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# Characterization of native parasitoid community associated with the invasive pest *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) in Cantabria (northern Spain)

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## Abstract

A survey of the native parasitoid community was conducted to characterize its possible use as biological control for Asian Chestnut Gall Wasp (ACGW) at two sampling sites in northern Spain. To this end, 500 ACGW galls were collected over five sampling dates between May and July 2017; 250 of them were dissected to estimate the parasitism rates and the remaining 250 galls were placed in emerging rearing boxes to collect adult parasitoids. Seven native parasitoid species belonging to six families (i.e. Eupelmidae, Eurytomidae, Ormyridae, Megastigmidae, Pteromalidae and Torymidae) were identified by morphological traits. All sampled species are considered as native parasitoids of gallers-oaks (*Quercus* spp.). The most abundant species were *Sycophila variegata* (Curtis), *Torymus auratus* (Müller) and *Sycophila biguttata* (Swederus), representing 70% of the identified parasitoids. In addition, the presence of these native parasitoids was associated with high parasitism rates, especially at the site that was surrounded by oak trees. Our findings highlight the important contribution of native parasitoids to pest regulation throughout the period of ACGW gall development.

## Keywords

Asian chestnut gall wasp, biological control, Chalcidoidea, chestnut pest, parasitism rate

## Introduction

Asian Chestnut Gall Wasp (*Dryocosmus kuriphilus* Yasumatsu; Hymenoptera: Cynipidae; ACGW), native to southern China, induces the formation of green- or red-coloured galls about 5-20 mm in diameter on new stems, petioles and leaves of chestnuts (*Castanea* spp.) (EPPO, 2005). It is considered one of the main pests of the genus *Castanea* worldwide, since the galls formed on the apical shoots cause abortion of the female flowers, significantly reducing fruit production by 50-80% in *C. sativa* (EPPO, 2005; Battisti *et al.*, 2014). However, infestation rarely causes plant death, only in cases of severe damage to seedlings or debilitated plants (Kato and Hijii, 1997; Cooper and Rieske, 2007; Gehring *et al.*, 2018).

ACGW is a univoltine species with parthenogenetic thelytokous reproduction (i.e. only females are known) (Kato and Hijii, 1993; EPPO, 2005; Abe *et al.*, 2007). Females usually lay ~100 eggs (about 3- 5 eggs per cluster) on chestnut buds that will prevent branches and leaves from being healthy the next year (EPPO, 2005). Adult develop shorth reproductive flight period (about ten days) that comprises the period between mid.June and late-July (EPPO, 2005).The eggs hatch in approximately 30-40 days and the larval stage completes its development in the following spring, when galls will begin to form (Viggiani and Nugnes, 2010). Depending on altitude, exposure, or host genotype, pupation occurs from mid-May to mid-July (EPPO, 2005).

Chemical control has been ineffective since ACGW development occurs inside galls (Cooper and Rieske, 2007). Tree management practices through pruning is not recommended because abandoned ACGW galls could be a refuge for some parasitoids species that help control the pest (Cooper and Rieske, 2007). Therefore, its control has focused on the search for resistant host genotypes (e.g. Moriya *et al.*, 2003; Dini *et al.*, 2012; Panzavolta *et al.*, 2012; Sartor *et al.*, 2015; Nugnes *et al.*, 2018), entomopathogenic fungi (e.g. Magro *et al.*, 2010; Graziosi and Rieske, 2015; Tosi *et al.*, 2015; Fernández *et al.*, 2018; Muñoz-Adalia *et al.*, 2019) and native parasitoids (e.g. Aebi *et al.*, 2006; Cooper and Rieske, 2007; Matošević and Melika, 2013; Kos *et al.*, 2015; Panzavolta *et al.*, 2018; Jara-Chiquito *et al.*, 2020). In this regard, in the late 1970s, a parasitoid native to China, *Torymus sinensis* Kamijo (Hymenoptera: Torymidae), was imported into Japan, which managed to control the pest below 30% shoot infestation after 6 to 18 years of release (Quacchia *et al.*, 2008). This Chinese univoltine ectoparasitoid is controlling ACGW infestation levels in all countries where it has been imported and has been postulated as one of the most effective tools in the control of ACGW in the short and medium term (Moriya et al., 2003). However, reported evidence of displacement and/or hybridizations of native parasitoids species, and attacks on non-target species in regions where the exotic parasitoid has been released calls for studying the potential of local natural enemies to control ACGW (Yara *et al.*, 2012; Ferracini *et al.*, 2017, 2018; Pogolotti *et al.*, 2019; Jara-Chiquito *et al.*, 2020).

