

## ENTOMOLOGY

# Biology and life table parameters of *Metaphycus marensis* Chirinos & Kondo, 2019, an encyrtid parasitoid of guava cottony scale in Venezuela

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## Abstract

The guava cottony scale, *Capulinia linarosae* (Hemiptera: Eriococcidae) is an important pest of guava, *Psidium guajava*, in Venezuela and northern Colombia. *Metaphycus marensis* (Hymenoptera: Encyrtidae) is a new primary parasitoid species

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recently described associated with this pest. Studies were conducted on oogenesis, life cycle, survival, daily fecundity and life table parameters of *Metaphycus marensis* Chirinos & Kondo (Hymenoptera: Encyrtidae): intrinsic rate of natural increase ( $r_m$ ), generation time ( $T$ ) and net reproductive rate ( $R_o$ ). Females of *M. marensis* are synovigenic and this parasitoid goes through four larval instars and completes its life cycle in about 12.7 days. Survival was of type I, where mortality was initially detected by encapsulation of eggs and larvae. *Metaphycus marensis* was able to multiply its population 28.7 times ( $R_o$ ) with  $r_m$  of 0.242 in 13.9 days ( $T$ ). The short generation time of the parasitoid in relation to its eriococcid host could represent a desirable attribute as a natural enemy. However, the low fecundity and the encapsulation by the host must be analyzed through field experiments in order to evaluate its effectiveness as a biological control agent for *C. linarosae*.

## Introduction

Guava, *Psidium guajava* L. (Myrtaceae) is a crop native to the Americas and is currently distributed in tropical regions of the world, with a production of approximately 1.2 million MT, where India and Pakistan contribute 50% of the production worldwide, followed by Mexico (25%) and the rest is produced by other countries, including Brazil, Colombia and Venezuela (Yam *et al.*, 2010). Guava fruit is consumed both fresh and processed and it has a high potential as a commercial crop that derives from its uses, e.g., juices, nectars and pulps (Yam *et al.*, 2010). The guava plant may be attacked and damaged by a wide range of insect pests, including, among others, scale insects. Within the latter, the most economically important insect species causing substantial crop losses worldwide belong to the families Pseudococcidae (mealybugs), Coccidae (soft scales) and Diaspididae (armored scales) (Miller *et al.*, 2002; Gullan & Cook, 2007; Mansour *et al.*, 2017); and some species of Eriococcidae (García Morales *et al.*, 2020).

The family Eriococcidae (Hemiptera: Sternorrhyncha: Coccoomorpha), the so called felt scales, is one of the four most species-rich scale insect families; these are phytophagous insects that feed on plant sap through their stylets and can infest one or several plant parts, e.g., fruits, leaves, main stems, roots or trunk (Miller, 2005). The Eriococcidae is most abundant in the southern hemisphere, where South America has a rich eriococcid fauna with 72 species in 24 genera (Hodgson *et al.*, 2013).

Within the Eriococcidae, the cottony guava scale, *Capulinia*

*linarosae* Kondo & Gullan, 2017, is the most important pest insect of guava crop in Venezuela and the Caribbean coast of Colombia, a pest that causes severe damage to branches, leaves and fruits (Chirinos *et al.*, 2017). The importance of this scale insect as a major guava pest has been documented for Venezuela (Chirinos *et al.*, 2017) and more recently for the Caribbean coast of Colombia (Kondo *et al.*, 2016).

*Capulinia linarosae* was first reported as a pest in Venezuela in 1993, devastating 600 hectares of guava cultivated areas in the Lake Maracaibo basin, the most important guava producing region in the country at that time (Chirinos *et al.*, 2017). Biological studies showed that each female is capable of laying 2400 eggs, which gives it a high biotic potential and explains the high populations reached during colonization of the guava crop in the absence of efficient and effective natural enemies (Chirinos *et al.*, 2017). This insect pest was reported recently in Colombia, where it causes considerable losses to guava in the Caribbean coast, involving both commercial crops and scattered backyard guava trees in the departments of Atlántico, Bolívar, Casanare, Cesar, Magdalena, Meta and Norte de Santander (Kondo *et al.*, 2016; Ramos, 2018; Chirinos & Kondo, 2019).

