

Article

Insect Communities Associated with Siam Weed: Evaluation after Three Decades of *Cecidochares connexa* Release as Biocontrol Agent

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Abstract: *Chromolaena odorata* is well known as an invasive weed, and its existence in agricultural habitats causes an undesirable effect on crop plants. The invasion of *C. odorata* alters local biodiversity and shapes the new trophic interaction with local herbivores and other insects. This research was conducted to study the insect communities associated with *C. odorata* and evaluate the success of the release of *Cecidochares connexa*, the natural enemy of *C. odorata*. Field research was conducted in two different geographical regions in Bogor Regency (Java) and South Lampung Regency (Sumatera), Indonesia. In each region, we selected five villages that have two land-use types (oil palm plantations and open area) and contain a high population of *C. odorata*. Observation of insects and natural enemies of *C. odorata* was conducted in each land-use type using two methods: suction sampling and gall collection, which were performed in 30 plants as sampling units. In total, we found 255 species of insects associated with *C. odorata*. The difference of region affected the abundance of insects but not their species richness. The species composition of insects showed difference between regions as well as between land-use types. There was a positive correlation between elevation and species richness of insects. In addition, the population of *C. connexa* (gall numbers) was significantly affected by regions and was found to be higher in Bogor and Lampung. The same pattern also was shown for its parasitoids (based on parasitized galls). We found a negative relationship between the number as well as parasitize galls and elevation. In conclusion, the presence of *C. odorata*, as well as its natural enemies, shape the new trophic interaction with local insects, and as consequence, its introduced natural enemies may not be effective to control the population of *C. odorata*.

Keywords: *Chromolaena odorata*; open area; oil palm; parasitoid wasp

1. Introduction

The Siam weed, *Chromolaena odorata*, is a well-known exotic invasive species which originates from tropical America and has become widespread in Asia. Based on herbarium record, *C. odorata* was first reported in India in 1872 [1,2] and then spread to Sumatera, Indonesia in 1934 [3]. The invasion of Siam weed in a new region can cause biotic homogenization and its existence in an agricultural area can be destructive to crop plants due to nutrient competition [4]. The replacement of native plants by *C. odorata* is due to the effect of allelopathy [5] and the ability of this invasive weed to change the soil quality [6] as well as the soil microbial community [7]. Therefore, the control of *C. odorata*, especially in agricultural areas, is very crucial to prevent the negative effect on crop plants and avoid economic loss. In Indonesia, the common methods of controlling *C. odorata* in plantations include a mechanical

approach (e.g., uprooting the weeds), slashing, and chemical control using herbicides [3]. However, these methods have not been reported successful due to the ability of *C. odorata* to recover rapidly. Tjitrosemito [3] found that Siam weed grows vegetatively during the wet season, flowers and sets a high number of fruits at the end of the wet season, and senesces during the dry season.

Considering the characteristics of *C. odorata*, the use of biological control agents that attack leaves, branches, and flowers is seen as a promising approach to control this weed. As a classical biological control approach, the natural enemies from the native range of Siam weed are introduced to the new invasion area. This method is considered to have several advantages, such as effective control of the weed population by way of hindering its ability to establish and spread to other areas, and also the consideration of control cost, which is lower than herbicide application [8–10]. In Indonesia, several natural enemies have been introduced to control Siam weed, e.g., *Pareuchaetes pseudoinsulata* Rego Baros (Lepidoptera: Arctiidae) from Guam in 1992 in North Sumatra. Yet, the introduction of *P. pseudoinsulata* was only able to suppress the population of Siam weed in North Sumatra and was not successful in other areas, such as Java [11]. Then in 1993, a gall fly, *Cecidochares connexa* Macquart (Diptera: Tephritidae) was introduced from Columbia into quarantine at the Indonesian Oil Palm Research Institute in North Sumatra and was released in 1995 in West Java [12,13]. A two years' post release evaluation of *C. connexa* indicated that the population of *C. odorata* went down to 37.2% and the production of seeded fruits decreased by about 50% in one season [3]. However, the effectiveness of *C. connexa* can be reduced due to parasitism and predation [1]. McFadyen et al. [13] reported that five years after the first releases at the sites, parasitism and predation can exceed 50%. Recent research in Java also reported a new complex interaction between *C. connexa* and local insects that may reduce the effectiveness of *C. connexa* to control *C. odorata* [14]. This is perhaps one of the reasons why *C. odorata* is still a problem in agricultural areas, especially plantations.

