Description and Biological Studies of a New Species of Metaphycus Mercet, 1917 (Hymenoptera: Encyrtidae), A Parasitoid of Capulinia linarosae Kondo & Gullan

Authors: Chirinos, Dorys T, and Kondo, Takumasa

Source: International Journal of Insect Science, 11(1)

Published By: SAGE Publishing

URL: https://doi.org/10.1177/1179543319857962

Description and Biological Studies of a New Species of *Metaphycus* Mercet, 1917 (Hymenoptera: Encyrtidae), A Parasitoid of *Capulinia linarosae* Kondo & Gullan

International Journal of Insect Science
Volume 11: 1–9

DOI: 10.1177/1179543319857962
SAGE

Dorys T Chirinos[1] and Takumasa Kondo[2]

[1]Facultad de Ingeniería Agronómica, Universidad Técnica de Manabí, Portoviejo, Manabí, Ecuador. [2]Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Centro de Investigación Palmira, Palmira, Colombia.

ABSTRACT: The guava cottony scale, *Capulinia linarosae* Kondo & Gullan (Hemiptera: Eriococcidae), is an important pest of guava, *Psidium guajava* L. (Myrtaceae) in northern Colombia and Venezuela. A species of *Metaphycus* (Hymenoptera: Encyrtidae) is the only known primary parasitoid associated with this insect pest. The parasitoid is herein described as *M. marensis* Chirinos & Kondo, sp. nov., based on morphological characteristics of the adult female and male. Biological studies on adult longevity, fecundity, host preference, and sex ratio were conducted. The maximum longevity of the female and the male were 8.0 and 6.5 days, respectively, when fed with diluted honey. On average, a fed mated female laid approximately 40 eggs. Adult females of *M. marensis* were shown to prefer to parasitize 11- to 15-day-old adult females of *C. linarosae* and do not parasitize first-instar nymphs of the host eriococcid. The female-to-male sex ratio of the parasitoid was 2.24: 1. When ovipositing females of *M. marensis* were given only small-sized individuals (second-instar nymphs) of *C. linarosae*, generally the resulting progeny was a single male wasp. This parasitoid species has arrhenotokous reproduction and is a facultative gregarious parasitoid. These results show a short adult longevity, as well as a relatively low fecundity of the female compared with studies conducted on other *Metaphycus* species. This study provides essential baseline information for future biological control programmes for *C. linarosae*.

KEYWORDS: Biological control, Coccoidea, natural enemies, *Metaphycus* species, taxonomy

RECEIVED: May 18, 2019. **ACCEPTED:** May 23, 2019.

TYPE: Original Research

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Takumasa Kondo, Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Centro de Investigación Palmira, Calle 23, Carrera 37, Continuo al Penal, 763531 Palmira, Valle del Cauca, Columbia. Email: takumasa.kondo@gmail.com

Introduction

The guava cottony scale, *Capulinia linarosae* Kondo & Gullan (Hemiptera: Eriococcidae),[1] has been considered an important pest of guava, *Psidium guajava* L. (Myrtaceae) in Venezuela since its appearance in 1993.[2,3] In Venezuela, it has caused heavy damage and severe infestations in the north-western part of the country, which accounted for 80% of the national production of guava at that time. At the beginning of its colonization, *C. linarosae* devastated about 600 ha due to its high reproductive rate and the lack of specific natural enemies.[2,3] This insect pest was reported recently in Colombia,[1,4] 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.[4]

C. linarosae is found on branches, leaves, and fruit of guava, and damage is caused by the insects feeding on the plant phloem.[2–4] When the guava cottony scale was detected for the first time in Venezuela, it was only associated with generalist predators and no specific and effective natural enemies were found.[5,6] Around 1995, a specific parasitoid of *C. linarosae* was detected, which was identified by Dr. John Noyes (Natural History Museum, London, UK) as an undescribed species of the genus *Metaphycus* (Hymenoptera: Encyrtidae),[6] and recorded as *Metaphycus* sp. in the Chalcidoidea database of the Natural History Museum, London, England.[7] This parasitoid was detected simultaneously in the states of Aragua and Zulia in the central and western part of Venezuela, respectively, and for this reason it was suggested that the parasitoid could have followed its host in its process of dispersion from the Venezuelan Amazon region where *C. linarosae* is thought to have originated.[2]

