INVENTORY AND IDENTIFICATION OF PARASITOID EGGS AND LARVAE OF *Spodoptera frugiperda* (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Spodoptera frugiperda is an invasive pest that invades Indonesia and attacks maize crops. Suppressing the development of pests needs various control methods, including biological control through the application of natural enemies. Therefore, this study was carried out to prepare the inventory and identified the natural enemies, especially parasitoids of *S. frugiperda*. This study was conducted on farmer's land in Purwobinangun Village, Sei Bingai Sub-District, Langkat District, North Sumatra Province, Indonesia. It was performed using a survey method by taking samples of eggs and larvae of pests *S. frugiperda* on maize through a purposive sampling (intentional sampling) method. The sampling was carried out on intercropping and monoculture maize crops. The collected eggs and larvae of *S. frugiperda* were taken to the Agriculture Faculty, Universitas Sumatera Utara plant pest laboratory to be reared until the parasitoids emerged. The result showed that two species of parasitoids were obtained namely *Telenomus* sp. and *Trichogramma* sp., with a level of parasitization of egg reached 80% for intercropping and 62.67% for monoculture. The larval parasitoids were obtained from three subfamilies of parasitoids, namely Cheloninae, Microgastrinae, and Campopleginae, with a level of parasitization around 13.2% for monoculture and 9.2% for intercropping.

Keywords: Egg and larval parasitoids, maize, *Spodoptera frugiperda*

ABSTRAK

Spodoptera frugiperda merupakan perosak invasif yang menceroboh masuk ke Indonesia dan menyerang tanaman jagung. Untuk menyekat perkembangan perosak, pelbagai kaedah kawalan diperlukan termasuk kawalan biologi dengan menggunakan aplikasi musuh semulajadi. Oleh itu, kajian ini telah dijalankan untuk menyediakan inventori dan mengecam musuh semulajadi khususnya parasitoid *S. frugiperda*. Kajian telah dijalankan di ladang milik petani di Kampung Purwobinangun, Mukim Sei Bingai, Daerah Langkat, Wilayah Sumatera Utara, Indonesia. Kajian dijalankan menggunakan kaedah tinjauan dengan mengambil sampel telur dan larva perosak *S. frugiperda* secara persampelan bertujuan (*intentional sampling*) pada tanaman jagung. Persampelan dijalankan pada tanaman jagung selingan dan monokultur. Kumpulan telur dan larva *S. frugiperda* yang telah dikumpul dibawa ke makmal perosak tumbuhan Fakulti Pertanian Universiti Sumatera Utara untuk diternak sehingga kemunculan parasitoid. Hasil kajian menunjukkan bahawa bagi kumpulan telur *S. frugiperda*, dua spesies parasitoid telah diperoleh iaitu daripada genus *Telenomus* sp. dan *Trichogramma* sp., dengan kadar parasitisasi mencapai 80% untuk tanaman selingan dan 62.67% untuk monokultur. Parasit larva didapati dari tiga subfamili parasitoid iaitu Cheloninae, Microgastrinae, Campopleginae. Kadar parasitisasi untuk seluruh famili parasitoid adalah 13.2% untuk monokultur dan 9.5% untuk tanaman selingan.

Kata kunci: Parasitoid telur dan larva, jagung, *Spodoptera frugiperda*

INTRODUCTION

Maize is an important food commodity after rice, widely cultivated in Indonesia. In other words, maize can be a staple food, and it is used as animal feed and industrial raw material (Rangkuti et al. 2014). Due to the high demand for maize yearly which is directly proportional to population growth, it is necessary to increase its production. However, maize production can decrease due to pests and diseases (Hanif & Susanti 2017). It was proved by a report in mid-2019 regarding roughly thousands of hectares of maize crops in Indonesia having been attacked by a new invasive pest of *Spodoptera frugiperda* (Maharani et al. 2019; Trisyono et al. 2019).

The armyworm *S. frugiperda* is an invasive pest from America and has spread to several countries. At the beginning of 2019, it was found in maize in the Sumatra area (Kementan 2019). It attacks the growing point of the plant, and failure can occur in the formation of shoots and young leaves of the plants if it cannot be controlled. Moreover, *S. frugiperda* larvae have high feeding ability; therefore, they actively eat when attacking the plant. As such, if the population is still low, it will be challenging to detect. The imago is a strong flier with a high cruising range (Wan et al. 2021).

