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Preliminary Study on the Distribution of the Introduced Gall-forming Fly, *Cecidochares connexa* (Macquart) (Diptera: Tephritidae) for the Biological Control of the Invasive Alien Weed *Chromolaena odorata* (L.) R.M. King & H. Rob. (Asteraceae) in the Philippines

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The distribution of *Cecidochares connexa* (Macquart), a biological control agent of the invasive plant *Chromolaena odorata* (L.) R.M. King & H. Rob. was determined around the three main islands – Luzon, Visayas, and Mindanao – in the Philippines. A total of 105 sites in 17 localities with *C. odorata* were surveyed for the presence of *C. connexa*. *Cecidochares connexa* was present at 82 sites in eight localities, limited to around Visayas and Mindanao. Some sites where the gall fly was reported were up to 400 km from the initial release sites around Davao, Mindanao. *Cecidochares connexa* was not found at any of the nine localities surveyed around Luzon. Visual observations showed that the gall fly is having some impact on *C. odorata*, as evidenced by dead branches and stems. These results show that *C. connexa* has firmly established in the country and that it has the ability to disperse long distances to new areas. It is likely that *C. connexa* will continue to disperse further with time. However, a more robust study regarding its presence in other parts of the country and its effectiveness as a biological control agent is needed.

Key words: impact, Luzon, Visayas, Mindanao

INTRODUCTION

Invasive alien species (IAS) are non-native organisms that have an adverse ecological and economic impact on the environment and the communities around them (Bruton & Merron 1985, De Silva 1989). Seventeen years ago, invasive species were estimated to cause more than $1.4 trillion annually in damage worldwide (Pimentel *et al.* 2001) and this is likely to have increased. For instance, the total annual losses that can be attributed to harmful non-indigenous species – including crop pests (*i.e.*, insects, weeds, and pathogens); molluscs (golden apple snail); rodents; animal diseases; and human diseases (*i.e.*, measles, malaria, cholera, dengue, human immunodeficiency virus, and Severe Acute Respiratory

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Syndrome) on agricultural systems, human health, and the environment for each of the 10 member countries of the Association of Southeast Asian Nations (ASEAN) was estimated at $33.2 billion – with $21.6 billion being the estimated annual total losses to crop production by non-native weeds, insects, and pathogens (Nghiem *et al.* 2013).

IAS are one of the major agricultural problems the Philippines has been battling for many years. One of these species is the neotropical weed *Chromolaena odorata* (L.) R.M. King & H. Rob. (Asteraceae), known locally as *hagonoy* (Oerke *et al.* 1994, Sinohin & Cuaterno 2003). It was first recorded in the Philippines on the Zamboanga Peninsula in the 1960s through traders from Borneo (Codilla & Metillo 2011). Subsequently, it was documented on Busuanga Island, Palawan; at Davao City; and San Jose, Mindoro Occidental (Pancho & Plucknett 1971, Aterrado & Bachiller 2002, Lariosa *et al.* 2003, Codilla & Metillo 2011), and is now recorded throughout the archipelago.

Chromolaena odorata is a serious invader of many crop lands, plantations, wastelands, rangelands, and reforested areas – infesting up to about 500,000 hectares (Aterrado & Bachiller 2002, Acasio 2003) and is considered one of the seven worst invasive species in the Philippines (Joshi 1989). *Chromolaena odorata* is also found in nearly 50 other tropical countries worldwide, causing major agronomic problems (Awanyo 2008, Zachariades *et al.* 2009).

Chromolaena odorata is known to shade out other vegetation and for having allelopathic properties that enables it to prevent the establishment of other plant species, which reduces plant biodiversity. *Chromolaena odorata* also interferes with natural ecosystem processes and can alter the integrity of natural plant communities (Goodall & Zacharias 2002). It has toxic properties that cause livestock poisoning and is a fire hazard during the dry season, affecting wildlife and animal husbandry (Joshi 1989, Timbilla & Braimah 1991). These characteristics of *C. odorata*, make it one of the world's 100 worst invasive alien plant species (Lowe *et al.* 2000).

