Preliminary Assessment of the Parasitism of *Comperiella calauanica* Barrion, Almarinez, and Amalin on *Aspidiotus rigidus* Reyne in Hidden Valley Springs Resort, Calauan, Laguna, Philippines

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ABSTRACT

The coconut scale insect (CSI), *Aspidiotus rigidus* Reyne (Hemiptera: Diaspididae), is one of the most devastating insect pests of coconut in the Philippines. It reached epidemic pest status in 2013 in the Southern Tagalog mainland (Calabarzon Region). Several control measures had been tried but did not reach the management level desired. However, in April 2014, an encyrtid parasitoid was first recorded in the Hidden Valley Resort in Calauan, Laguna, to have direct association with *A. rigidus*. It was later described as a new species and named as *Comperiella calauanica* by Barrion, Almarinez, and Amalin (Hymenoptera: Encyrtidae). To assess the potential of this parasitic wasp against *A. rigidus*, a survey of the presence and establishment of this wasp was conducted at the Hidden Valley Springs Resort. Forty randomly selected frond-forming coconut saplings were chosen, and one leaflet per sapling was excised for the evaluation. Each leaflet was divided into three equal-size segments for counting the unparasitized and *C. calauanica*–parasitized *A. rigidus*. The average percentage parasitism of *C. calauanica* on *A. rigidus* was computed as 85.52% ± 1.28. Results suggest the potential of *C. calauanica* as a primary biological control agent of *A. rigidus*. Moreover, *C. calauanica* may be exploited for mass production and field augmentation for the management of *A. rigidus*.

Keywords: coconut insect pests, biological control, parasitoid, coconut palm, pest control

INTRODUCTION

The Philippines is one of the leading suppliers of organic coconut products in the world market. As an industry, organic coconut products serve as one of the economic backbones for the Philippines. However, the recent infestation of the coconut scale insect (CSI) has reached epidemic proportions and is a major threat to the industry (Almarinez et al., 2014). The scale was first recorded in Barangay Balele near Tanauan, Province of Batangas, Philippines, and rapidly spread throughout the Southern Tagalog mainland in the Calabarzon Region (Watson et al., 2014). By 2014, the scale insect had infested over 2 million coconut trees, causing about 65% death of trees while fruit yields have reportedly dropped to an average of about 60% (FAO, 2014).

Infestations of this scale insect lead to fruits forming less nutmeal, sour-tasting water in the nuts, and, under severe infestations, death of the tree in six months or less. Because of its recent outbreak in the Philippines, it is now included in the Cooperative Agricultural Pest Survey (CAPS) Target: Tropical Pest List of 2015 (Molet, 2015).

Aspidiotus rigidus Reyne (Hemiptera: Diaspididae) is an armored scale insect closely related to *Aspidiotus destructor* Signoret, to which it was first described as a subspecies (Reyne, 1947). It was, in fact, misidentified as A. destructor when it was first observed in the Philippines in 2009. Morphologically, *A. rigidus* displays a hard sclerotized cuticle prosoma, which is one of its defining characteristics in comparison with *A. destructor* (Miller & Davidson, 2005; Reyne, 1947, 1948; Watson et al., 2014). Some of the key differences of *A. rigidus* from *A. destructor* include a more rigid and tough cuticle, a life cycle about 1.5 times longer, and an egg laying pattern that is crescent shaped (Watson et al., 2014). The detailed biology of *A. rigidus* was documented by Reyne (1948), and additional ecological information was reported by Kalshoven (1981). The female life cycle is 45–55 days and has a reproductive potential of 25–50 eggs per female. Although males are present, reproduction is through parthenogenesis (Kalshoven, 1981; Reyne, 1948; Watson et al., 2014). It has a multiplication rate of 5–10 times with around 8 generations per year (46 days generation time), relatively longer than that of A. destructor. Although male *A. rigidus* are present in high numbers, their role is still not known. *Aspidiotus rigidus* is usually found on the lower surface of coconut-palm leaves, blocking the stomata of the leaf and resulting in the discontinuation of photosynthesis. Only in cases of heavy infestation can *A. rigidus* trigger behavior where it can attack almost all the green parts of the plant and feed on the green tissue. This feeding on the sap causes the leaf desiccation and fall. Also, the coconut fruit forms less nutmeat and produces sour coconut water. Various preventive measures have been done to lessen the spread of the scale insect, including the application of chemical pesticides, burning of the wilting leaves of the coconut, and biological control (Watson et al., 2014). The use of biological control was given priority in the management of *A. rigidus* because it offers long-term economic and sustainable pest management, reducing human and environmental impact of insecticides (Orr, 2009; Samways, 1989) and possibly resulting in a host–pest equilibrium ultimately reducing crop damage (Johnson, 2000; Walton, 1980).