Farmers use pesticides to control pests, despite many reports about the adverse effect of using pesticides. However, it is yet to be able to change the attitudes and behavior of farmers in cultivation. Natural enemies associated with *S. frugiperda* have been widely reported, such as the egg parasitoid of *Telenomus remus* (Kenis et al. 2019), *Trichogramma pretiosum*, *T. atopovirilia* (Jaraleño-teniente et al. 2020), the larval parasites of *Coccygidium melleum*, *Eriborus* sp. (Kalleshwaraswamy et al. 2019), *Aleoides* sp, *Chelonus* sp., *Cotesia* sp., *Glyptapanteles* sp., *Homolobus* sp., and *Meteorus* sp. (Molina-Ochoa et al. 2004), and predatory of *Orrius similis* (Zeng et al. 2021) *Podisus nigrispinus* (Malaquias et al. 2015), *Eocanthecona furcellata*, *Andralus spinidens* and *Micraspis discolor* (Jamil et al. 2021). This group of pathogens' natural enemies is *Metarhizium rileyi* (Lubis et al. 2020; Siahaan & Mullo 2021) and *Nomuraea rileyi* (Ginting et al. 2021).

North Sumatra lacks of information on parasitoids associated with *S. frugiperda*, considering this pest is invasive in Indonesia. In addition, no any inventory and monitoring of parasitoids that infesting *S. frugiperda* from North Sumatra Province, Indonesia. Therefore, this study was conducted to obtain information about local parasitoids associated with *S. frugiperda* pests. Subsequently, it will enrich knowledge for controlling *S. frugiperda* pests in North Sumatra province.

MATERIALS AND METHODS

The study was conducted on maize farmers' land in Purwobinangun village, Sei Bingei sub-district, Langkat district, North Sumatra province, Indonesia. It was performed using an observation method by taking samples of groups of eggs and larvae of pests *Spodoptera frugiperda* through purposive sampling (intentional sampling) on maize crop. The sampling was carried out on intercropping and monoculture maize crops. Groups of eggs and larvae were taken from maize crop around 5-50 days after planting (DAP). The leaves infested with egg groups were cut off and put into bottles in which one bottle contained one egg group. The egg groups were reared until hatching into larvae. If there were groups of eggs parasitized, the rearing would continue after the removal of larvae from the tube until the parasitoid imago emerged, and the level of parasitization was then calculated. Parasitoids that emerged from *S. frugiperda* eggs are put into bottles containing 70% alcohol solution for preservation and will then be identified.

Figure 1. Raring of *S. frugiperda* egg groups until egg parasitoids emerged

Larvae of *S. frugiperda* were collected from maize plants grown in intercropping and monoculture maize plants. The larvae taken were from instar 3 to instar 6. Each instar larva was reared in a separate plastic box. The larvae were fed maize leaves as needed and replaced every day. Larvae were reared until adult parasitoids appeared, and their level of parasitization was calculated. The emerging larval parasitoids were preserved in a 70% alcohol solution and then identified. The rearing of eggs and larvae was carried out in the laboratory of plant pests, Faculty of Agriculture, Universitas Sumatera Utara.

Figure 2. Rearing of *S. frugiperda* larvae until the emergence of parasitoids

The parasitization rate was calculated based on the formula:

$$\text{Percentage of parasitized egg group} = \frac{\text{the number of parasitized egg group}}{\text{s the number of egg groups}} \text{ x } 100\%$$

$$\text{Percentage of parasitized larvae} = \frac{\text{the number of parasitized larvae}}{\text{the number of larvae}} \text{ x } 100\%$$

The parasitoid imago that emerged was identified based on its morphological characteristics according to Goulet and Huber (1993) and Borror et al. (1992).

RESULTS AND DISCUSSION

Egg Parasitoids

The results of identifying egg parasitoids that parasitized the eggs of *S. frugiperda* belong to the order Hymenoptera and the family Scelionidae with the genus *Telenomus* and Trichogrammatidae with the genus *Trichogramma*.