Chromolaena odorata can be controlled by mechanical, chemical, and biological means (Rusdy 2015), with mechanical control being the most common method used. However, this method is labor-intensive and expensive (Groves 1989, Zachariades *et al.* 1999). Chemical control can be used but has significant negative impacts on the environment and is rarely effective in the long term, as repeated applications are necessary (Luwum 2002, Rusdy 2015). Biological control, on the other hand, is a natural way of controlling IAS. Classical biological control is the use of co-evolved host-specific natural enemies to control the invasiveness of a species (McFadyen *et al.* 2003).

Several biological control agents have been utilized to control *C. odorata.* The two most common are the leaf-feeding moth *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Erebidae) and the stem-galling fly *Cecidochares connexa* (Macquart) (Diptera: Tephritidae) (Zachariades *et al.* 2009). *Pareuchaetes pseudoinsulata* spread into the Philippines unaided into the Palawan Islands in 1985 and subsequently spread throughout the country (Waterhouse 1994, Muniappan *et al.* 2005). However, no study has ever been conducted regarding its effectiveness as a biological control agent in the country.

Cecidochares connexa is part of a species complex native to tropical South America (Prado *et al.* 2002) and was first utilized as a biological control agent for *C. odorata* in Indonesia, where it was imported from Colombia in 1993. Specimens of the insect that was tested and released in Indonesia were lodged with the Australian National Insect Collection, Canberra (McFadyen *et al.* 2003). Specimens were also lodged at IOPRI, Marihat Research Station, Sumatra, Indonesia where the research was undertaken. Following host-specificity testing against 55 species representing 17 families and its subsequent field release in Indonesia (McFadyen *et al.* 2003), a small colony of *C. connexa* was imported from Indonesia into the Philippines in 1999. Additional host-specificity testing was conducted in the Philippines at the quarantine confinement facility at the Philippine Coconut Authority – Davao Research Center (PCA-DRC) from Aug 1999 to Mar 2000 (Aterrado & Bachiller 2002). The tests confirmed *C. connexa* was highly specific and should be an effective biological control agent of *C. odorata*. However, despite being host-specific, it was approved for release only around PCA-DRC. Monitoring confirmed its establishment at some release sites but no further studies were conducted.

This study aimed to determine the current distribution of *C. connexa* in the Philippines, 18 years after it was first released in the country. Information from this study may also provide assistance with the management of *C. odorata* using *C. connexa*.

MATERIALS AND METHODS

Distribution of *Cecidochares connexa*

The distribution of *C. connexa* was determined by sampling sites where *C. odorata* was present on the main islands of Luzon, Visayas, and Mindanao, as well as some smaller nearby islands. Ninety-one sites around the Philippine Coconut Authority – Davao Research Center (PCA-DRC) were included in the study because it is the original point of release of *C. connexa* in 2000. PCA-DRC served as the reference point from which to measure the distance of the range extension of *C. connexa* to other parts of the country.

At each site, *C. odorata* plants were examined by two people for a maximum time of 30 min for the presence of galls on stems. For some sites, there was a high density of galls, which were very evident and so the searching time was shorter. As galls indicated the presence of *C. connexa*, the standard methods for the collection of insects using beating sheets and insect nets were not used in this study. Samples of galls were collected and some were dissected to confirm that the galls were caused by *C. connexa*. Adult *C. connexa* that emerged from the galls were curated for future reference and lodged at the Philippine National Museum of Natural History, Ermita, Manila, Philippines for future reference, and one of the authors determined the tephritid biocontrol species with voucher specimens lodged at the Ecosciences Precinct, Brisbane, Australia.

Chromolaena odorata samples were taken from representative sites and preserved using the standard method of drying and pressing and permanently stored in the Biology Laboratory of Adamson University, San Marcelino Street, Ermita, Manila, Philippines for future reference.

The locations of *C. odorata* and *C. connexa* were tabulated and mapped using Quantum GIS ver. 2.18.9. The distance of range extension of *C. connexa* from its point of release was measured using Google Earth ver. 7.3.0.