A biological control program of invasive weeds can be unsuccessful if the natural enemies fail to adapt to the new habitat, and are therefore less effective than in their area of origin. In addition, natural enemies with a broad host range can cause a non-target impact through physical and functional replacement of local species [15]. The presence of a new species in a new habitat also alters the community structure and trophic level dynamics, and may affect the stability of ecosystems [16]. Thus, the introduction of exotic species should be reviewed by considering the conservation perspective. If no alternative approach is available, the introduction of biological control agents must go through a rigorous screening process to determine its potential effectiveness against the target weed and impact on non-target organism. In addition, monitoring the new region is necessary to avoid negative impacts on local biodiversity [17]. With long-term monitoring, biodiversity degradation or native species extinction in an ecosystem will be detected especially in the region of the introduced natural enemies [18,19].

The objectives of this study were to assess the insect communities associated with *C. odorata* and to evaluate, three decades post-release, the population levels of *C. connexa* in Indonesia. The research is part of a monitoring and evaluation study to investigate the factors affecting the decrease of biological control agent effectivity to control invasive weeds. The results can be used as a recommendation for classical biological control strategy in Indonesia and for biodiversity conservation efforts especially related to the management of invasive species in tropical regions. This is to avoid the reluctance of government and other stakeholders to pursue weed biological control, due to poor perceptions of biological control [10].

2. Materials and Methods

2.1. Research Site and Plot Selection

This research was conducted in two geographical regions in West Java (Java Island) and Lampung (Sumatera) to compare the diversity in different islands. In West Java, we selected the research area in Bogor Regency, which was the historical release site for *C. connexa*, whereas in Lampung, we selected a

research area in South Lampung Regency, as a representative area in which *C. connexa* has never been released, which is geographically isolated and far from the release sites both in Java and Sumatera (North Sumatera). In each research area, five villages were chosen that fulfill the existence of two land-use types, i.e., oil palm plantations and open area, and have a large population of *C. odorata* (Figure 1). Open areas were defined as areas without trees, not being cultivated by crops and dominated by grass and bushes. We preferred to choose oil palm plantation due to this weed causing serious problems in plantations, and especially oil palm ones. In addition, this weed is also suitable to habitats with less tree cover. In each land-use type, we selected 30 plants of *C. odorata* as sampling units for the observation and sampling of insects.