Currently, this *Metaphycus* species is the only known primary parasitoid associated with *C. linarosae* in Venezuela,[8] and has not been reported yet in Colombia. Earlier experimental evaluations showed the effectiveness of this parasitoid as a natural biological control agent of this important pest.[6] Given the importance of *C. linarosae* as a pest of guava crop in Colombia and Venezuela, it is important to know the effective biological control agents. *Metaphycus* sp. is a primary parasitoid found in Venezuela, and studies on its taxonomy and biology are needed. Species of the genus *Metaphycus* are associated with members of the superfamily Coccoidea (Hemiptera: Sternorrhyncha) as solitary or gregarious parasitoids.[9,10] Some species of *Metaphycus* have been successful in regulating populations of their hosts.[10–12]

Herein we use morphological features of adult females and adult males to describe and illustrate the *Metaphycus* sp. associated with *C. linarosae* as a primary parasitoid. In addition, information on adult longevity, fecundity, preference for host age and stage for parasitization, and female/male sex ratio of the *Metaphycus* species are provided.

Materials and Methods

Morphology and species description

Observations were made on the external morphology of *Metaphycus* sp. using specimens collected in the field at the following localities, Maracaibo (10°41′17.47″N, 71°38′14.89″W), Mara (10°56′25.84″N, 71°50′75.48″W; 10°58′06.33″N, 66°08′47.69″W), and Sucre (09°15′48.53″N, 71°08′11.01″W), in Zulia state, Venezuela. Parasitized individuals of *C. linarosae* collected on guava branches were placed in transparent gelatin capsules until the emergence of the adult parasitoid. Most specimens were preserved in 98% ethyl alcohol and kept in the freezer with some stored dry in plastic petri dishes with cotton at the base. A number of the alcohol-preserved specimens were mounted following the techniques described by Noyes.[13] Some individuals were point-mounted without previous treatments and others were mounted on slides in Hoyer's mounting medium (distilled water 50 cc, gum Arabic 30 cc, chloral hydrate 200 cc and glycerin 20 cc) or Euparal. Except for the antenna, which was used to determine the distribution pattern of coloration, the specimens for slide mounting were previously cleared by boiling them for about 10 to 20 minutes in potassium hydroxide (10% w/v).

Depository

UZM: Museum of Arthropods, Faculty of Agronomy, University of Zulia, Maracaibo, Venezuela.

Host and parasitoid cultures

The study was carried out at the Phytosanitary Technical Unit, Faculty of Agronomy, University of Zulia, in Maracaibo, Venezuela. The temperature was maintained at 26.7°C (range: 24-28°C) and relative humidity 79.9% (range: 71%-80%). Approximately 200 small guava plants were cultivated, used to maintain cultures of the host, *C. linarosae*, and the parasitoid, *Metaphycus* sp.; the rest were used in the experiments.

Guava plants were infested with 10 egg masses (approximately 150 eggs per mass) in order to have a constant supply of eggs, immature, and adult individuals of *C. linarosae*. Once infested, the plants were placed inside cylindrical cages, each 81 cm high x 48 cm wide, consisting of a structure of five iron bars, equidistant and perpendicularly tied at their ends to two rings and covered with organdy cloth. The guava plants were left inside the cages for about 30 days. The eriococcids reared in this way were used for obtaining eggs, infesting new plants, replacing dying cultures, or used in experiments with the parasitoid. To start and maintain the parasitoid cultures, parasitized individuals of *C. linarosae* brought from the field were separated and placed in clear gelatin capsules until emergence of the adult parasitoids. Once emerged, the wasps were sexed and fed with honey diluted in distilled water (1:1 v/v), in a glass vial 4.5 cm high x 1.3 cm wide, and then exposed to cultures of *C. linarosae* for parasitization. When adults of the parasitoid were about to emerge, plants maintained under laboratory conditions and infested with approximately 50 to 100 individuals of *C. linarosae* were covered with a clear plastic cylindrical cage of 2 L capacity and 15 cm high x 10 cm wide with the top covered with organdy cloth. The cage with the infested plants had a hole in the middle of its sidewall, 1.5 cm in diameter, from which 2 to 3 previously mated parasitoid females were introduced from a glass vial. The female parasitoid wasps were fed with drops of honey diluted in distilled water, which were placed along the branch of the plant. *C. linarosae* produces small amounts of honeydew, however, because of the abundant waxy threads that covers its body, the parasitoids do not feed on its honeydew. The wasps remained inside the cages until their death. The branches were checked after 8 to 9 days in order to detect parasitized individuals of *C. linarosae*, which were easily identified by the brown coloration developed by the eriococcid host. The parasitized individuals of *C. linarosae* were placed in gelatin capsules until the emergence of the parasitoids, which were used to maintain the parasitoid cultures or for conducting the experiments.