*Metaphycus marensis* Chirinos & Kondo, 2019 (Hymenoptera: Encyrtidae) is so far the only species of primary parasitoid associated with *C. linarosae*, which was recently described based on specimens from Venezuela (Chirinos & Kondo, 2019). This parasitoid is one of the natural biological control agents of this pest for which there is limited information in the literature and its potential as a control agent remains to be elucidated. Chirinos & Kondo (2019) pointed out that *M. marensis* is an arrhenotokous species and the females prefer to parasitize young adult hosts.

Species of the genus *Metaphycus* play an important role in the natural regulation of their insect hosts and many species have been successfully used in biological control programs against agricultural pests, especially in soft scales of the genera *Saissetia* and *Coccus* (Guerrieri & Noyes, 2000). Some 30 species of *Metaphycus* have been introduced to 40 countries for the control of approximately 22 species of scale insects (Guerrieri & Noyes, 2000).

This paper presents novel biological information on *M. marensis*, which in addition to serving as a basis for insect breeding protocols, can be used to help understand the parasitoid-host ecological dynamics under field conditions and the potential of this parasitoid for managing *C. linarosae* in South America.

## Materials and Methods

The study was carried out under controlled laboratory conditions (temperature: 26.7°C; relative humidity 79.9%, 12:12 h light/darkness) at University of Zulia, in Maracaibo, Venezuela. The culture conditions of the host and the parasitoid were as described in Chirinos & Kondo (2019).

### Effect of feeding on oogenesis

In our study, 60 mated female individuals of *M. marensis*, both unfed (30 individuals) and fed (30 individuals) with honey diluted in distilled water, were dissected 0, 24, 48, 72 and 96 hours after they emerged from the pupa. The total number of ovarioles was counted for each pair of ovaries. Also, the total number of eggs (i.e., fully developed and undeveloped eggs) was counted in both ovaries.

### Duration of the parasitoid life cycle

To observe the development of the parasitoids, daily dissections of the eriococcid host were made. Twenty infested guava plants with young adult females of *C. linarosae* (11-15 days after molting) were

exposed to two virgin or mated female parasitoids for 24 hours. During the first four days, dissections were done every 12 hours and afterwards every 24 hours from the fifth day until the end of the cycle. In each observation, five to seven eriococcid individuals were dissected per plant, which resulted in the evaluation of 10 to 15 parasitoids. When all the parasitoids reached the pupal stage, the parasitized eriococcid hosts were removed from the plant and placed in clear gelatin capsules until the emergence of the adult parasitoids.

Some of the parasitized hosts were dissected daily to observe the sclerotization of the parasitoid pupae. Some of the parasitoid eggs, larvae and pupae were mounted on slides using Hoyer's slide-mounting medium following the method of Anderson (1954) in order to measure their greatest length and width. In the case of larvae, the width of the cephalic capsule and the length of the mandibles were also measured to determine the number of larval stages. Measurements were taken using image analysis software, Image-Pro Plus 6.0, Media Cybernetics®.

### Parasitoid daily fecundity

Fifty mated female parasitoids were released inside a cage containing plants with cultures of *C. linarosae* in order to count the daily number of eggs laid on the eriococcids. Thus, each guava plant with eriococcid hosts (about 200 females) was changed every 24 hours. This was repeated until the death of the female. When the female parasitoid was removed from each plant, all the eriococcid individuals found on the plant were dissected daily and the number of eggs laid was counted for each parasitized individual.

### Parasitoid survival ( $I_x$ )

Each of 12 plants with 15 females of *C. linarosae* were exposed for 24 hours to two previously mated females of *M. marensis*. The observations took place every 12 hours post-exposure for the first four days and were subsequently made every 24 hours until the end of the cycle. Causes of mortality of *M. marensis* that were taken into account included the encapsulation of eggs and larvae, immobility and necrosis of larvae and drying of pupae. Observations were made every day on about 15 parasitoids that were obtained from dissections of the scales, counting the number of live parasitoids among the total observed. The parasitoid life table parameters were calculated following the previously referred method (Krebs, 1978): Net reproductive rate ( $R_0$ ), Generation time ( $T$ ) and Intrinsic rate of natural increase ( $r_m$ ).