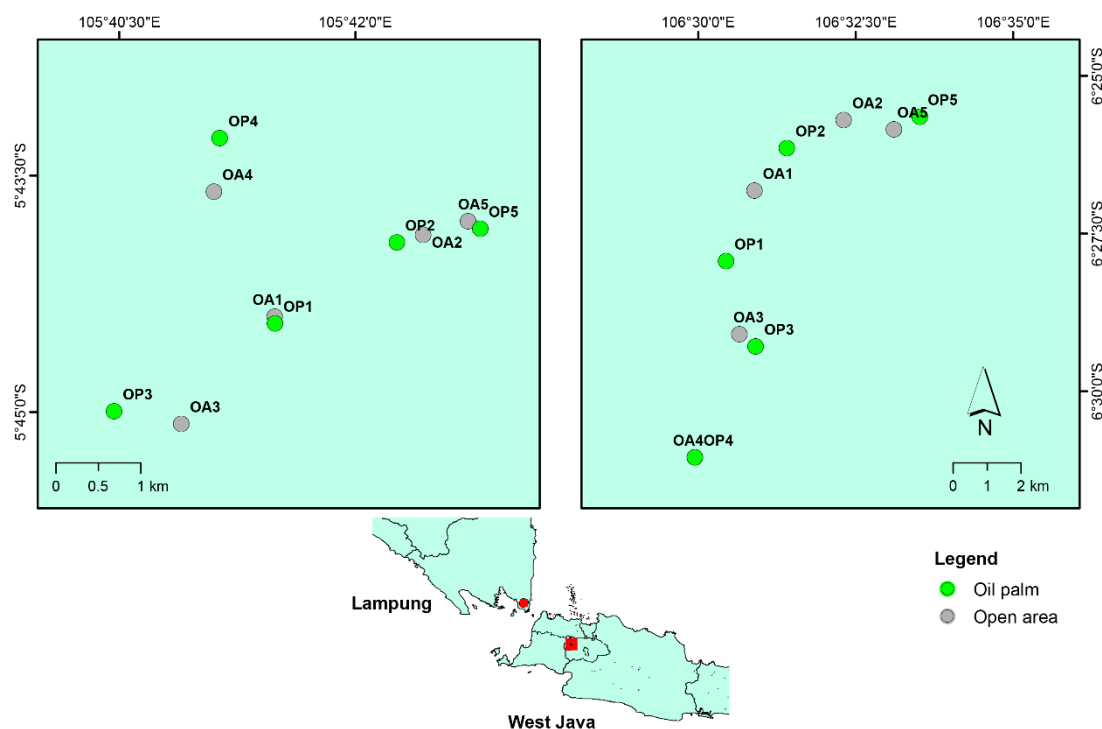


Figure 1. Map of the research area for insect observation in *C. odorata* in Bogor Regency, West Java (right) and South Lampung Regency, Lampung (left). The letters followed by numbers indicate site codes. The letters indicate land-use type: OA = open area, OP = oil palm; and the numbers (1–5) indicate villages as replications. Villages of Bogor Regency: 1 = Argapura, 2 = Bangun Jaya, 3 = Cantik Manik, 4 = Mekar Jaya, and 5 = Rengasajar. Villages of South Lampung Regency: 1 = Klaten, 2 = Ngandri, 3 = Pasuruan, 4 = Ruang Tengah, and 5 = Tetaan.

2.2. Sampling and Identification of Insects

Field research was conducted during the rainy season from November 2018 to January 2019. We conducted two different sampling methods to cover insect diversity associated with *C. odorata* and the population of its natural enemy (*C. connexa*) as well as other insects associated with *C. connexa*. The sampling of insects in *C. odorata* was conducted using the suction method with a FARMCOP. The FARMCOP is a suction sampler for insects that was developed by Carino, et al. [20] and is commonly used to sample brown planthoppers in the rice fields. Before sampling, we randomly selected 30 plants with similar size and architecture. Each plant was then covered by a net and all insects were sucked for 5 min. The collected specimens were then stored in plastic vials containing 70% alcohol for later identification in the laboratory. The sampling by the FARMCOP was conducted in the morning between 7 AM and 9 AM and with sunny weather.

For sampling of the natural enemy of *C. odorata*, we observed and collected the galls in *C. odorata*. The galls are a symptom of the abnormal development of stems or stalks due to attacks by *C. connexa*. In each land-use type, we randomly selected 30 plants with similar size and architecture that were not

the same plants that were sampled using the FARMCOP. From each plant, all galls were collected by cutting the stalks and taken to the laboratory. Each gall was put in a plastic container (diameter = 6 cm, height = 10 cm) and observed for one month. Observations were done for the emergence of parasitoids or other insects. Toward the end of the observation period, each gall was dissected, and the number of gall flies inside the galls were counted.

Samples of insects collected using both methods was initially sorted to the order level and later identified to the family and genera level using several relevant taxonomic literatures, e.g., Borror, et al. [21], CSIRO [22], McAlpine [23], Goulet and Huber [24], and Bolton [25]. All specimens were then determined to the morphospecies level based on their morphological characteristic, and if possible were identified to species level by comparing with voucher specimens at the Laboratory of Insects and other Arthropod Taxonomy, Indonesian Institute of Science.

2.3. Data Analysis

Species accumulation curves were used to compare insect species richness between different regions and land-use types based on plant numbers of *C. odorata* as a sampling unit. To assess the completeness of sampling, the Chao estimator [26] was calculated for each region and each land-use type. Analysis of variance (ANOVA) was used to compare the species richness and abundance of insects between different regions and land-use types. The relationship between the species richness and abundance of insects and elevation was analyzed using regression analysis. Data of insect abundance were transformed to $\log(x + 1)$ to achieve normal distribution. Differences in insect community structure were quantified using the Bray–Curtis index for dissimilarity based on species abundance. The dissimilarity matrix was then reduced to a two-dimensional ordination using non-metric multidimensional scaling (NMDS) [27]. The effects of region and land-use type on species composition of insects were assessed by analyses of similarity (ANOSIM) [28].