The observations for the experiments and the morphological characteristics of the new species were made with a Leica S8 Apo Stereo Microscope with Apochromatic Optics, Microsystems, Wetzlar, Germany, with 10X to 120X magnification.

Experiments

Adult longevity of the parasitoid

Twenty infested guava plants with young adult females of *C. linarosae* (15 days after moulting) were exposed to 2 to 3 mated parasitoid females for 24 hours. The plants were covered with organdy cloth bags (33 cm high x 12 cm wide), with an opening at the end tied to the container by means of a nylon cord. 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. The emerged adults were sexed and evaluated under four different conditions: (a) non-fed females and males, (b) non-ovipositing fed females, (c) fed males, and (d) ovipositing fed females. Evaluations for conditions (a), (b), and (c) were carried out in 10 mL glass vials; honey diluted in distilled water (1:1 v/v) was added to vials b and c as a source of food. For evaluations for condition (d), adults were kept in cages and exposed daily (until their death) to guava branches with females of *C. linarosae* under similar climatic conditions and fed with honey diluted in distilled water (1:1 v/v). About 520 specimens (260 female and 260 male parasitoids) were used to determine their longevity, for both unfed and honey-fed individuals. The longevity of the females was recorded for both egg-laying and non-egg-laying individuals.

Fecundity of the parasitoid

Fifty adult females of *Metaphycus* sp. at 2 days after emergence were paired with males of the same age to mate. Mating lasted for about 5 to 10 seconds. Mated females were then released inside a cage that contained plants with cultures of *C. linarosae*

in order to count the daily number of eggs laid on the eriococcids. For this purpose, each guava plant with eriococcid hosts (about 200 females of 11-15 days after emergence) was changed every 24 hours; this was repeated until the death of the female parasitoid. All the eriococcid individuals found on the plants were dissected daily and the number of eggs laid by each parasitoid was determined.

Parasitoid preference for the stage and age of the host for parasitization

In order to determine the preferred host age and stage for oviposition by the parasitoid female, plants infested with *C. linarosae* of overlapping generations (mixed ages) and later plants infested with individuals of uniform ages were used. Two to three mated female parasitoids at 2 days after emergence were used. The adult males also were 2 days post-emergence in order to provide individuals of the same age as the adult females. Parasitoids were fed with honey diluted in distilled water (1:1 v/v).

Obtaining overlapping generations of the host. Three plants infested with *C. linarosae* were used per replicate (20 replicates). The overlapping generations of *C. linarosae* were composed by first-instar nymphs, second-instar nymphs and adult females of different ages. In order to obtain branches with overlapping generations of *C. linarosae*, 120 eriococcid eggs that were about to hatch were placed on each guava branch; this was repeated four times at eight-day intervals to guarantee a plenty number of adult females and nymphs of all ages.

Seven to eight days after the eriococcids were exposed to the parasitoid wasps, parasitized individuals of *C. linarosae* were separated according to their growth stage in order to calculate the percentage of parasitized individuals for each eriococcid growth stage ([number of *C. linarosae* individuals parasitized at a particular stage/total number of parasitized *C. linarosae* individuals in all stages] x100). Subsequently, parasitized individuals were placed individually in transparent gelatin capsules until the emergence of the parasitoids. Upon emergence, the number and sex of the parasitoids was determined, and the number of parasitoids per host was estimated for each stage of development of *C. linarosae*. Also, the proportions of female and male parasitoids were determined.

Field information also was obtained for the localities of Mara (10°56′25.84″N, 71°50′75.48″W) and Sucre (09°15′48.53″N, 71°08′11.01″), Zulia state. Thirty samples composed of guava branches infested with *C. linarosae* were collected and brought to the laboratory where the parasitized individuals were processed in the same way as described above.