Telenomus (Hymenoptera: Scelionidae)

Telenomus parasitoid imago has a body length of 0.5 mm, black color, 11-12 segments of antennae, and 9-10 segments of flagellum. All male antennae segments are elongated, and the last segments of funicular are slightly enlarged while the female antennae are angled. The hindwing, which has a prominent groove, is smaller than the forewing and has one small hook. The hindwing's submarginal vein reaches the hamuli, while the forewing's submarginal vein reaches the wing's anterior tip (Borror et al. 1992; Goulet & Huber 1993; Polaszek & Kimani 1990).

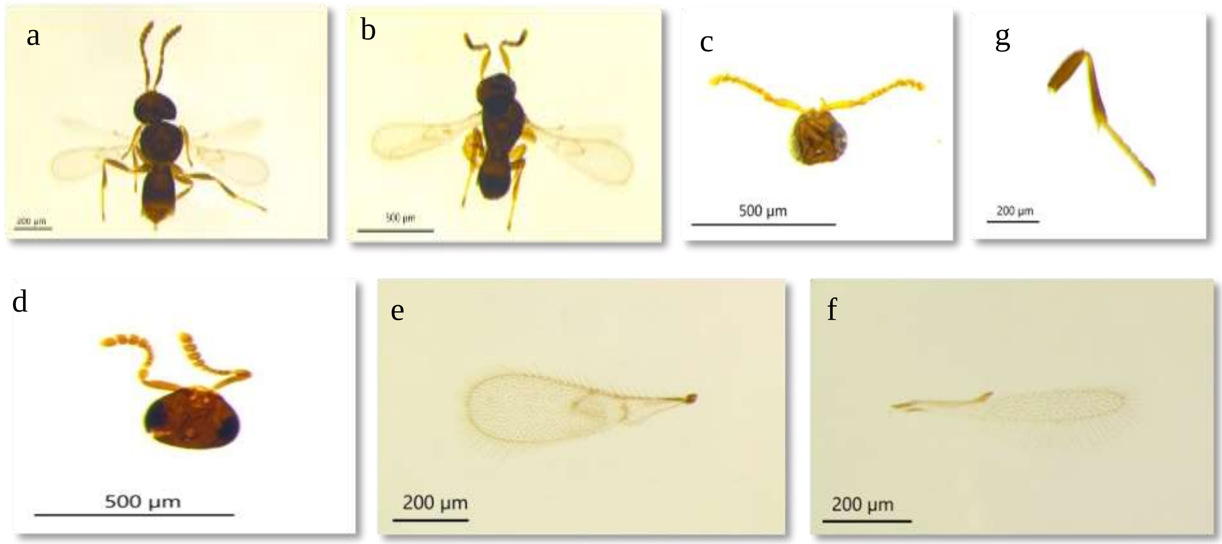


Figure 3. *Telenomus* sp parasitoid; (a) adult of male , (b) adult of female, (c) antennae of male, (d) ,Antennae of female, (e) forewing, (f) hindwing, (g) legs

***Trichogramma* (Hymenoptera: Scelionidae)**
Trichogramma parasitoid imago has a small yellowish-brown body measuring 0.2 mm with a fused abdomen and thorax. The wings are transparent with line formation and surrounded by many long feathers (tassels) at the tip of the wings. The shape of the arista antenna is shorter than the head, and metasoma has 2-3 flagellum consisting of funiculus and clavus, and the tarsus consists of 3 segments. The female antennae are club-shaped, short-haired, and nearly hairless, while the male antennae are straight and overgrown with hair (Borror et al. 1992; Goulet & Huber 1993; Nagarkatti & Nagaraja 1971).