To evaluate the release of *C. connexa* to *C. odorata*, ANOVA was used to compare the gall numbers and parasitized galls between regions and land-use types. Regression analysis was then used to analyze the relationship between gall numbers as well as parasitized galls and elevation. Data of gall numbers and parasitized galls were approximately normal following $\log(x + 1)$ transformation. All analyses were performed using R statistical software [29], utilized the BiodiversityR package to calculate the Chao species estimator, created the species accumulation curve [30], used the ggplot2 package to visualize the boxplot and regression plot [31], and used the vegan package for analysis of NMDS and ANOSIM [32].

3. Results

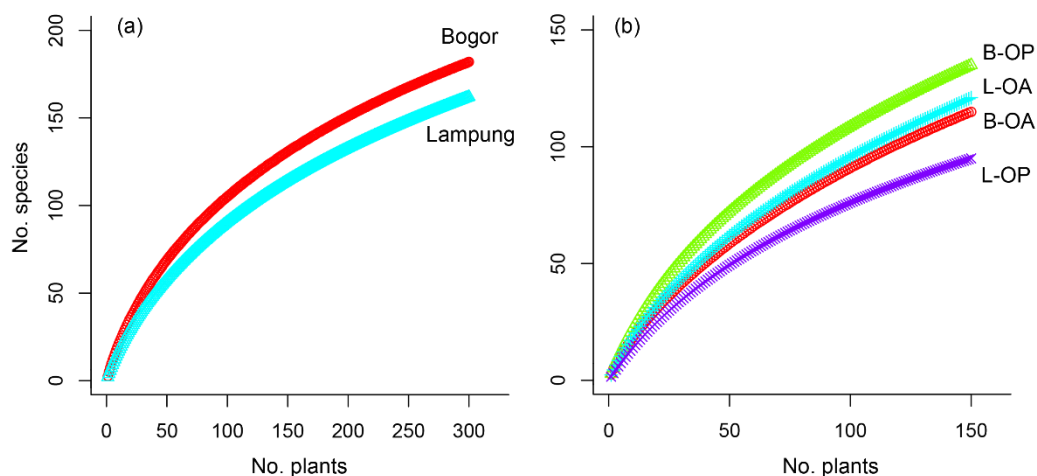
3.1. Insect Diversity Associated with *C. odorata*

In total, we found 255 species and 13,325 individuals of insects associated with *C. odorata*, including 181 species and 10,944 individuals from Bogor and 161 species and 2381 individuals from Lampung (Table 1). The most abundance of insects (91.4%) were *Aphis gossypii* (Hemiptera: Aphididae) with 10,168 individuals (76.3% of total individuals) and ants (Hymenoptera: Formicidae) with 2005 individuals (15.1%) with *Dolichoderus* sp as the most dominant species (1091 individuals). The most species-rich were hymenopteran, which were found with 124 species within the total area (48.6% from total species).

Table 1. Insects diversity associated with *C. odorata* collected by vacuum methods both in Bogor and Lampung. S: species richness, N: number of individuals.

No	Order	Bogor				Lampung				Entire Area	
		Open Area		Oil Palm		Open Area		Oil Palm		S	N
		S	N	S	N	S	N	S	N	S	N
1.	Araneae	13	31	15	41	11	15	9	13	25	100
2.	Blattodea	0	0	1	1	0	0	1	1	2	2
3.	Coleoptera	14	29	9	17	6	16	5	6	25	68
4.	Diptera	16	40	24	118	13	69	9	48	30	275
5.	Hemiptera	14	2352	13	6755	13	162	12	1193	32	10,462
6.	Hymenoptera	45	1159	61	299	65	357	50	450	124	2265
7.	Lepidoptera	2	8	0	0	2	2	1	1	3	11
8.	Mantodea	2	7	3	33	3	10	1	2	3	52
9.	Odonata	0	0	0	0	1	1	0	0	1	1
10.	Orthoptera	8	22	7	28	5	12	5	19	9	81
11.	Thysanoptera	0	0	1	4	1	3	1	1	1	8
Total										255	13,325

We found that Bogor had a higher number of insect species (182 species, 64.6% of the Chao estimated species) than Lampung (162 species, 57.8% of the Chao estimated species) (Figure 2a). Based on land-use type, the oil palm plantations of Bogor had the highest insect diversity (135 species, 55.0% of the Chao estimated species), whereas the lowest diversity was found in the oil palm plantations of Lampung (95 species, 56.4% of the Chao estimated species) (Figure 2b).