### Statistical analysis

The variables were transformed with the square root function  $\sqrt{x}$  and analyzed through the General Linear Model, and means were separated using the Least Squares method (LSM,  $P < 0.05$ ). All the statistical analyses were done with the statistical program SAS (SAS Institute Inc. 2009).

## Results

### Effect of feeding on oogenesis

Although host-feeding by the female parasitoid wasp was not observed, *M. marensis* is a synovigenic species that needs to feed on sources of carbohydrates in order for her eggs to mature. In the dissection of the ovaries, it was observed that each ovary is composed of three ovarioles (Figure 1A). For non-fed females, dissections were made only up to 24 hours because these wasps generally failed to survive beyond that time. For honey-fed females, dissections were performed until 96 hours after the emergence of

the adult, a time that allowed observation of the differential development of the eggs. For newly emerged adult females (0 hours), only 1.7 developed eggs were detected on average per female, a value that increased 14 times after 96 hours.

The total number of eggs was similar and independent ( $P < 0.05$ ) of the feeding condition (Table 1). No significant differences were observed ( $P < 0.05$ ) in the number of eggs between fed and unfed females. However, significant differences in the number of developed eggs over time were observed for honey-fed females ( $P < 0.05$ ).

### Duration of the different stages and life cycle

#### Egg

In *M. marensis*, the eggs are stalked (Figure 1B) and measure  $160 \pm 15 \mu\text{m}$  long x  $67 \pm 4 \mu\text{m}$  wide, excluding the stalked, which

measures  $75 \pm 9 \mu\text{m}$  in length. Eggs lack an aeroscopic plate which is inserted in the fatty body at the caudal part of the body of the host (Figure 1C). The eggs are not fixed internally and can be easily detached, leaving them free inside the hemocoel.

#### Larva

Larvae are of the encyrtiform type, pass through four larval instars and mandibles are evident in all instars (Figures 1D-G, Figure 2) as well as head capsule width (Table 2).