Impact of *Cecidochares connexa*
Visual impact of the gall fly on *C. odorata*, was assessed in terms of physical plant health (*i.e.* whether plants and/or branches were moribund) and recorded with a camera. Impact assessment was based on those in Baars and Heystek (2003) as there were no comparable studies for *C. odorata* and gall-forming insects. The categories were: (0) No damage – no galls were observed and no characteristic signs of new or old damage on the plants; (1) Slight – 1–5 galls were seen per plant, with minimal damage (*e.g.*, very few moribund twigs present); (2) Moderate – 6–10 galls were seen per plant with moderate damage (*e.g.*, numerous moribund twigs and/or branches); and (3) Abundant – plants with 10+ galls present, with many moribund branches.

RESULTS

Distribution of *Cecidochares connexa*
A total of 105 sites in 17 localities with *C. odorata* were sampled for the presence of *C. connexa* around the three main islands in the Philippines. Nine localities were sampled around Luzon, three localities around Visayas, and five localities around Mindanao (Figure 1). Seven localities were on the island of Luzon and one locality each on the nearby islands of Mindoro and Palawan. For Visayas, there was one locality on each of the islands of Negros Oriental, Bohol, and Cebu. Around Mindanao, the localities surveyed were Davao City – which incorporated the PCA where *C. connexa* was released (27 sites) – Davao

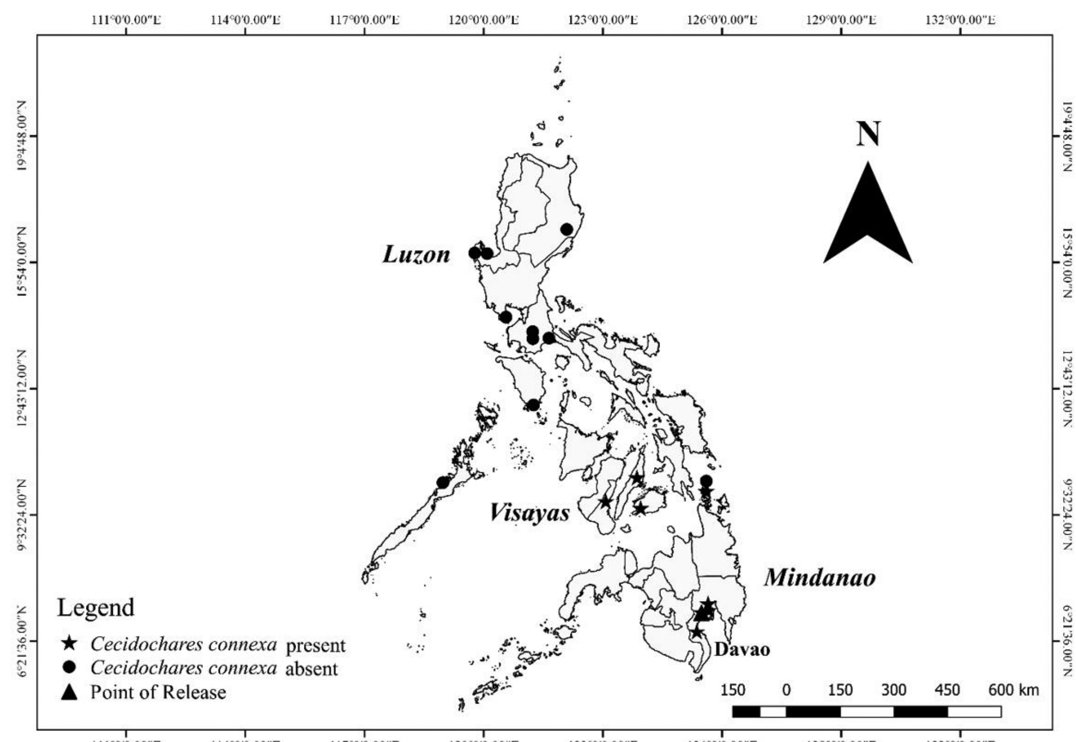

Figure 1. Map showing the sites at which *C. odorata* was surveyed around Luzon, Visayas, and Mindanao and whether *C. connexa* was present or absent.

del Norte (24 sites), Davao del Sur (16 sites), Samal Island (24 sites), and Dinagat Island (two sites).