**Figure 2.** Species accumulation curve of insect diversity based on the number of *C. odorata* plants. (a) Insect diversity between Bogor and Lampung, (b) insect diversity between different land-use types in Bogor and Lampung. B-OP = oil palm in Bogor, B-OA = open area in Bogor, L-OP = oil palm in Lampung, L-OA = open area in Lampung.

Based on ANOVA, we found different abundances of insects between Bogor and Lampung ($F_{1,17} = 9.596$, $p = 0.007$), but not for species richness ($F_{1,17} = 0.754$, $p = 0.397$) (Figure 3a,b). Land-use type did not affect the diversity of insects in *C. odorata* ($p > 0.05$). In addition, we found a positive relationship between elevation and species richness of insects ($R^2 = 0.35$, $p = 0.006$) and no correlation with insect abundance ($R^2 = 0.04$, $p = 0.657$) (Figure 3c,d).

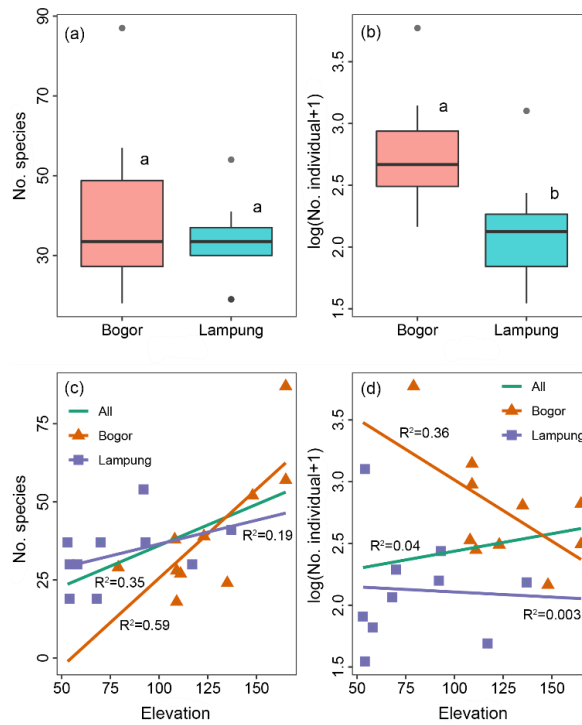


Figure 3. Difference of (a) species richness and (b) individual of insects between Bogor and Lampung and the relationship between elevation (m above sea level) and (c) species number and (d) individual insects. Data of insect individual were transformed to $\log(x + 1)$ to achieve normal distribution. Boxplots with different letters was significantly different at $p < 0.05$ according to Tukey’s test.

Based on ANOSIM, we found that the similarity of insect species composition was significantly different between regions ($R = 0.39, p = 0.002$) and as visualized in the ordination results of the NMDS analysis, also separated between Bogor and Lampung (Figure 4a). By contrast, the species composition of insects between land-use types did not differ ($R = -0.05, p = 0.718$), indicated by the overlap of plot groups within a region (Figure 4b).