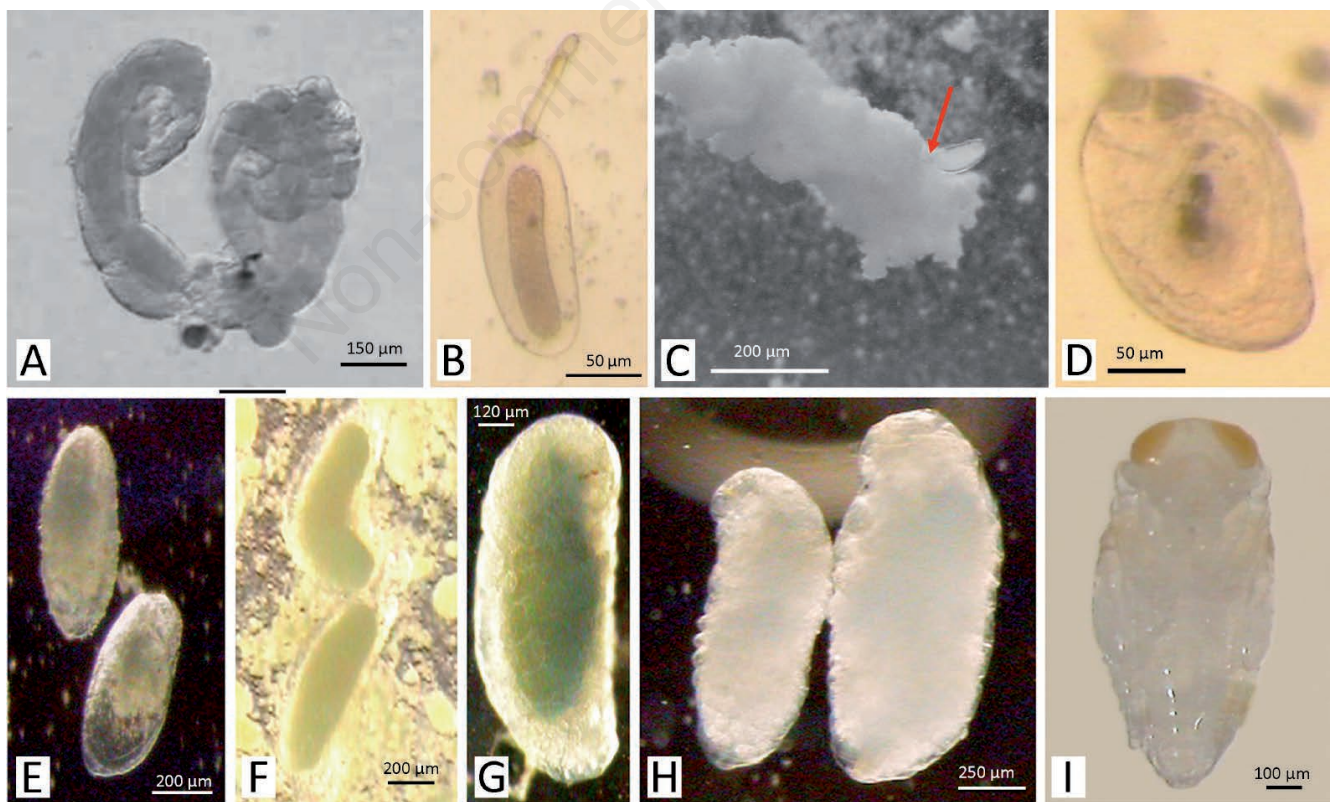
#### First-instar larva

Once it hatches, the first-instar larva moves freely inside the host's hemocoel. The first-instar larva (Figure 1D) tends to be spheroidal, each  $183 \pm 7 \mu\text{m}$  long x  $165 \pm 30 \mu\text{m}$  wide. This stadium lasted approximately 24 hours.

**Table 1. Number of developed eggs counted in the ovaries of *M. marensis* over time in both unfed and honey-fed adult females.**

Time (hours)	Honey-fed females			Unfed females		
	Total number of eggs	Developed eggs	N.	Total number of eggs	Developed eggs	N.
0	$41.0 \pm 0.9$ a	$1.7 \pm 0.1$ a	30	$42.0 \pm 0.4$ a	$1.6 \pm 0.7$ a	30
24	$39.2 \pm 1.4$ a	$14.4 \pm 2.6$ b	30	$41.4 \pm 0.9$ a	$1.7 \pm 0.2$ a	11
48	$41.2 \pm 2.2$ a	$20.7 \pm 1.9$ c	30	-	-	-
72	$42.0 \pm 0.3$ a	$22.0 \pm 2.3$ cd	30	-	-	-
96	$40.7 \pm 1.5$ a	$24.1 \pm 1.9$ cd	30	-	-	-

N = number of females dissected. Means followed by different letters within the same column are significantly different ( $P < 0.05$ ).



**Figure 1. Ovarioles and immature stages of *M. marensis*. A. Ovarioles. B. Egg. C. Egg with stalked inserted in fat body (see arrow). D. First-instar larva. E. Second-instar larvae. F. Third-instar larvae. G. Fourth-instar larva. H. Prepupae. I. Pupa.**

**Second-instar larva**

The second-instar larva (Figure 1E) is elongate, each  $514 \pm 129 \mu\text{m}$  long and  $231 \pm 18 \mu\text{m}$  wide. This stadium lasted approximately 24 hours.

**Third-instar larva**

The third-instar larva (Figure 1F) is elongate each  $636 \pm 147 \mu\text{m}$  long and  $239 \pm 36 \mu\text{m}$  wide. The mandibles are amber in color and more sclerotized compared with the previous instars. This stadium lasted approximately 24 hours.

**Fourth-instar larva**

The fourth-instar larva (Figure 1G) is elongate,  $949 \pm 181 \mu\text{m}$  long,  $446 \pm 88 \mu\text{m}$  wide. This stadium lasted 2.5 to 3.0 days.