Chestnut forests from temperate regions are ecosystems of high ecological and landscape value. These stands are located in regions of special nature protection recognized by the European Union's Habitats Directive (92/43/EEC). From the point of view of the health of these forests, climate change represents a threat, since insects respond positively to temperature and their abundance may peak at warm temperatures, encouraging pest outbreaks or disturbing trophic interactions (Pureswaran *et al.,* 2018). However, biological control agents (i.e. parasitoids) could also benefit from environmental changes, both in terms of finding more resources (hosts) in a wider time window (Tougeron and Tena, 2019). In this regard, native parasitoids can be used in biological control to reduce ACGW infestation levels, particularly where the presence of facultative hyperparasitoids could delay, or even temporarily suppress, the establishment of *T. sinenis* (Murakami and Gyoutoku, 1995; Cooper and Rieske, 2007, 2011; Panzavolta *et al.*, 2013).

In Spain, ACGW was firstly recorded in 2012 in Catalonia region (Jara-Chiquito *et al.*, 2016), being currently present in almost the whole distribution range of *Castanea sativa* Mill. in Spain (Nieves-Aldrey *et al.*, 2019). In 2019, the Ministry of Agriculture, Fisheries and Food (MAPA) authorized the release of *T. sinensis* as a biological control organism of ACGW throughout the territory. However, the role of native parasitoids in the effective control of ACGW in Spain remains uncertain due to the significant lack of research on this topic. In consequence, the aim of this study was to characterize the native parasitoid community and its potential use in the biological control of ACGW in northern Spain.

## Materials and methods

Two *C. sativa* stands (Site 1 and 2) were surveyed in Cantabria (northern Spain). Site 1 (Table S1) includes a 15 to 20 year old chestnut forest, located in a riparian zone, with hardly any human intervention and forming a mixed forest with *Quercus robur* L.. At this site, ACGW galls were collected from a representative witness tree previously used for biocontrol studies with entomopathogenic fungi (Muñoz-Adalia *et al.*, 2019). Following the criteria mentioned for Site 1, another chestnut witness tree was selected for collecting the galls from Site 2 (Table S1). This site was a 25 to 30 year-old chestnut grove located on a steep slope (52.5%), surrounded by a Monterey pine (*Pinus radiata* D. Don) plantation for timber purposes and strong timber extraction and fire prevention management.

The beginning of the sprouting of the chestnut trees began in the second half of April and the first galls were appreciated from the end of April-beginning of May in the branches of the chestnut trees. Galls were collected from May to the end of July 2017 (31/05, 14/06, 3/07, 13/07 and 25/07) at both sites. On each sampling date, 50 current season galls were randomly collected per tree at 5 m height. Of the 50 galls per tree collected monthly, 25 galls were dissected under a stereo-microscope (MOTIC SMZ–168 SERIES) and the remaining 25 galls were placed in cardboard rearing boxes. Gall dissection at each sampling date allowed the evaluation of the following categories of ACGW developmental stages: [L] larvae; [P1] white pupa, [P2] black pupa, and [A] adult. Empty larval chambers [E] and the presence of different developmental stages of the parasitoids (larvae, pupae and adults) were also recorded and counted. ACGW specimens (larvae, pupae and adults) and parasitoids in larval and pupal stage were stored in the laboratories of the Zoology area of the University Campus of Palencia (University of Valladolid). The galls placed in cardboard rearing boxes provided with extractable skylights (transparent plastic containers with screwed cap, Ø35 mm x 40 mm) were kept at room temperature (25-28 ºC) and checked daily to collect emerged adult parasitoids until end-July.