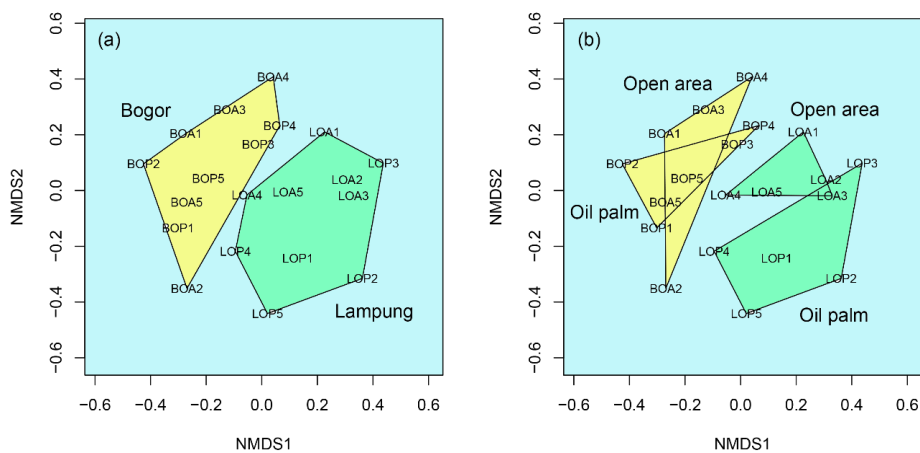


Figure 4. Two-dimensional non-metric multidimensional scaling ordination (NMDS) of insect species composition by *C. odorata* based on a Bray–Curtis dissimilarity index. Plots are grouped by (a) different regions and (b) land-use types. The letters followed by the number indicated plot codes. The first letter indicates the region: B = Bogor, L = Lampung; the second and third letters are land-use type: OA = open area, OP = oil palm; and the numbers (1–5) are replications.

3.2. Evaluation of the Release of *C. connexa* as the Natural Enemy of *C. odorata*

The evaluation of the release of *C. connexa* was measured based on the number of galls and the parasitized galls in *C. odorata* that were found both in Bogor and Lampung. In total, we found 2218 galls from all research areas. The number of galls varied and ranged from 0 to 109 galls per plant with an average of 3.69 galls per plant. The gall number in Lampung was significantly higher than in Bogor ($F_{1,17} = 7.787$, $p = 0.013$; Figure 5a). The number of galls that were parasitized by parasitoids was also higher in Lampung than in Bogor ($F_{1,17} = 12.026$, $p = 0.003$; Figure 5b). There was a negative relationship between elevation and gall numbers ($R^2 = 0.27$, $p = 0.019$; Figure 5c) as well as parasitized galls ($R^2 = 0.20$, $p = 0.046$; Figure 5d).