Members of family Diaspididae like *A. rigidus* are believed to have various natural enemies such as ants, coccinellids, and encrytids (Muma, 1971; Reyne, 1947, 1948; Samways, 1989; Watson et al., 2014; Yarpuzlu et al., 2008). Species in the genus Comperiella (Hymenoptera: Encyrtidae) are found throughout the world parasitizing various hosts (Table 1). Previous surveys of natural enemies of *A. rigidus* in Hidden Valley Springs Resort, in Calauan, Laguna, and a demonstration farm of the Philippine Coconut Authority (PCA) in Alaminos, Laguna, found *Comperiella* sp. directly associated with *A. rigidus*, with a report of up to 80% parasitization having been observed in San Pablo City and Los Baños, Laguna (Almarinez et al., 2014). Careful morphological examination of the specimens revealed that the encyrtid parasitoid was a new species, later described as *C. calauanica*, whose specific epithet was based on the type locality (Barrion et al., 2016). Quantifying the parasitization level is important to verify the direct association of the parasitoid to its host for inclusion in the Integrated Pest Management (IPM) Program of *A. rigidus*. Thus, this study aims to assess the direct association of *C. calauanica* on *A. rigidus* by examining the parasitization level in the area where the endoparasitoid was first recorded in Calauan, Laguna.

Table 1. Hosts and Distribution Range of the *Comperiella* Species Complex (Based on Noyes, 2014, and Barrion et al., 2016).

Species	Hosts	Distribution
Comperiella aspidiotiphaga	*Aoidiniella orientalis* (Newstead)	India
Comperiella bifasciata	*Aodiniella aurantii* Maskell *Aodiniella citrina* Coquillett *Aodiniella taxus* Leonardi *Aspidiotus camelliae* Signoret *Aspidiotus kryptomeriae* Kuwana *Aspidiotus destructor* Signoret *Aspidiotus orientalis* (Newstead) *Aspidiotus* sp. *Chrysomphalus dictyospermi* Morgan *Chrysomphalus ficus* Ashmed *Chrysomphalus* sp. *Nuculapis abietis* Schrank *Quadraspidiotus gigas* Thiem & Gernek	China, Fiji, Hungary, India, Indonesia, Japan, Mauritius, Philippines,** South Africa, Spain, Taiwan, USA, and USSR
Comperiella calauanica	*Aspidiotus rigidus* Reyne	Philippines
*Comperiella eugeniae**	Unknown	Madagascar
Comperiella indica	*Aspidiotus tamarindi* Green	India
Comperiella lemnisciata	Aodiniella orientalis (Newstead)	China, India, Pakistan
*Comperiella pia**	*Unknown*	Australia
Comperiella ponticula	*Unknown*	South Africa
Comperiella unifasciata	*Aspidiotus destructor* Signoret *Pseudaonidia duplex* Cockrell	Fiji, India, Indonesia, Japan

*Suspected synonyms of *C. bifasciata.* **Introduced to the Philippines from Japan.

MATERIALS AND METHODS

Description of Study Site

The study was conducted at the Hidden Valley Springs Resort located in Barangay Perez, Calauan, Province of Laguna, Philippines (GPS coordinates of 14°09′N 121°19′E). Hidden Valley Springs Resort is a private resort with an agricultural and natural ecosystem and included as one of the main tourist areas registered by the Philippine Department of Tourism. It is a 60-hectare property with coconut trees included in its natural landscape. All coconut trees, including wild-growing coconut seedlings and saplings scattered throughout this property, were infested by *A. rigidus*.

Collection of Samples

One whole leaflet from each of 40 randomly selected, wild-grown coconut saplings (Laguna Tall variety) was collected and placed individually into plastic Ziploc® containers, which were then transported to the laboratory for processing. All collected leaflets were handled very carefully to assure that *A. rigidus* remained intact on the leaf surface.

