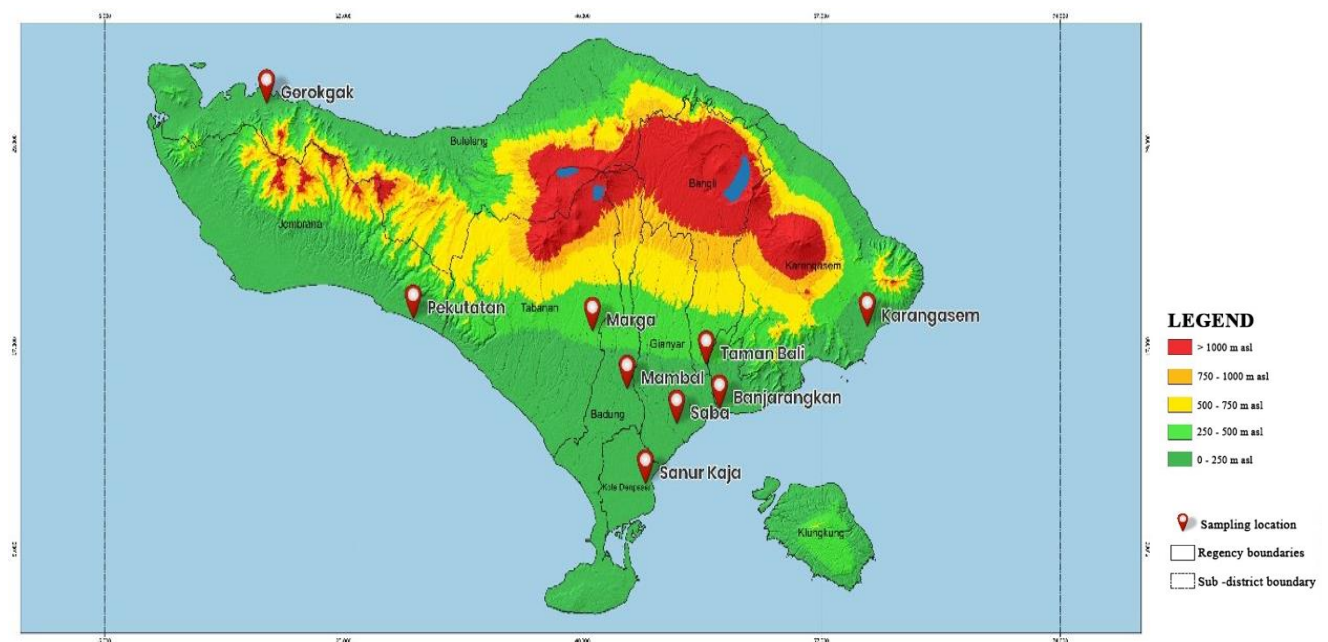


Figure 8. Distribution map of egg and larval parasitoid *Spodoptera frugiperda* in Bali, Indonesia

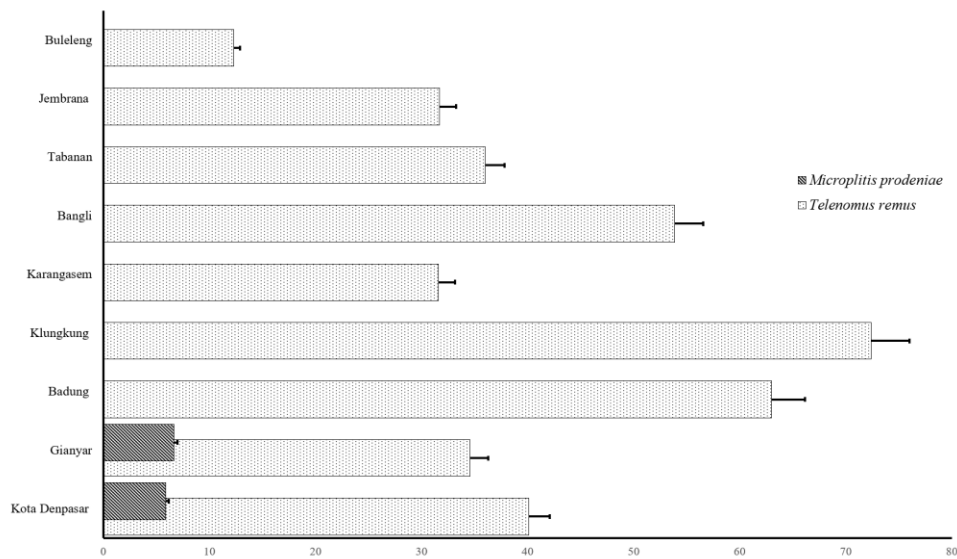


Figure 9. Percent parasitism of *Telenomus remus* and *Microplitis prodeniae*

Percent parasitism of the *S. frugiperda* parasitoid

The highest percentage of *T. remus* parasitism was found in Klungkung (72.4%), followed by Badung (63%), Bangli (53.9%), Denpasar (40.1%), Tabanan (36.04%), Gianyar (34.56%), Jembrana (31.7%), and Karangasem (31.6%), while the level of parasitoid parasitization was lowest in Buleleng (12.3%) (Figure 9). The difference in parasitization rates from each district in Bali is due to differences in cropping patterns in each district. Klungkung and Badung districts tend to have polyculture cropping patterns in their areas by combining corn plants with Fabaceae family plants such as *Vigna radiata* and *Arachis hypogaea*, this is what causes the high level of parasitization in the area. Meanwhile, in Bangli, Denpasar, Gianyar, Jembrana and Karangasem districts, the planting pattern in these areas is monoculture by only planting corn on cultivated land. The highest percentage of parasitism of *M. prodeniae* parasitoids on *S. frugiperda* larvae in Bali Province was found in Karangasem with a parasitism percentage of 6.66%, then in Denpasar with a parasitism percentage of 5.88%. Furthermore, in Gianyar, Badung, Klungkung, Bangli, Jembrana, Tabanan and Buleleng, no *M. prodeniae* was found. These data show that *T. remus* is able to adapt to all areas in Bali while *M. prodeniae* is only found in two locations, namely Karangasem and Denpasar. Thus, *T. remus* is superior in controlling *S. frugiperda* when viewed from its ability to adapt. *T. remus* is a very potential and effective parasitoid as a mortality factor for *S. frugiperda* eggs in the field. Oktaviani et al. (2022) stated that *T. remus* has the potential as a natural enemy to control *S. frugiperda* with a parasitization rate of *S. frugiperda* eggs reaching 91%. *T. remus* can parasitize 78% of *S. frugiperda* eggs compared to *Trichogrammatoidea* sp. which is only able to parasitize 25% of *S. frugiperda* eggs, so *T. remus* is suitable for augmentative release against *S. frugiperda* in Africa (Saidou et al. 2020).

The presence of the parasitoid *S. frugiperda* in Bali has never been reported before. The discovery of *M. prodeniae* as a larval parasitoid and *T. remus* as an egg parasitoid of *S. frugiperda* in Bali can be used as initial information regarding the types of natural enemies of *S. frugiperda*. The percentage parasitism and distribution of *T. remus* found in all districts in Bali indicate that *T. remus* can adapt and can be used as a recommendation as a biological control agent for the *S. frugiperda* pest in Bali.

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