For each date and site the following rates were calculated: 1) parasitism rate (Pr: parasitized chambers*100/total chambers); 2) parasitism rate, including chambers with parasitoid exuviae [Pe: (parasitized chambers + empty chambers with exuvial remains)*100/total chambers] and; 3) overall mortality rate of ACGW [Pef: (parasitized chambers + empty chambers with exuvial remains + empty chambers with presence of fungal mycelium + empty chambers with presence of dead specimens)*100/total chambers]. Hyperparasitism rate was also obtained for each site (Hr: hyperparasitized chambers*100/total chambers containing parasitoids).

Parasitoids adults were identified using the dichotomous keys by Thuróczy and Askew (unpublished data) and were deposited in the JP-V entomological collection of the University of Barcelona.

Prior to statistical analysis, the normality and homocedasticity of the data was checked with the Shapiro-Wilk and Levene tests. As the mean parasitism rate data were normal and their variances equal, the difference between the sampling sites was analyzed by the T-test (n=5), using the R enviroment (RStudio Team, 2019).

## Results

Overall, larvae (L), white pupae (P1), black pupae (P2) and adults (A) of ACGW were observed from May to mid-July, end of May to beginning of July, mid-June to end of July, and during the entire month of July, respectively (Figure 1). Development stages of ACGW were completed earlier at Site 2 than at Site 1 (Figure 1). Parasitoids larvae and pupae were present throughout the studied period at both sites (Table 1). Twenty-six specimens of native parasitoids, belonging to seven species and six families, were collected directly from cardboard rearing boxes or by gall dissection (Table 2). Five species were found at Site 1, while four species were detected at Site 2. The most abundant species were *Sycophila variegata* (Curtis), *Torymus auratus* (Muller) and *Sycophila biguttata* (Swederus), which represented 70% of the individuals collected (Table 2). At Site 1, *S. variegata* and *Bootanomyia dorsalis* (Fabricius) emerged first in early June, followed by *Mesopolobus tibialis* (Westwood) (Figure 1). *Sycophila variegata* reappeared in early and late July (Figure 2) whereas *S. biguttata* was only observed in mid-July along with *B. dorsalis*, which was recurrent during the sampling period. Finally, *Eupelmus urozonus* emerged at the end of July (Figure 2). At Site 2, *T. auratus* was collected from mid-June to mid-July, co-occurring with species such as *S. variegata*, *S. biguttata* and *O. pomaceus* during July (Figure 2).

Pr were high (up to 44.68%) and hardly changed at Site 1 during the five sampling dates, but at Site 2, rates decreased progressively until the end of July, when this rate increased to values similar to those observed in mid-June. (Table 3). Mean Pr at Site 2 (16.49 ± 2.81%) was significantly lower than at Site 1 (39.23 ± 2.19%) ($p<0.001$). Hr was 5.69% and 1.69% at Site 1 and 2, respectively.

The percentage of empty larval chambers was similar for both sites (27% and 25% at Site 1 and 2 respectively). Most of these empty chambers were associated with adult emergency holes, mainly at Site 2 (Table 1). However, we have also detected larval chambers with the presence of fungal mycelium, exuvial remains or dead specimens in both sites (Table 1). Considering empty chambers with exuvial remains as parasitized, mean Pe values reached $41.94 \pm 2.07$ (Site 1) and $17.74 \pm 3.04$ (Site 2) (Table 3). Finally, mean Pef showed an overall mortality of the ACGW close to 50% for Site 1 and approximately 23% for Site 2 (Table 3).