Three segments, each of 6.35 cm in length and representing the base, middle, and distal regions, were cut from each leaflet and placed in a plastic petri plate. The petri plate was sealed using a paraffin film to avoid desiccation of the leaflet segments while waiting to be observed for the unparasitized *A. rigidus* or parasitized *A. rigidus* with *C. calauanica*. The degree of parasitization was determined on the whole area of each leaflet segment (whose width was variable). The degree of parasitism was determined on the whole area of each leaflet segment (the width of which was variable). The plastic petri plates were placed in the freezer (at approximately 0°C) overnight to halt the development of both the *A. rigidus* and the *C. calauanica*. Samples were collected in late June 2014.

Insect Counting

After 24 hours, the petri plates with the leaf segments were removed from the freezer for counting of parasitized and unparasitized *A. rigidus*. Counting of insects was done manually under a Nikon SMZ-800® dissecting microscope. Parasitization by *A. rigidus* was recorded, based on the presence of all observable developmental stages of *C. calauanica*, such as larvae, pupae, and exit holes for adults. The late larval and early pupal stages of *C. calauanica* can be observed by the presence of reddish-brown meconial pellets (Fig. 1) surrounding the immature parasitoid inside the mummified scale insect, which is produced shortly before pupation.

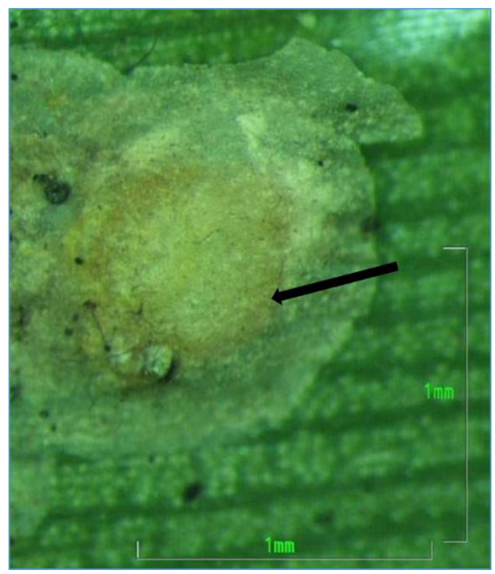

Figure 1. Larval stage of endoparasitoid *C. calauanica* in a mature coconut scale insect (CSI), *A. rigidus*. Arrow points to the accumulation of reddish-brown meconium surrounding the margin produced by the larva inside the mummified host insect. Photograph by D. Amalin and B. J. Almarinez.

The pupal stage of *C. calauanica* exhibited a large black pupa inside the scale (Fig. 2). The presence of a small oval exit hole (Fig. 3) indicated that the adult parasitoid had already emerged from the scale insect. Unparasitized *A. rigidus* were live third nymphal and adult stages (Fig. 4), which are the developmental stage preferred by *C. calauanica* for oviposition. In addition, the adult sex ratio of *C. calauanica* was estimated by counts of adult females and males that were able to emerge in 19 plates prior to freezing. Sexing of emergent parasitoids was done using a dissecting microscope or a magnifying glass and was based on morphological differences between male and female *C. calauanica* described by Barrion et al. (2016). Out of 40 petri plates, 26 were randomly selected for insect counting due to manpower limitations. Additionally, the leaflet segments in the remaining 14 plates that were stored for more than 24 hours exhibited physical changes that made it difficult to count the insects.

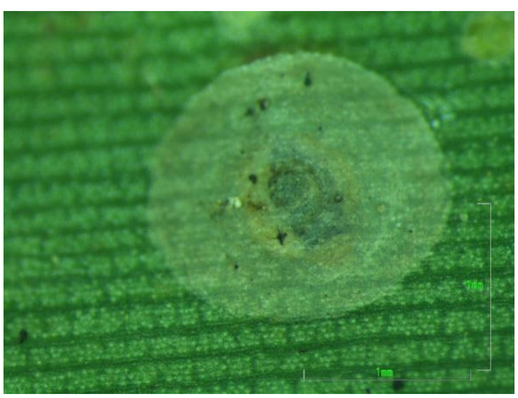

Figure 2. *Aspidiotus rigidus* nymph with a late pupal stage of *C. calauanica*. Arrow points to the black pupa inside the mummified (dead) host. Photograph by D. Amalin and B. J. Almarinez.