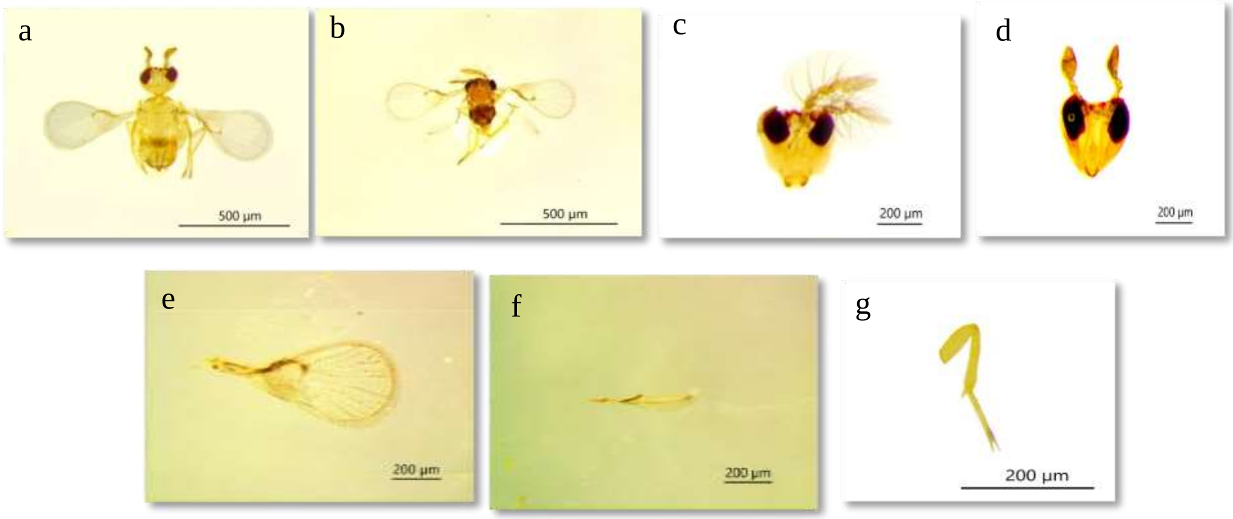


Figure 4. *Trichogramma* sp parasitoid; (a) adult of male, (b) adult of female, (c) antennae of male, (d) antennae of female, (e) forewing, (f) hindwing, (g) legs

Larval Parasitoids
Based on the results of the identification of larval parasitoids *S. frugiperda*, three subfamilies of parasitoids from the order Hymenoptera were found: Cheloninae, Microgastrinae, and Campopleginae.

Subfamily Cheloninae (Hymenoptera: Braconidae)
Cheloninae is small to medium-sized wasps (body size 1.8–6.0 mm); the overall body is black, the forelimbs and middle limbs are yellowish-orange, and the base of the abdomen is slightly yellowish (Figure 5a). It has filiform antennae (each segment is the same size) and 25-28 segments (Figure 5c). Tarsus has 5 tarsomeres. The mesosoma and metasoma are separated (Figure 5a) (Borror et al. 1992; Goulet & Huber 1993).

The forewings are marginally shorter than the pterostigma, and the radial cells of the forewings are along with the post-marginal ones. The RS vein (radial sector) usually reaches the tip of the wing; the 1-SR+M vein and the 2m-cu vein (medial branch) of the forewing are absent. The hindwings have only slight venation (Figure 5b). Metasoma at tergum 1-3 fused to form a carapace that covered the soft upper part of the metasoma (Figure 5a) (Borror et al. 1992; Goulet & Huber 1993).

During rearing, the larvae of *S. frugiperda* parasitoid subfamily Cheloninae emerged from the larval body of the 4th instar host with the condition of the dead host in the form of white parasitoid larvae less than 2 days later forming a cocoon (pupa) and the pupa stage until the imago emerged for about 10 to 12 days. The Cheloninae parasitoid that emerged from the host's body was only 1 tail or solitary, and it was an egg-larval parasitoid.