Cecidochares connexa was found at all three localities (Negros Occidental, Bohol, and Cebu islands) around Visayas and all five localities around Mindanao (Figure 1). Around Mindanao, *C. connexa* was common at Basilisa, Dinagat Island and widespread throughout the four localities in the south (Table 1). *Cecidochares connexa* was not found at any site around Luzon (Figure 1). The list of

Table 1. Description of *Chromolaena odorata* plants and the presence of *Cecidochares connexa* at each locality sampled in the Philippines.

Locality	Presence/ absence of *C. connexa* (+/-)	Description
(Luzon region) Brgy. Tappa, San Mariano, Isabela, Luzon Island	–	Scattered stands of *C. odorata*. Sandy soil. Well-lit areas and outside the vegetation. No galls were observed.
(Luzon region) Sual, Pangasinan, Luzon Island	–	*C. odorata* abundant. Present beside roads, in residential areas and near the shore. Plants had thin, glossy leaves and slender stems. No galls were observed.
(Luzon region) Agno, Pangasinan, Luzon Island	–	Healthy *C. odorata* abundant along the sea shore, inside mango plantations, beside the road, and attached to elevated rock substrate. No galls were observed. Feeding marks similar to that caused by *P. pseudoinsulata* were observed on some *C. odorata* plants.
(Luzon region) Limay, Alangan, Pilar, Bataan, Luzon Island	–	Patchy distribution of *C. odorata* plants. *C. odorata* plants observed in this area have thin branches and tall stands (about 2 m height). No galls were observed.
(Luzon region) University of the Philippines, Los Baños, Laguna, Luzon Island	–	Patchy distribution of *C. odorata*. Most plants were 1–3 m high and found along the trail at Mt. Makiling, but never inside the forest. No galls were observed.
(Luzon region) Mt. Malarayat, Lipa, Batangas City, Luzon Island	–	*C. odorata* was recorded at 705 m. *C. odorata* plants had a patchy distribution. They were found along the mountain trail and near trees but never within the forest. No galls were observed.
(Luzon region) Tayabas City, Quezon Island	–	*C. odorata* abundant. Large thickets were found near banana plantations. No galls were observed.
(Luzon region) Bulalacao, Mindoro Island	–	*C. odorata* abundant. Inside or near coconut plantations and rice fields. No galls were observed.
(Luzon region) Sitio Gawid, Brgy. Caruray, San Vicente, Palawan Island	–	Patchy distribution of *C. odorata*. Scattered along the coastal area and besides coconut trees. Large thickets seen on the hillsides. No galls were observed.
(Visayas region) Holcim Silica Quarry, Ayungon, Negros Oriental Island	+	Patchy distribution of *C. odorata* plants were observed beside a silica mining quarry. Galls were observed on some plants, in the upper portion of the stems.
(Visayas region) Tarsier Wildlife Sanctuary, Corella Road, Tagbilaran, Bohol Island	+	*C. odorata* shrubs were located outside the Tarsier Wildlife Sanctuary. Abundant number of galls was present on a lot of *C. odorata* plants, mostly in the apical meristem.
(Visayas region) Cebu City, Island	+	*C. odorata* is widely distributed in this area and galls were observed.
(Mindanao region) PCA, Davao City (Point of Introduction)	+	*C. odorata* is widespread in this area, being found at 27 sites. They were found near coconut, cacao, and mango plantations and along the road. Galls were present at 20 sites, on plants from the apical stem to the lowermost trunk. Most *C. odorata* plants observed with galls were either dying or already withered.
(Mindanao region) Davao del Norte	+	Dense thickets of *C. odorata* occurred mostly in abandoned areas and near coconut and rice plantations. Galls were present on plants at all 24 sites where *C. odorata* was found.
(Mindanao region) Samal Island, Davao del Norte	+	Large thickets of *C. odorata* were widely spread on the island. They were found present along the road, near coconut plantation and residential areas, and open and elevated areas. Galls were present at all 24 sites, on plants, from the apical stem to the lower trunk of the plant. The stems were either withered or died.
(Mindanao region) Davao del Sur	+	Large thickets of *C. odorata* plants were found beside banana trees and along the road at 16 sites. *C. odorata* plants with numerous galls were found at 10 sites.
(Mindanao region) Basilisa, Dinagat Island	+	Abundant *C. odorata* plants were observed around Basilisa, on the lower side of the hill. *C. connexa* galls were observed on most *C. odorata* plants. The *C. odorata* stems were withered. There was one healthy *C. odorata* plant observed at Loreto but no galls were observed.