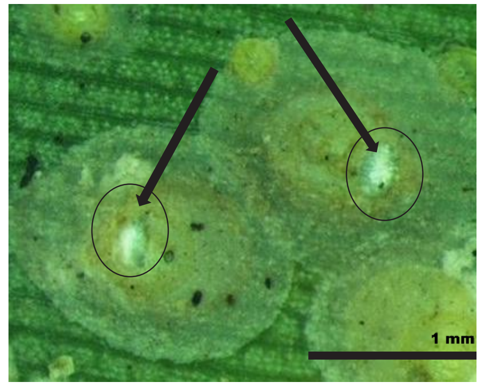

Figure 3. Mummified, parasitized *A. rigidus* with exit holes made by emergent *C. calauanica*. Arrows point to the exit holes. Photograph by D. Amalin and B. J. Almarinez.

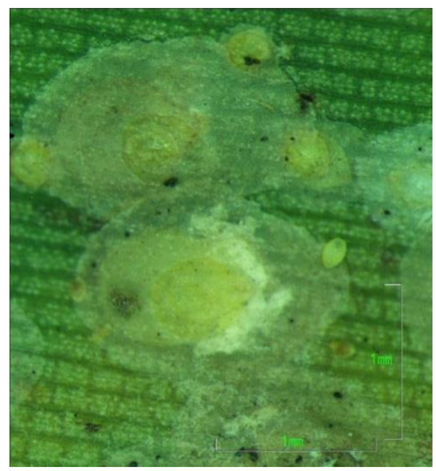

Figure 4. Unparasitized *A. rigidus*. Arrow points to a mature, unparasitized female bearing no sign of parasitization (e.g., immature parasitoid and/or reddish-brown meconium deposit). Photograph by D. Amalin and B. J. Almarinez.

Data Analysis

Percent parasitization of *C. calauanica* was estimated by dividing the total number of parasitized *A. rigidus* (as shown by the total number of *C. calauanica)* by the total number of unparasitized and parasitized *A. rigidus* multiplied by 100 in each segment of each leaflet. The equation used is shown below:

$$\% \text{ Parasitism} = \frac{\text{Numberof parasitized } A.rigidus}{\text{Number of parasitized } A.rigidus + \text{Number of unparasitized } A.rigidus} \times 100$$

RESULTS AND DISCUSSION

The percent parasitism computed from the count per leaf segment is shown in Table 2. The computed percent parasitism was above 85%, which denotes a high level of parasitism by *C. calauanica* on *A. rigidus*. Out of all the adults that emerged, 19 were male and 94 were female (on the average, 1 male:5 females). The observed female-biased sex ratio could be suggestive of a possible facultative thelytoky in *C. calauanica*. A thelytokous parasitoid is expectedly more efficient in parasitizing (and eventually managing populations of) its host, since most, if not all, of the offspring produced are females that can subsequently lay eggs and parasitize more of the individuals remaining in the host population.

Table 2. Percent parasitism of coconut scale insect *Aspidiotus rigidus* by *Comperiella calauanica* per Leaf Segment.

Replicate	Leaf Segment 1	Leaf Segment 2	Leaf Segment 3	Average*
1	66.67 (6/9)	86.67 (13/15)	78.57 (22/28)	77.30
2	77.27 (17/22)	76.00 (19/25)	82.76 (24/29)	78.68
3	90.00 (45/50)	88.00 (44/50)	82.61 (19/23)	86.87
4	86.67 (26/30)	56.52 (13/23)	83.87 (26/31)	75.69
5	86.84 (33/38)	57.89 (11/19)	73.68 (28/38)	72.81
6	76.47 (39/51)	80.52 (62/77)	83.64 (46/55)	80.21
7	85.00 (34/40)	93.33 (28/30)	95.00 (19/20)	91.11
8	89.36 (42/47)	90.16 (55/61)	**	89.76
9	94.59 (35/37)	**	97.06 (33/34)	95.83
10	77.27 (17/22)	85.19 (23/27)	90.48 (19/21)	84.31
11	82.14 (23/28)	73.91 (17/23)	84.00 (21/25)	80.02
12	94.74 (18/19)	82.35 (14/17)	90.00 (27/30)	89.03
13	90.00 (27/30)	92.68 (38/41)	91.30 (21/23)	91.33
14	86.84 (33/38)	84.62 (44/52)	85.00 (34/40)	85.49
15	100.00 (9/9)	86.96 (20/23)	73.33 (11/15)	86.76
16	88.24 (15/17)	81.25 (13/16)	92.00 (23/25)	87.16
17	100.00 (9/9)	86.67 (26/30)	95.12 (39/41)	93.93
18	81.82 (27/33)	96.97 (32/33)	90.91 (30/33)	89.90
19	63.46 (33/52)	77.55 (38/49)	84.48 (49/58)	75.17