**Prepupa**

Once the parasitoid larva has consumed all the contents of the host, the integument of the parasitized eriococcid stays yellowish, but it turns brown in the following 24 hours. At this stage, the parasitized eriococcids are commonly referred to as “mummies”. Along with the formation of cells, the parasitoid expels the meconium in the form of yellowish granules or “pellets”. Immediately after, the parasitoid becomes a prepupa, which is whitish in color (Figure 1H). The prepupa is the final phase of the fourth instar, when the larva prepares to transform into a pupa. The prepupal stage lasts less than 24 hours.

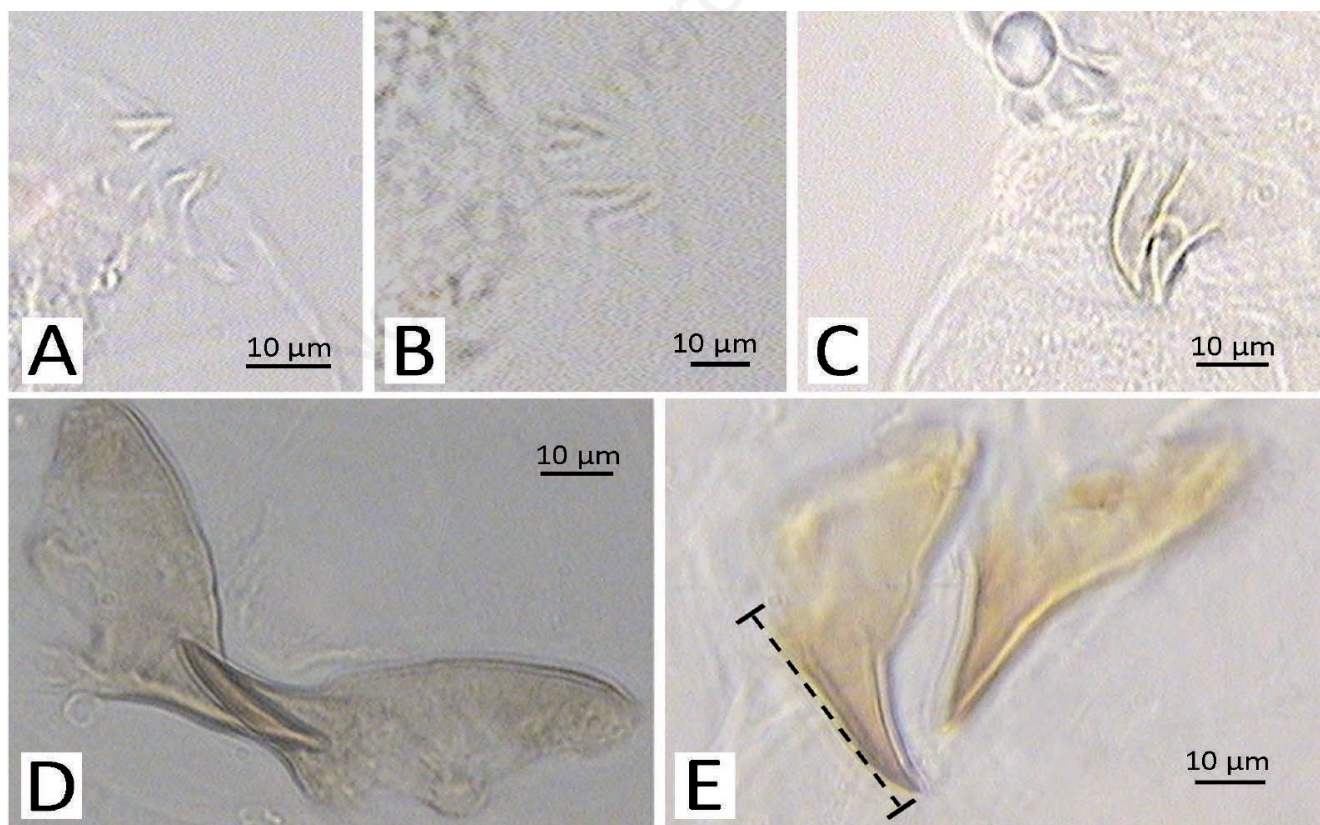
**Pupa**

The pupa (Figure 1I) is of the exarate type; it is whitish during the first two days and then turns brown and finally bright black in color. The eyes are whitish during the first 24 hours, then change to