Obtaining uniform ages of the host. To start the experiments, 65 young adult females of *C. linarosae* (5-10 days after emergence) were selected from the plants of the culture, removing all existing eggs with a fine brush. The females were marked with an entomological pin placed next to each individual. In order to obtain insect hosts of uniform age, the eriococcid adult females were left for a period of 24 hours to oviposit and then approximately 150 eggs were removed with a fine brush and placed on the plant used as the experimental substrate, approximately in the middle of the stem. The first-instar nymphs that emerged from these eggs were kept in the rearing cage to grow until they reached the age and instar at which they were exposed to the parasitoids.

Second-instar nymphs of *C. linarosae* were divided into two age categories: young second-instar nymphs (females and males) (2 days after moulting) and older second-instar females (4 days after moulting). Adult females were divided into the following age ranges: 1 to 5 days, 6 to 10 days, 11 to 15 days, 16 to 20 days, 21 to 25 days, 25 to 30 days and 30 to 35 days after moulting. Although an adult female can live for up to 45 days or more, after 35 days they became very flaccid and shrivelled, and therefore were discarded for the purpose of this study. As the eriococcid hosts showed parasitization symptoms (brown integument), they were removed from the plant and placed in gelatin capsules. Five to seven days later, the adult parasitoids emerged and were sexed and counted.

For this experiment, a supply of *C. linarosae* individuals for the 10 different age ranges (as described above) were provided; a plant was used for each of the age range (10 plants in total); a total of seven replicates were performed. The preference of the parasitoid for age and sex of the host was determined in the same way as for the experiment with overlapping generations.

Sex ratio of the parasitoid

In order to determine the type of reproduction and the sex ratio of the parasitoid, guava plants with cultures of *C. linarosae* were exposed to virgin and mated parasitoid females during a period of 24 h. A total of 20 replicates were made over time, evaluating three plants per condition (virgin or mated female parasitoids) for each replicate. In addition, for eriococcids parasitized by *Metaphycus* females, the number of parasitoids that emerged per individual was estimated.

Statistical analysis

The variables, adult parasitoid longevity, percentage of parasitized individuals by host stage, individuals separated by age ranges of the host (see above), as well as the number of females and males of the parasitoid, and their sex ratio were previously transformed with the square root function ($\sqrt{x+1}$) and subsequently analysed through the General Linear Model, and mean comparisons were carried out using the Least Squares method ($P < 0.05$), for which the SAS® statistical programme was used.

Results and Discussion

Metaphycus marensis *Chirinos & Kondo sp. nov*

Diagnosis

Adult female. Length is, including ovipositor, 0.8 to 1.3 mm (Figures 1A and B). Head uniformly dark brown in coloration,

Figure 1. Adult wasps of *Metaphycus marensis* Chirinos & Kondo sp. nov. (A) Adult female on an ovisac of *C. linarosae*, (B) Close-up of adult female showing exserted ovipositor, and (C) Adult male. Photos by D.T. Chirinos.