20	86.49 (32/37)	86.67 (13/15)	100.00 (12/12)	91.05
21	80.00 (4/5)	100.00 (4/4)	100.00 (5/5)	93.33
22	67.92 (36/53)	72.06 (49/68)	72.86 (51/70)	70.95
23	85.71 (24/28)	82.61 (19/23)	100.00 (19/19)	89.44
24	100.00 (17/17)	100.00 (24/24)	87.23 (41/47)	95.74
25	94.59 (35/37)	87.50 (56/64)	92.31 (60/65)	91.47
26	81.48 (66/81)	86.46 (83/96)	74.68 (59/79)	80.87
Grand average			**85.55%**	
Standard error (SE)			**1.42**	
% Parasitism (±SE)			**85.55 ± 1.42**	

Note. Numbers in parentheses indicate raw counts of parasitized/parasitized + unparasitized *A. rigidus*.
*Average percent parasitism per replicate. **Unusable sample.

Reyne (1947) previously reported that *C.* infesting coconut palms in Sangi Island, Indonesia. He observed that *C. unifasciata* was absent in areas where *A. rigidus* was absent and was very common in Java, Indonesia, where *A. rigidus* was prevalent. He examined 578 samples of coconut leaves infested with *A. rigidus* colonies, and he found that 86% of the colonies with CSI were parasitized by *C. unifasciata*. Parasitism of 80%–90% was observed in some colonies of *A. rigidus*, but the average percent parasitism was much lower. He obtained 4.9% parasitism from observations from July to August 1929, 2.6% in October 1929, and 5.6% in December 1929.

Although a low percentage parasitism was obtained, it was observed that *C. unifasciata* had spread into 92% of the samples he distributed over Sangi Island within a span of 2.5 yr (Reyne, 1947). He then concluded that *C. unifasciata* would not be able to control an outbreak of *A. rigidus* due to low percentage of parasitism but can slow down the rate of increase to some measure.

CONCLUSION

The results of this study suggest that the native Philippine *Comperiella* appears to exhibit a higher rate of parasitism compared to *C. unifasciata* on *A. rigidus*. High parasitization rates by *C. calauanica* suggest that it is a promising biological control agent. Biologically based IPM of *A. rigidus* may include conservation measures by providing a natural enemy habitat for *C. calauanica* and need-based application of selective insecticides. Finally, *C. calauanica* may be a candidate species for mass production and field augmentation. Further assessment of a wider scope is needed to determine the role of the high parasitism of *C. calauanica* in the management of *A. rigidus* in CALABARZON, Southern Tagalog Region. The result of this present study can be used in other areas of new invasion of *A. rigidus* to avoid pest outbreak.

ACKNOWLEDGEMENT

We thank Mr. Guillermo B. Roxas and Mr. Luigi Roxas (owners) for allowing this study to be conducted at the Hidden Valley Springs Resort, Calauan, Laguna, Philippines. We thank Dr. Manuel Pescador (Prof. Emeritus, Florida A&M University, Tallahassee, Florida, USA) and Dr. Rufina N. Ward (Research Entomologist [retired], Alabama A&M University, Hunstville, Alabama, USA) for reviewing this manuscript. We are grateful to De La Salle University, the Philippine Coconut Authority, and the Quezon Agricultural Experimental Station for financial and technical support of this study. We also thank Dr. Benjamin Legaspi, Jr. for helpful discussions. This paper is based on an undergraduate thesis dissertation by the senior author. This article presents the results of research only. The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by De La Salle University and the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

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