## Discussion

The parasitoid community associated with ACGW in newly-formed galls collected in Cantabria was characterized. Seven parasitoids species have been detected four years after the first occurrence of the gall wasp in this area (Fernández *et al.*, 2018) (Figure 2; Table 2). All these species have been previously cited in Spain by Jara-Chiquito *et al.,* 2016; Pérez-Otero *et al.,* 2017 and Jara-Chiquito *et al.,* 2020), as well as in other countries such as China, Japan, Korea or Italy (Aebi *et al.*, 2006; Quacchia *et al.*, 2013; Panzavolta *et al.*, 2013, 2018). The presence of different species throughout all the sampling periods suggests an existing temporal complementarity among the natural enemies in the control of ACGW. For instance, at Site 1, adults of species such as *S. variegata* or *B. dorsalis* were recurrent during the months of June and July, occasionally accompanied by species such as *M. tibialis, E. urozonus* or S. *biguttata* (Figure 2). On the contrary, *T. auratus, S. biguttata* or *O. pomaceus* appeared at the same time during some of the July samplings from Site 2 (Figure 2). This temporal complementarity between natural enemies in the control of ACGW has already been observed in previous studies and suggests that some species find the same "window of vulnerability" to attack ACGW, despite being different regions and/or countries (e.g. Matošević and Melika, 2013; Panzavolta *et al.*, 2018; Bonsignore *et al.*, 2019; Jara-Chiquito *et al.*, 2020). In this sense, are consistent with those provided by Bonsignore et al., (2019) who reported that species such as *T. auratus* or *B. dorsalis* parasitize ACGW later than the formation of the pupa phase of ACGW (Figure 2). However, our results contrast with the mentioned study about *S. variegata* at least for the first gall sampling at Site 1 (Figure 2). This species has been collected from galls since the first moment of sampling, when only larvae of ACGW were found in Site 1 (Figure 1). This suggests an early attack of this species on ACGW at some point during its long flight period (approximately 10 months) (Jara-Chiquito *et al.*, 2020). Most of the parasitoids identified in this study have a wide host range and a prolonged flight period so that the same species could attack at more than one moment during the development period of ACGW (Jara-Chiquito *et al.*, 2020). In fact, although the number of specimens is very low, *B. dorsalis* was detected in two different periods (Figure 2). This observation supports an aspect already known in the bibliography referring to the fact that *B. dorsalis* is a polyphagous species of cynipid galls that presents several annual generations (Jara-Chiquito *et al.*, 2020). The same could be said for *S. variegata*, *S. biguttata* or *M. tibialis* (Jara-Chiquito *et al.*, 2020).

The presence of these native parasitoids was associated with high parasitism rates, especially at Site 1 (close to 40%; Table 3). The higher parasitism rates at Site 1 could be related to the presence of oak trees in the surroundings. Oak forests may promote the transfer of parasitoids into ACGW-infected  chestnut stands (Aebi *et al.*, 2006; Quacchia *et al.*, 2013; Bonsignore *et al.*, 2019). Our results seem to point towards a greater control of ACGW populations (with higher parasitism rates) in mixed stands with *Quercus* spp. species than in mixed stands with *Pinus* spp. This contrasts with the results obtained by Fernandez-Conradi *et al.*, (2018) who did not observe differences in parasitoid abundance as a function of stand composition. In consequence, the possible role of mixed stands as a source of parasitoids of intertest in biocontrol of ACGW deserves future studies.

The diversity of native parasitoid species in our work indicates that ACGW galls abundance represents a massive unexploited resource available to a variety of parasitoids species that have a similar phenology to *D. kuriphilus* as previously mentioned by Quacchia *et al.*, (2013). As gall develops, several aspects of gall morphology (e.g. gall size, wall thickness) change dramatically. These changes can lead to the parasitoids to lose the opportunity to exploit the gall (Stone and Schönrogge, 2003). Such is the case of ACGW and, indeed, a mismatch between the phenology of gall development of ACGW and emergence times of native natural enemies (Matošević and Melika, 2013; Quacchia *et al.*, 2013; Colombari and Battisti, 2016) or a short ovipositor (Murakami, 1981; Cooper and Rieske, 2011) seems to be the reasons behind the low parasitation rates reached in some countries. However, a diverse parasitoid guild in species that have an appropriate phenology and a flight period that coincides with the vulnerable period of ACGW can obtain high parasitism rates (Jara-Chiquito *et al.*, 2020). The phenological mismatch between ACGW and native parasitoids seems to be compensated by a greater diversity of polyphagous parasitoids that can attack for several moments during gall development (even several generations of a single species could exploit ACGW. In addition, some parasitoids have developed adaptive traits as a result of attacking a wide diversity of oak galls and may help exploit a greater number of ACGW galls. In fact, the adaptive traits of the first-generation of *T. auratus* with different ovipositor sheaths´ length (specimens with short- and long-) can be useful for the successful control of the pest, considering that this parasitoid was the second most abundant species detected (Table 2). By contrast, we have also detected cases of hyperparasitism and species that may act as facultative hyperparasitoids such as *E. urozonus* or *M. tibialis* according to Panzavolta *et al.*, (2018) and Jara-Chiquito *et al.*, (2019) and could have an antagonistic effect on pest control in future years.