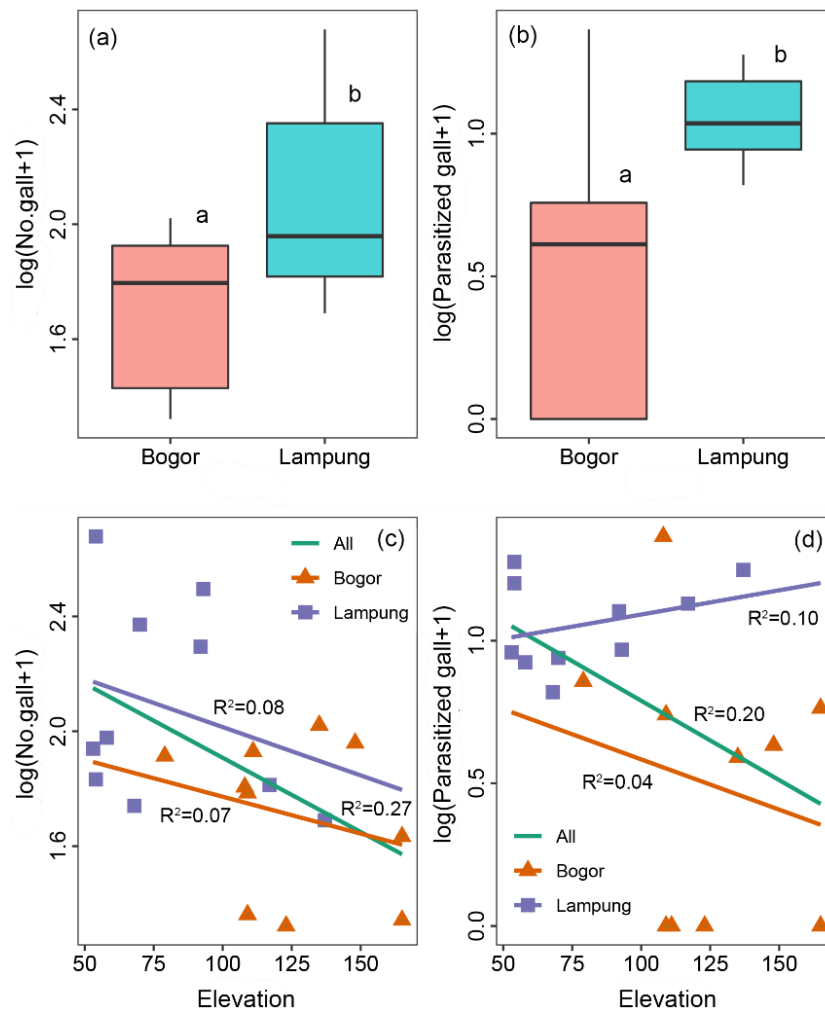


Figure 5. Difference of (a) gall numbers and (b) parasitized gall between Bogor and Lampung and the relationship between elevation (m above sea level) and (c) gall numbers and (d) parasitized gall. Data were transformed to $\log(x + 1)$ to achieve normal distribution. Boxplots with different letters are significantly different at $p < 0.05$ according to Tukey's test.

Based on galls reared, *C. odorata* was found to be attacked by two gall flies species, i.e., *C. connexa* (introduced species) and *Lonchaea* sp (local species, Diptera: Lonchaeidae). *Lonchaea* sp was only recorded in Lampung and with only five individuals from both open areas and oil palm plantations. In addition, we found eight families and 13 species of parasitoid wasps that emerged from the galls (Table 2). The species richness of parasitoid wasps was higher in Lampung (11 species) than in Bogor (four species). The most dominant parasitoid wasp was *Quadrastichus* sp (Eulophidae), recorded in all research areas.

Table 2. Species richness and abundance of gall flies and associated parasitoid wasps that emerged from gall samples. OA = open area, OP = oil palm plantation.

No.	Family	Species	Bogor		Lampung		Entire Area	Role
			OA	OP	OA	OP		
DIPTERA								
1.	Tephritidae	<i>Cecidochares connexa</i>	13	36	194	227	470	Gall fly
2.	Lonchaeidae	<i>Lonchaea</i> sp			4	1	5	Gall fly
HYMENOPTERA								
1.	Bethylidae	<i>Bethylidae</i> sp		1			1	Parasitoid
2.	Braconidae	<i>Braconidae</i> sp1			3	7	10	Parasitoid
3.		<i>Braconidae</i> sp2			1	2	3	Parasitoid
4.	Encyrtidae	<i>Encyrtidae</i> sp			1		1	Parasitoid
5.	Figitidae	<i>Eucoilinae</i> sp1			1	1	2	Parasitoid
6.		<i>Eucoilinae</i> sp2			1		1	Parasitoid
7.		<i>Eucoilinae</i> sp3			1		1	Parasitoid
8.	Eulophidae	<i>Quadrastichus</i> sp	14	8	52	102	146	Parasitoid
9.	Eupelmidae	<i>Eupelmidae</i> sp				1	1	Parasitoid
10.	Eurytomidae	<i>Eurytomidae</i> sp1			1	3	4	Parasitoid
11.		<i>Eurytomidae</i> sp2			1		1	Parasitoid
12.	Pteromalidae	<i>Pteromalidae</i> sp1	2	1	1	4	8	Parasitoid
13.		<i>Pteromalidae</i> sp2		1			1	Parasitoid

4. Discussion

In the present study, we found a higher diversity of insects associated with *C. odorata* than in the native area of *C. odorata*. In Trinidad and other areas of the native range of *C. odorata*, 225 insects and mites were found associated with the weed [33]. Our results showed that *C. connexa*, the introduced natural enemy of *C. odorata*, still exists in Bogor Regency at the release site and even has dispersed to areas where it has never been released, such as South Lampung Regency. This indicates that *C. connexa* is well adapted and has established itself in the tropical region and can spread in a wide range of geographical areas, although its effectiveness to control the population of *C. odorata* is to be questioned due to parasitism and predation by local insects. In general, the parasitism and predation levels of *C. connexa* on *C. odorata* remained low, although in a certain conditions the parasitism reached 50% of a sample of 18 galls at one site [13]. *C. connexa* has been reported to be established easily from small founder populations, and once established, its population can rapidly build up and spread [12]. The population of *C. connexa* in South Lampung may come from North Sumatera as a release site in Sumatra [13]. Based on Desmier de Chenon et al.'s study [11], *C. connexa* can spread over 200 km within 5 years following release. However, the population in South Lampung may have also come from West Java, which is the closest distance from the release site although it is separated from it by the Sunda Strait.