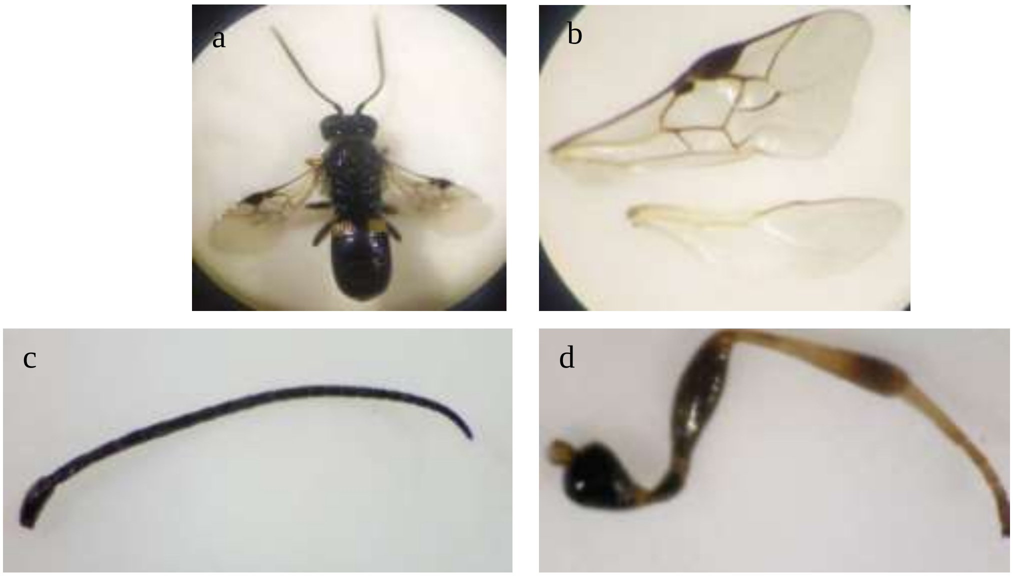


Figure 5. Body parts of subfamily parasitoids Cheloninae (100x magnification); (a) whole body, (b) wings, (c) antennae, (d) legs.

Subfamily Campopleginae (Hymenoptera: Ichneumonidae)

The body is 7.0-7.3 mm, the head and thorax are black, the forelegs, middle legs, and spurs are yellow, hind legs are brown with yellow trochantellus (Figure 6a). It has a long filiform antenna (each segment is the same size) and consists of 2,8-44 segments (Figure 6c) (Fitton et al. 1988).

Furthermore, the forewings have slender stigmas and open and closed areolets. The forewings have 2m-cu veins; costal cells from the leading edge of the forewings lack tight with dense Sc and R veins, and the hindwings have closed sub-basal cells (Figure 6b). The tarsus consists of 5 tarsomeres (Figure 6e). Metasomal segment 1 (tergum 1) is usually long and slender, extending apically compared to other metasomal segments. Viewed laterally, metasomal segments 3 and 4 are taller and broader, and the ovipositor is short (Figure 6d) (Goulet & Huber 1993; Fitton & Rotheray 1982).

During rearing, the larvae of *S. frugiperda* parasitoid of subfamily Campopleginae emerged from the body of the dead 5th instar host larva in the form of a parasitoid pupa. The pupa was brown-speckled and large; there was a fine thread at the tip of the pupa, and it hung on the wall of the plastic container. The development period of the pupa until the imago emerged was about 7 days. The campopleginae parasitoid that emerged from the host's body was only one tail or solitary.

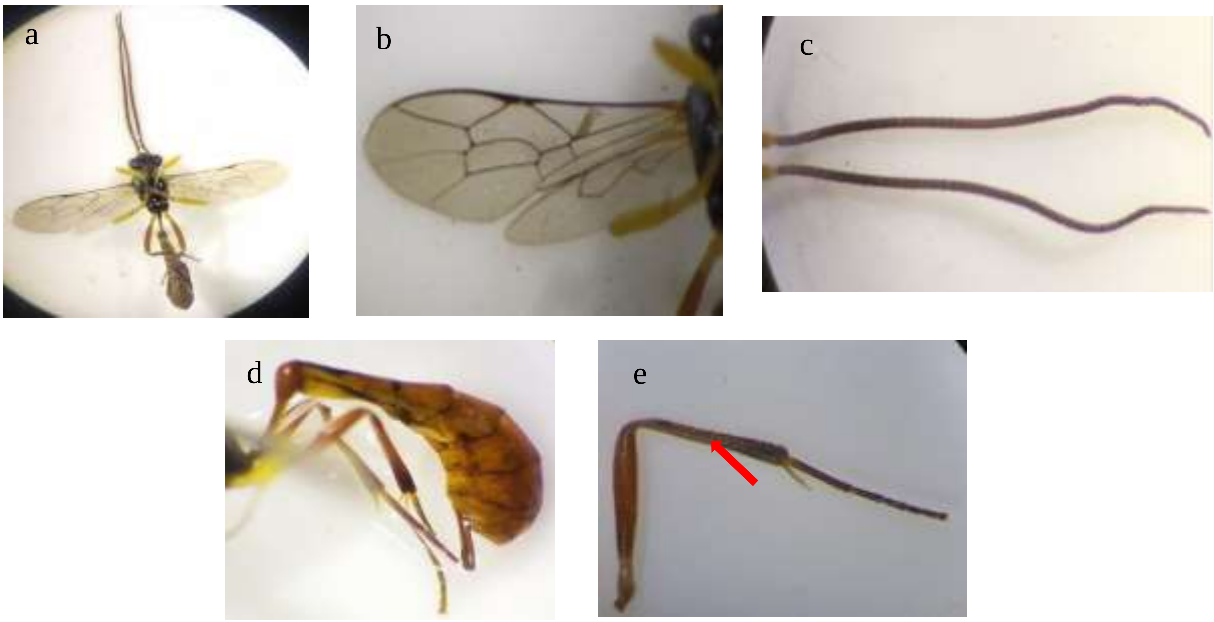
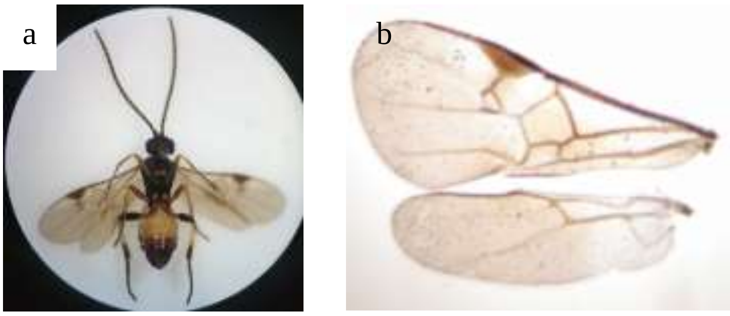