Larval chambers containing dead specimens whose cause remains unknown were detected at Site 1 (7.79%) and Site 2 (11.25%) (Table 1). The presence of dead specimens in chambers has been linked to premature death of chamber inhabitants (either *D. kuriphilus* or parasitoids) caused by early strains of parasitoid species or by fungi present inside larval chambers (Cooper and Rieske, 2010). In fact, we also found 14.29% (Site 1) and 6.25% (Site 2) of empty larval chambers containing fungal mycelium (Table 1). Potentially entomopathogenic fungi such as *Colletotrichum acutatum* Simmonds, *Gnomoniopsis castanea* Tamietti, *Cladosporium cladoporioides* Fresenius or *Fusarium* spp. have already been isolated from necrotic and asymptomatic galls in the same sampling area (Fernández *et al.,* 2018; Muñoz-Adalia *et al.*, 2019). These observations are consistent with the Pef values reported here (48.24 and 22.62% at Sites 1 and 2, respectively; Table 3) which could highlight a complementary role of native fungi as biocontrollers, although the role of these fungi in ACGW biocontrol may vary from year to year even when climatic conditions are favorable for their development.

In summary, our results highlight the important contribution of native parasitoids to pest regulation throughout the period of ACGW gall development. However, our study comprised an annual cycle of *D. kuriphilus* in a single area providing a screenshot of the whole colonization process. Subsequently, it would be interesting to analyze these results on a longer time scale, given the inter-annual fluctuations in the parasitism rate and the number of native parasitoid species associated with the ACGW (Panzavolta *et al.*, 2018). The role of species such as *S. variegata*, *S. biguttata* or *T. auratus* in the ACGW control should be studied in further detail. Sequential sampling throughout the life cycle period from larva to adult of ACGW allowed us to detect other sources of natural control, including potential entomopathogenic fungi. Lastly, in a context of climate change, warmer temperatures, longer growing seasons and greater climate uncertainty are expected to change the seasonal strategies (phenology) of insects in temperate regions (Tougeron *et al.*, 2020). Shifts in food web interactions (host-parasitoids-other species) could result in a mismatch of existing interactions and establishment of new ones, or in changes in the frequencies of interactions (Thierry *et al.*, 2019; Tougeron and Tena, 2019). Therefore, the characterization of the native parasitoid community acting on forest pests in temperate regions should be the first step to predict how global warming will act on the host-parasitoid system, since slight climatic modifications could generate cascading effects that affect all trophic levels of the community.


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## Conflict of interest statement

All authors have no conflict of interest to declare.

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Table 1. Characterization of ACGW gall content. Developmental stages of native parasitoids (L: larvae, P: pupae, A: adult), empty larval chambers and number of chambers per gall during sampling periods at both sites.

| | Sampling event | Galls dissected | Parasitoids | | | Empty chambers | | | | | | No. chambers/gall * |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | L | P | A | Fungal mycelia | Exuvial remains | Dead specimens | Exit holes | No exit holes | Total | |
| Site 1 | 31/05/2017 | 25 | 22 | 0 | 0 | 4 | 2 | 0 | 0 | 0 | 6 | 2.20 ± 0.27 |
| | 14/06/2017 | 25 | 23 | 6 | 1 | 0 | 3 | 0 | 0 | 0 | 3 | 2.48 ± 0.31 |
| | 03/07/2017 | 25 | 16 | 6 | 2 | 0 | 1 | 1 | 3 | 11 | 16 | 2.6 ± 0.31 |
| | 13/07/2017 | 25 | 11 | 10 | 1 | 5 | 1 | 1 | 16 | 6 | 29 | 1.88 ± 0.21 |
| | 25/07/2017 | 25 | 8 | 11 | 2 | 2 | 0 | 4 | 15 | 2 | 23 | 2.2 ± 0.20 |
| | Total | 125 | 80 | 33 | 6 | 11 | 7 | 6 | 34 | 19 | 77 | 2.27 ± 0.12 |
| Site 2 | 31/05/2017 | 25 | 15 | 5 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2.28 ± 0.30 |
| | 14/06/2017 | 25 | 2 | 6 | 4 | 0 | 3 | 0 | 0 | 0 | 3 | 2.36 ± 0.29 |
| | 03/07/2017 | 25 | 4 | 3 | 2 | 0 | 0 | 1 | 11 | 1 | 13 | 2.84 ± 0.46 |
| | 13/07/2017 | 25 | 0 | 4 | 2 | 5 | 0 | 6 | 11 | 0 | 22 | 2 ± 0.22 |
| | 25/07/2017 | 25 | 3 | 3 | 1 | 0 | 1 | 2 | 38 | 0 | 41 | 3.12 ± 0.33 |
| | Total | 125 | 24 | 21 | 9 | 5 | 4 | 9 | 60 | 2 | 80 | 2.52 ± 0.15 |