**Table 2. Mandible length (Mandible-L) and head capsule width (HC-W) of *Metaphycus marenensis* larval stages.**

Larval stage	N.	Mandible-L ( $\mu\text{m}$ )	N.	HC-W ( $\mu\text{m}$ )
L1	35	$7 \pm 1$	38	$32 \pm 5$
L2	36	$16 \pm 3$	41	$71 \pm 2$
L3	47	$24 \pm 3$	49	$182 \pm 4$
L4	43	$33 \pm 3$	43	$234 \pm 3$

N= number of measured individuals.



**Figure 2. Mandibles of the larvae of *Metaphycus* sp. A. First-instar larva. B. Second-instar larva. C. Third-instar larva. D. Fourth-instar larva. E. Mandibles of the fourth-instar larva.**

amber and later turn brown after another 24 hours. The female pupa measures  $1020 \pm 158 \mu\text{m}$  long x  $464 \pm 89 \mu\text{m}$  wide and the male pupa  $757 \pm 162 \mu\text{m}$  x  $376 \pm 99 \mu\text{m}$  wide.

No significant differences were found in the duration of the life cycle between female and male individuals ( $P < 0.05$ ) although the duration of the male lasted a little less in the pupal stage than that of the female parasitoid (Table 3). The duration of the life cycle of *M. marensis* ranged from 12 to 13 days.

### Parasitoid daily fecundity

Figure 3 shows the distribution of the average oviposition of adult females of *M. marensis* throughout its lifetime. Although *M. marensis* is a sinovigenic species, the period of oviposition did not last beyond six days, most of the oviposition occurring from the second to the fourth day.

### Parasitoid survival ( $I_x$ )

The mortality observed at the beginning of the life cycle (Figure 3) was mainly due to the encapsulation of eggs and first-instar larvae by the host. Subsequently, the mortality observed after 12 days corresponds to that of the adult stage. The survival curve of *M. marensis* is of type I, where the highest mortality (after encapsulation) occurred later in life.

### Population parameters

For each generation that lasted 13.9 days ( $T$ ), the parasitoid multiplied its population by 28.7 times ( $R_0$ ) and the mean value for the intrinsic rate of natural increase ( $r_m$ ) was 0.242 female per day.

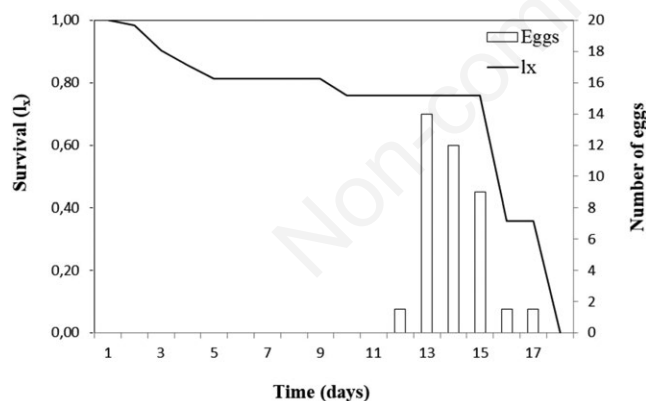


Figure 3. Survival ( $I_x$ ) and daily oviposition of *Metaphycus marensis* adult females on *Capulinia linearosae*.

## Discussion and Conclusions

### Effect of feeding on oogenesis

*Metaphycus marensis* is a synovigenic species and needs to feed on sources of carbohydrates to be able to complete the development of eggs within their ovarioles, as occurs with other species of this genus (Guerrieri & Noyes, 2000; Kapranas & Luck, 2008; Kapranas *et al.*, 2010, 2011). Synovigenic parasitoid wasp species emerge with a small number of developed eggs and throughout their lifetime the rest of the eggs mature gradually (Gordh *et al.*, 1999).