Figure 6. Body parts of subfamily Campopleginae parasitoids (100x magnification); (a) whole body, (b) wings, (c) antennae, (d) abdomen, (e) legs

Subfamily Microgastrinae (Hymenoptera: Braconidae)
The body is 2.5-3 mm long, the head, antennae, and thorax are black, and some of the tergum and sterna are yellow (Figure 7a). It has filiform antennae (each segment is the same size) and 16 yellow segments (Figure 7d). Tarsus has 5 tarsomeres (Figure 7c). The forewings contain the stigma and areolet. The RS vein does not reach the wing margin, and the 2m-cu vein on the forewings is absent. The hindwings only have slight venation (Figure 7b). The abdomen is oval, Tergum segment 1 is longer than the other segments, and has a hidden ovipositor (Goulet & Huber 1993).

During rearing, the larvae of *S. frugiperda* parasitoid of subfamily Microgastrinae emerged from the body of the surviving 4th instar larvae in the form of brown pupae, and the development period of pupae to imago was about 5 to 6 days. The parasitized host died 1 to 2 days after the parasitoid left the host's body. Host larvae still were alive over time and stiffened until they died and hardened brown for approximately a day.



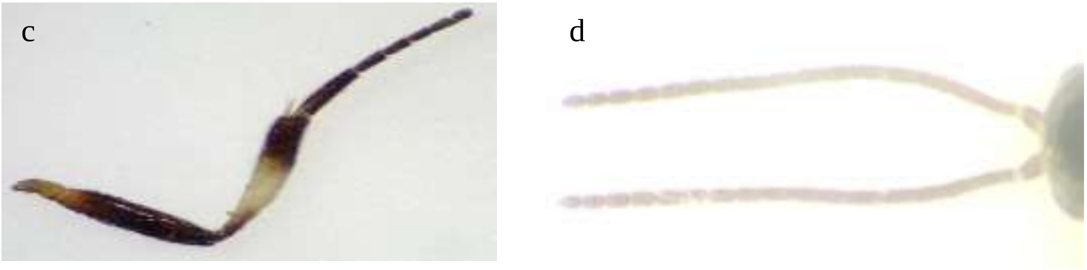


Figure 7. Body parts of the parasitoid of subfamily Microgastrinae (100x magnification); (a) whole body, (b) wings, (c) legs, (d) antennae. No scale?

Egg and larval group parasitization rate

Egg groups were collected from monoculture maize and intercropped maize. The parasitization rate of egg groups in intercropped maize was higher than in monoculture maize (Table 3). In comparison, the larval parasitization rate of intercropped maize was lower than monoculture maize (Table 4).