*Values are means ± standard error (SE)

Table 2. Description of parasitoid community associated with *Dryocosmus kuriphilus* during the samplimg period. R: rearing; D: dissection.

| Family | Parasitoid species | Site 1 | | | | | Site 2 | | | | | Relative Abundance % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R | | D | | Subtotal | R | | D | | Subtotal | |
| | | ♂ | ♀ | ♂ | ♀ | | ♂ | ♀ | ♂ | ♀ | | |
| Eupelmidae | *Eupelmus urozonus* Dalman* | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 7.6 |
| Eurytomidae | *Sycophila biguttata* (Swederus) | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 3 | 3 | 15.3 |
| | *Sycophila variegata* (Curtis) | 1 | 3 | 1 | 2 | 7 | 0 | 0 | 0 | 1 | 1 | 30.7 |
| Ormyridae | *Ormyrus pomaceus* (Geoffroy) | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 7.6 |
| Megastigmidae | *Bootanomyia dorsalis* (Fabricius)* | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 7.6 |
| Pteromalidae | *Mesopolobus tibialis* (Westwood) | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 3.8 |
| Torymidae | *Torymus auratus* (Muller) | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 2 | 7 | 26.9 |
| Total | | 4 | 3 | 2 | 4 | 13 | 3 | 1 | 3 | 6 | 13 | |

* *Bootanomyia dorsalis* and *Eupelmus urozonus* are considered as species complex.

Table 3. Values of parasitism rate (Pr), parasitism rate, including chambers with parasitoid exuviae (Pe) and overall mortality rate of ACGW (Pef) for each sampling site and date.

| | Pr (%) | Pe (%) | Pef (%) |
|---|---|---|---|
| Site 1 | | | |
| Date | | | |
| 31/05 | 36.36 | 40.00 | 47.27 |
| 14/06 | 25.64 | 25.64 | 25.64 |
| 03/07 | 43.08 | 47.69 | 47.69 |
| 13/07 | 18.31 | 22.54 | 22.54 |
| 25/07 | 40.00 | 41.82 | 43.64 |
| Mean* | 39.23 ± 2.19 | 41.94 ± 2.07 | 48.24 ± 2.42 |
| Site 2 | | | |
| Date | | | |
| 31/05 | 25.64 | 25.64 | 25.64 |
| 14/06 | 18.31 | 22.54 | 22.54 |
| 03/07 | 14.04 | 14.04 | 15.79 |
| 13/07 | 8.47 | 8.47 | 27.12 |
| 25/07 | 16.00 | 18.00 | 22.00 |
| Mean* | 16.49 ± 2.81 | 17.74 ± 3.04 | 22.62 ± 1.95 |

*Values are means ± SE of five sampling date for each site.