### Duration of the different stages and life cycle

Within the *Metaphycus* genus, males generally emerge a little earlier than females, as reported previously (Kapranas *et al.*, 2013; Kapranas & Tena, 2015). For other species of *Metaphycus*, the duration of the life cycle is quite variable depending on the species, ranging from 13 to 35 days (Blumberg, 1997; Guerrieri & Noyes, 2000; Stauffer, 2003). The life cycle of *C. linearosae* determined under the same laboratory conditions of this study lasts more than 22 days (Chirinos *et al.*, 2004), which is longer than the duration of this parasitoid. A shorter development time of the parasitoid with respect to the host's development time is considered one of the attributes of an effective natural enemy since more generations of the parasitoid could occur and thus increase their populations more quickly (Debach, 1964).

### Parasitoid daily fecundity

The oviposition period was shorter compared to other species within the same genus (Bernal *et al.*, 1999; Guerrieri & Noyes, 2000). This could have negative implications for the degree of biological control by *M. marensis* on *C. linearosae*. The most effective species for biological control are those that can live for a longer time because they can reproduce at low population densities of their insect host (Gordh *et al.*, 1999).

However, low fertility is not always considered a limiting factor when deciding the efficiency of a parasitoid. Lane *et al.* (1999) pointed out that the low fecundity of a parasitoid should not be a limiting factor for its selection as a biological control agent, since other bio-ecological characteristics of the parasitoid as well as its host may influence the success of a biological control program. If the parasitoid has a shorter life cycle than its insect host, as in the case of *M. marensis*, the practical capability to regulate the populations of its host insect may be high, despite its comparatively low fecundity (Lane *et al.*, 1999). As for host insects with sedentary habits, phytophages such as "hemipterans" are especially susceptible to parasitization because they are relatively easy to find due to the increase in the searching efficiency of the parasitoids, which compensates for deficiencies such as low fecundity. It is for this reason that biological control programs against phytophagous pests have had considerable success.

Table 3. Duration of the life cycle of *M. marensis*.

	N.	Egg	Larva	Pupa	Life cycle
♀	883	1.6±0.2	5.6±0.7	5.7±0.5	12.9±0.1 a
♂	387	1.6±0.1	5.6±0.2	5.4±0.	12.5±0.1 a

Means with the same letter within the same column do not differ significantly ( $P < 0.05$ ). N = number of individuals evaluated.

## Parasitoid survival ( $I_x$ )

Encapsulation of parasitoid eggs by *Coccus hesperidum* L. (Hemiptera: Coccidae) has been reported for several species of *Metaphycus*, e.g., *Metaphycus luteolus* Timberlake (Kapranas *et al.*, 2009), *M. flavus* (Howard) (Tena *et al.*, 2009; Kapranas *et al.*, 2012) and *M. angustifrons* Compere (Kapranas *et al.*, 2011).

## Population parameters

Although the  $R_0$  is considerably lower than that of its host ( $R_0$ : 1090), the  $r_m$  (0.20) (Chirinos *et al.*, 2004) was slightly lower, because of its short generation time. In addition, the efficiency of a parasitoid should be considered within the context of the insect-host-parasitoid relationship. The total oviposition rate of a female *M. marenensis* is about 40 eggs, with an average of 2.2 eggs laid per host (Chirinos & Kondo, 2019), thus a single parasitoid can parasitize up to 18 eriococcid hosts during its reproductive life.

The short generation time of *M. marenensis* compared to that obtained for *C. linerosae* indicates that the development time of the parasitoid could be 3.2 times shorter, which can be considered as an attribute of a suitable natural enemy. However, the phenomenon of encapsulation by the host coupled with the relatively low longevity and fertility of the adult female parasitoid must be analyzed through field experiments in order to define its importance as a biological control agent. Since *M. marenensis* is the only primary parasitoid associated with *C. linerosae*, this study which deals with the biological aspects of *M. marenensis* constitute one of the first contributions for the understanding of the role of this primary parasitoid in the regulation of populations of *C. linerosae*, the major arthropod pest that affects the guava orchards in Venezuela and Colombia.

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