Table 3. Percentage of egg groups of *S. frugiperda* parasitized in monoculture and intercropping maize

Practise	The number of groups of eggs collected	The number of groups of eggs that are parasitized	Percentage of parasitized egg groups (%)
Monoculture	75	47	62.67
Intercropping	60	48	80.00

Table 4. Parasitization rate of *S. frugiperda* larvae in monoculture and intercropping maize

Practise	The number of larvae collected	The number of parasitized larvae	Percentage of parasitized larvae (%)
Monoculture	151	20	13.2
Intercropping	42	4	9.5

DISCUSSION

Egg parasitoids *Trichogramma* sp. and *Telenomus* sp. are egg parasitoids that are often found parasitizing eggs from insect pests, including *Spodoptera litura* (Widihastuty 2013), *Spodoptera frugiperda* (Agboyi et al. 2020; Jaraleño-Teniente et al. 2020), *Chilo auricilius* (Subandi et al. 2017), and *Mythimma separata* (Hou et al. 2018). The group of *S. frugiperda* eggs was found more in monoculture agroecosystems than in polycultures, and parasitic egg clusters were also higher in intercropped maize than in monoculture maize (Table 3). The high and low percentage of parasitoid parasitization in the field is influenced by the abundance of natural enemies (Winsou et al. 2022) and the balance of the agroecosystem (Abang et al. 2021). Polyculture agroecosystems have a more diverse abundance of natural enemies than monoculture agroecosystems (Lal et al. 2019). Therefore, the more complex the community, the more species present; the more interactions, the more stable the system (Lisdayanti & Nurkomar 2022).

The larval parasitoids obtained from the results of this study were from the families Braconidae and Ichneumonidae. Shylesha et al. (2018) found that the larval parasitoids of *S. frugiperda* were *Campoletis chlorideae* (Hymenoptera: Ichneumonidae), *Glyptapanteles creatonoti* (Hymenoptera: Braconidae). The larval parasitization level obtained from this study was around 13.2%. According to Rongkok and Pasaru (2021), the parasitization rate of *S. frugiperda* larvae in Donggala Regency was around 11.25%. The level of larval parasitization in monoculture was higher than in polyculture. Thus, the number of individual pests found in polycultures is less than the number of pests in monocultures. In an agroecosystem, the population development of natural enemies will usually follow the development of herbivorous insects (Grettenberger & Tooker 2020). In addition, the larval stage collected also determines the level of parasitization. During the study, the larvae collected were 3 to 6 instar larvae. The early instar stages (instars 2 and 3) were usually more preferred by parasitoids to be parasitized than the last instars (instars 4, 5, and 6). It was because the early instars usually lived in groups. Therefore, the larval parasitoids found it easier to find a host to parasitize than the last instars, whose behavior had begun to be solitary and spread. This finding statement is similar to da Silva et al. (2017), who found that the larval parasitoid of *S. frugiperda* prefers to parasitize the 3rd instar larvae more than the other instars.

CONCLUSION

The egg parasitoids associated with *Spodoptera frugiperda* were from the Trichogrammatidae family and *Telenomus* genus, while the larval parasitoids belonged to Braconidae and Ichneumonidae. Intercropping maize can optimize the presence of the egg parasitoid of *S. frugiperda* so that it can be an alternative pest control through habitat management. This inventory of *S. frugiperda* parasitoids will enrich the types of natural enemies that exist and will improve the implementation of IPM in maize. The farmers are expected to be wiser in using pesticides so that the existence of these natural enemies can be maintained and make an excellent contribution for controlling corn pests. However, a comprehensive taxonomy study is highly needed for species identificaton on each parasitoid species.

AUTHORS DECLARATIONS

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Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Declarations
No ethical issue required for this research

Data Availability Statement
My manuscript has data related to Tobing et al. research on Inventory and Identification of Parasitoid Larvae On Spodoptera frugiperda (Lepidoptera: Noctuidae) data

Authors Contributions

MCT, AZS, W, and SFS conceived this research and designed experiments; MCT, AW, and TTH participated in the design and interpretation of the data; MCT, AZS, W, and SFS performed experiments and analysis; MCT and W wrote the paper and participated in the revisions of it. All authors read and approved the final manuscript. All authors read and approved the final manuscript

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