Figure 1

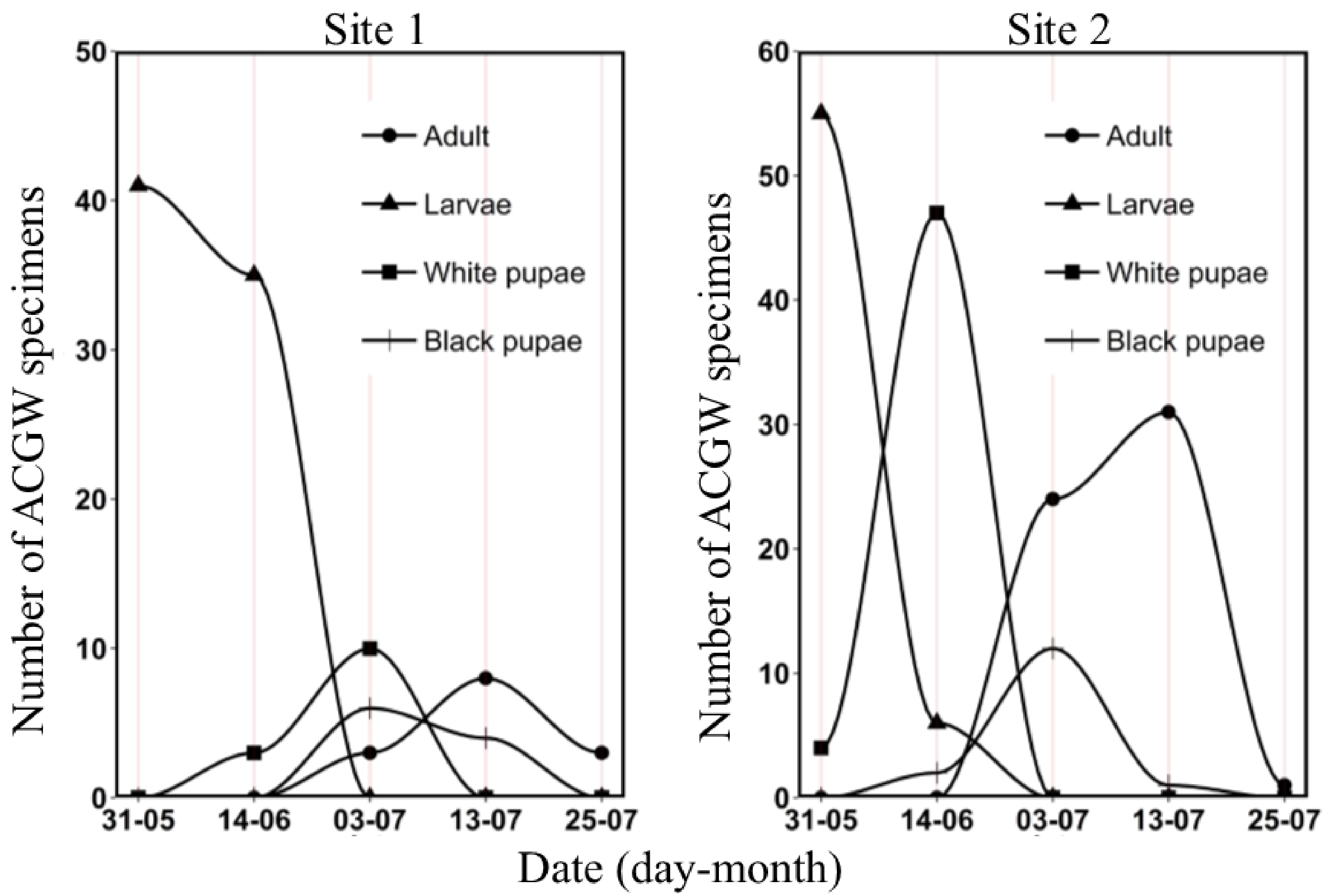
Site 1
Site 2
Number of ACGW specimens
Adult
Larvae
White pupae
Black pupae
0
10
20
30
40
50
60
31-05
14-06
03-07
13-07
25-07
Date (day-month)