localities and islands where *C. connexa* was recorded, as well as the health of *C. odorata* at each site are recorded in Table 1. It should be noted that during the surveys, no adult *C. connexa* were encountered. However, mature galls were collected and *C. connexa* emerged from these galls. Adults that emerged were curated and lodged at the Philippine National Museum of Natural History with catalogue numbers PNMNH 13654-13656. Figure 2 shows an adult and larva of *C. connexa*, as well as an intact gall.

Since *C. connexa* was released in 2000, it has spread from the release sites around PCA, Davao to over 400 km, covering several islands in the regions of Visayas and Mindanao (Table 2).

Figure 2. (A) Adult of *C. connexa* (photo from C. Wilson, Australia); (B) swelling of the stem of *Chromolaena odorata* due to the presence of *Cecidochares connexa*; (C) exposed larva of *C. connexa*.

Table 2. Distance travelled by *Cecidochares connexa* from the Philippine Coconut Authority, where it was field released.

Location	Distance (km)
PCA to Samal Island (Mindanao region)	20.18
PCA to Davao del Norte (Mindanao region)	31.62
PCA to Davao del Sur (Mindanao region)	55.41
PCA to Tagbilaran, Bohol (Visayas region)	336.07
PCA to Basilisa, Dinagat Island (Mindanao region)	336.34
PCA to Ayungon, Negros Occidental(Visayas region)	407.05
PCA to Cebu City (Visayas region)	416.70*

*Farthest distance travelled

Impact of *Cecidochares connexa*

Cecidochares connexa was observed in all areas surveyed around Visayas and Mindanao. Galled plants tended to have withered or moribund stems (Table 1; Figure 3). However, further studies that include the quantification of the effects and assessment of the impact of *C. connexa* to *C. odorata* are needed.

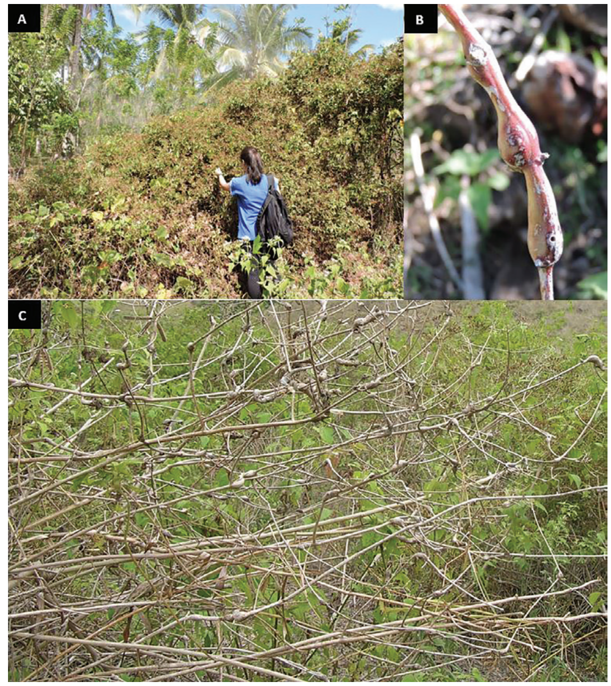

Figure 3. (A) Large thicket of dead shrub of *Chromolaena odorata* due to heavy infestation of galls inside PCA, Davao City; (B, C) moribund stems of *C. odorata* showing galls.