One interesting finding is the new association that *C. connexa* has with local parasitoids, which may affect its effectiveness to control *C. odorata*. This finding suggests that this new association is a factor that needs to be taken into consideration for classical biological control in the future [34]. The most dominant parasitoid was *Quadrastichus* sp. This genus is known for plant gall wasp species, such as *Quadrastichus erythrinae*, the Erythrina gall wasp [35], and also as for parasitoids, such as *Quadrastichus mendeli*, a parasitoid of the eucalyptus gall wasp (*Leptocybe invasa*) [36]. In addition, *C. odorata* also has a new association with local gall fly species, *Lonchaea* sp [37] and *A. gossypii*. As the most abundant herbivore to be associated with *C. odorata*, aphids were found attacking the shoot tips. It appears there is a competition between *C. connexa* and *A. gossypii* due to the fact that the gall fly uses the shoot tips for laying eggs and the larvae develop within galls at the nodes of the plant. In this research, we did not investigate the effect of aphids on *C. odorata* as well as *C. connexa*. However, previous research by Watanabe, et al. [38] revealed that the parasitism of aphid, *Macrosiphoniella yomogicola*, significantly decreased the number of inflorescences of the mugwort (*Artemisia montana*). The presence of several

herbivores may be effective to control *C. odorata*, which was also shown in the simultaneous occurrence between *C. connexa* and *P. pseudoinsulata* that significantly reduced the performance of *C. odorata* [39,40].

The interaction of *C. odorata* with *C. connexa*, local herbivores, and other insects was affected by region and elevation, but not by land-use type. This is due to the ability of *C. odorata* to establish itself and become dominant in different land-use types in sunny and open areas (e.g., disturbed areas, grasslands, and plantations) while not being able to bloom under the shaded conditions of undisturbed forest [1]. Elevation was reported to affect the distribution of *C. odorata*. In its native range, *C. odorata* can grow from the coast to an elevation of 1000–1500 m asl [41] and in Asia, *C. odorata* can be found up to 1000 m asl [42]. The population density of *C. connexa* is also affected by elevation, and it can be more effectively controlled by *C. odorata* in lower elevations, especially in areas with a short dry season [13]. At higher elevations and cooler areas, the abundance of *C. connexa* is lower due to the fact that the population tends to build up slowly [1]. The same pattern was also shown for associated parasitoids—the parasitism of *C. connexa* becomes lower at higher elevations. By contrast, the species richness of insects associated with *C. odorata* tends to increase with increasing elevation. In general, the diversity of insects tends to increase with increasing elevation, though the maximum species richness occurs at intermediate elevations [43]. However, there is a complex interplay between local ecological interactions that determines the effect of elevation on the species richness of insects [44].

The interaction between *C. odorata* and local insect diversity as well as *C. connexa* with local parasitoids indicates a consequence of the presence of new species in a new habitat that may provide ecosystem services or disservices. As a host-specific herbivore [13,45], the new interaction may impair the existence of *C. connexa* and cause the failure to control *C. odorata* [17,46], as well as possibly support ecosystem disservices due to non-target impacts [47]. The results of this research showed the importance to understand the long-term effect of the introduction of natural enemies on their ability to control their target species [48]. This research revealed that introduced biological control agents encounter reduced effectiveness to control invasive weeds due to new interactions with local insects diversity (e.g., natural enemies).

5. Conclusions

Our research results revealed that the elevation and geographical area affect the insect communities associated with *C. odorata* as well as its natural enemy, *C. connexa*. We recorded a new association of local gall fly, *Lonchaea* sp., and *A. gossypii* (the most abundant herbivore) with the Siam weed. We also found a new association between *C. connexa* and 13 species of parasitoid wasps that are dominated by *Quadrastichus* sp. These results indicate that the release of introduced biological control agents of invasive weeds will have reduced effectiveness as a consequence of new associations with local insects. Thus, long-term studies to determine the effect of trophic interaction on biological control agents are needed to conserve the population and effectivity of biological control agents.

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