Figure 2

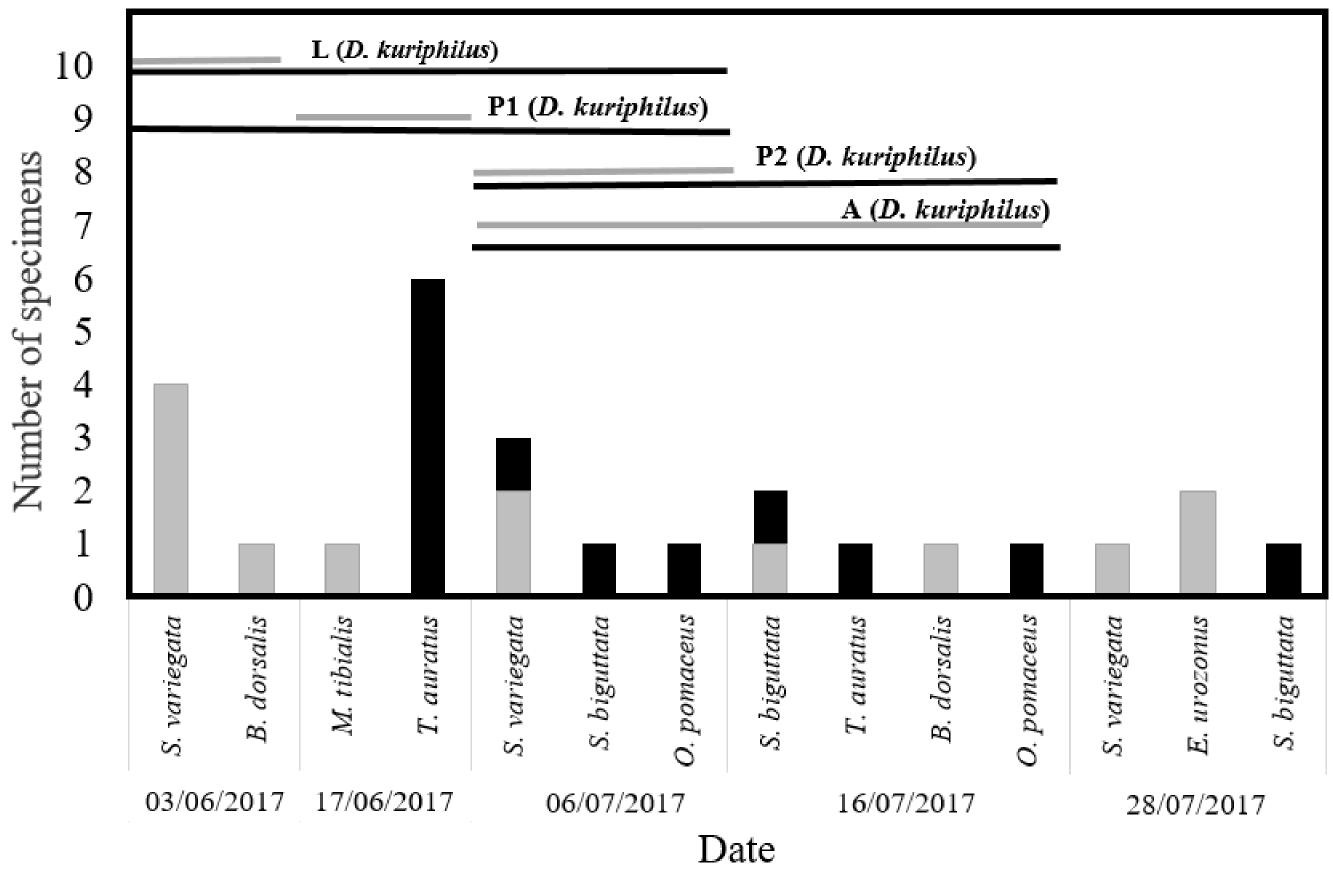
L (D. kuriphilus)
P1 (D. kuriphilus)
P2 (D. kuriphilus)
A (D. kuriphilus)
Number of specimens
10
9
8
7
6
5
4
3
2
1
0
S. variegata
B. dorsalis
M. tibialis
T. auratus
S. variegata
S. biguttata
O. pomaceus
S. biguttata
T. auratus
B. dorsalis
O. pomaceus
S. variegata
E. urozonus
S. biguttata
03/06/2017
17/06/2017
06/07/2017
16/07/2017
28/07/2017
Date

Figure 1. Developmental stages of ACGW at each sampling site (triangle- larvae; square-white pupae; crosses- black pupae; circle- adult) from gall dissection at each sampling date (vertical bars).

Figure 2. Developmental stages of the ACGW (L=larvae, P1=white pupae, P2=black pupae, A=adult) in the study area and number and species of native parasitoids collected (grey-Site 1 and black-Site 2) during the studied period.