DISCUSSION

This is the first study conducted to confirm the presence and spread of the biological control agent *C. connexa* introduced in 1999 by the Philippine Coconut Authority – Davao Research Center to control the invasive plant species *C. odorata*. This study verified that *C. connexa* has established in the country and is now found in numerous localities in the Davao region and at several sites on other islands in the north of Mindanao and central Visayas. The farthest site from the point of release that *C. connexa* was found was at Cebu City, about 400 km away.

Visual inspections of *C. odorata* found that branches and stems which were galled were usually withered and death of branches, stems, and plants common. This suggests that the gall fly is having some impact on *C. odorata*.

The spread and distribution of *C. connexa* over the last 18 years in the Philippines is not unusual. *Cecidochares* *connexa* was reported to have spread over 100 km in Papua New Guinea (PNG) over seven years (Day *et al.* 2013a) and about 1000 km in West Africa over 13 years (Aigbedion-Atalor *et al.* 2018). The spread in the Philippines is noteworthy, as *C. connexa* has spread to numerous islands and where the distribution of *C. odorata* is patchy.

Other weed biological control agents have also managed to disperse and spread to other islands from where they were released. The leaf-mining fly *Calycomyza lantanae* (Frick) (Diptera: Agromyzidae), a biological control agent for *Lantana camara* L. (Verbenaceae), was released in only three countries (Australia, Fiji, and South Africa) but is now found in 29 countries – including seven countries in the Pacific and 12 countries in Asia (Winston *et al.* 2014). The herringbone leaf-mining fly *Ophiomyia camarae* Spencer (Diptera: Agromyzidae), another biological control agent for *L. camara*, spread from South Africa to Madagascar in eight years, to Ethiopia in nine years, and to La Réunion Island in 13 years (Winston *et al.* 2014).

The ability of biological control agents to move and spread to other regions where the target weed is present is considered a desirable trait, as it circumvents active re-distribution programs. However, in cases where weeds are patchy or found on islands or if the biological control agent is relatively sedentary, it may be necessary to assist in the spread of the biological control agent to overcome such geographical barriers or to speed up its spread (Harley & Forno 1992).

Since *C. odorata* is found widespread on some islands in the Philippines (Joshi 1989, Aterrado and Bachiller 2002, Acasio 2003), it may be advantageous to move galls to other islands in the Philippines such as Luzon, where it is not already present to help manage *C. odorata*.

Anecdotal observations found that *C. odorata*, which was heavily galled by *C. connexa*, possessed withered branches or stems. In some cases, branches, stems, or even whole plants had died due to the effect of *C. connexa*. These results support similar observations in other countries such as PNG, Guam, and Timor Leste where *C. connexa* has established (Day *et al.* 2013a, 2013b; Reddy *et al.* 2013). In these countries, the presence of *C. connexa* significantly reduced infestations of *C. odorata*. Furthermore, in parts of PNG, *C. odorata* is now considered under control (Day *et al.* 2013a).

However, further studies that include the quantification of the effects and assessment of the impact of *C. connexa* to *C. odorata* in the Philippines are needed.

CONCLUSIONS AND RECOMMENDATIONS

Chromolaena odorata is widespread in the Philippines, with the gall fly currently found on two of the main islands *i.e.*, Visayas and Mindanao. *C. connexa* has been able to extend its distribution from the point of its introduction in PCA, Davao City some 18 years ago by 400 km. From the field observations, *C. connexa* appears to be having an impact on *C. odorata*, with many plants having withered or dead stems and branches. The full impact of *C. connexa* on *C. odorata* is still to be determined.

We recommend a more robust study to determine the full distribution of both *C. odorata* and *C. connexa* in the Philippines and the efficiency of *C. connexa* as a biological control agent to *C. odorata*. Lastly, since another biological control agent *Pareuchaetes pseudoinsulata* for *C. odorata* was observed during the study, it would be beneficial to conduct surveys regarding its distribution and effects on *C. odorata* as well.

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