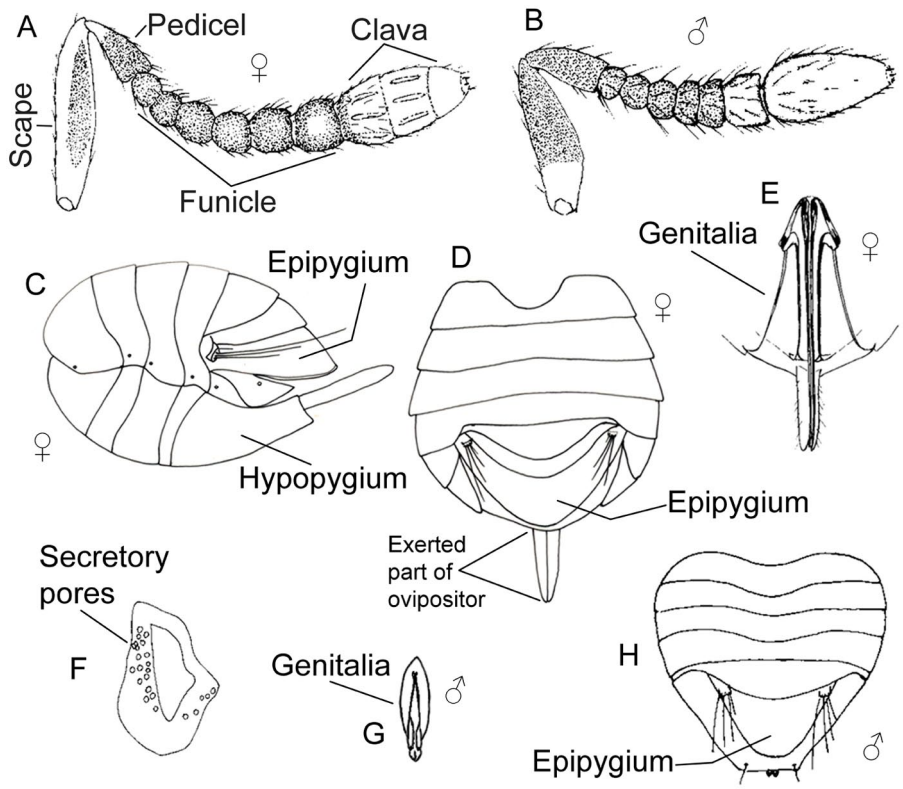

Figure 2. (A) Antenna of the adult female, (B) Antenna of the adult male, (C) Gaster of adult female (lateral view), (D) Gaster of adult female (dorsal view), (E) Female genitalia, (F) Torulus of male antenna with secretory pores, (G) Male genitalia, and (H) Gaster of adult male.

antenna bicoloured, with 11 segments (formula 1163: 1 scape, 1 pedicel, 6 funicle, 3 clava), scape mostly dark brown, pedicel dark brown, pale yellow at its base, all segments of funicle dark brown, but F6 pale yellow in the centre, clava with part of basal segment dark brown (Figure 2A). Thorax dark brown; legs yellow, fore wing hyaline; gaster dark brown, alternated with yellow coloration near intersegmental areas, with sternites mostly yellow. Head about 3X as wide as frontovertex; scape 0.17X length, mandible 3-dentate; palp formula 3-3; notaular lines nearly reaching 0.5X across mesoscutum; ovipositor strongly exserted; hypopygium reaching about 0.5X length of gaster (Figure 2 C).

Male. Length of air-dried specimens is 0.40 to 0.70 mm (Figure 1 C). Body similar in shape to female. Antenna with scape mostly dark brown, pedicel dark brown, F1 to F5 dark brown, F6 and clava pale yellow (Figure 2B), toruli associated with secretory pores with distribution as illustrated in Figure 2H; gaster relatively shorter than that of the female (Figure 2 F); genitalia long and ellipsoidal (Figure 2G).

Description

Adult female. Length of air-dried specimens, from frons to tip of ovipositor sheath, is 0.8 to 1.3 (Holotype 1.1) mm. Head uniformly dark brown in coloration, not variegated or with spots, about 3X as wide as frontovertex; mandible 3-dentate, dark brown; palp formula 3-3; lacinia and galea transparent, cardo and stipe of dark brown coloration; and labium transparent. Head width 3.05(2.88-3.28)X length of frontovertex; malar sulcus 1.87(1.60-2.20)X length of malar space; POL-posterior ocellar line 2.20(1.50-2.80)X the length of OOL – ocular-

ocellar line; ocelli forming an equilateral triangle, diameter of lateral ocellus 2.33(2.20-3.00)X the length of OOL, diameter of medium ocelli, twice the distance that separates it from the lateral ocellus; large compound eye 1.70(1.56-1.93)X its width; antenna (Figure 2A) bicoloured, with 11 segments (formula 1163: 1 scape, 1 pedicel, 6 funicle, 3 clava); scape mostly dark brown, width 0.17(0.14-0.19)X its length; pedicel dark brown, pale yellow at its base, width 0.52(0.40-0.66)X its length; pedicel length 0.34(0.33-0.39)X the length of 3 basal funicular segments combined; all segments of funicle dark brown, but F6 pale yellow in the centre, 3 basal funicular segments subequal in width, the 3 apical segments gradually widening, width of apical segment 1.55(1.33-1.67)X the width of basal segment; and clava 3-segmented, ovate, with part of basal segment dark brown, its width 0.35(0.31-0.46)X its length, length of clava 0.67(0.64-0.71)X length of funicle.

Thorax. Dark brown coloration; notum sculpture reticular; notaular lines reaching about 0.5X across mesoscutum; anterior and posterior wings hyaline, anterior wings with speculum, disc and rib cell 4, veins of yellowish coloration; and anterior and posterior legs yellowish in colour, with globose coxae and media approximately quadrangular. Width of scutellum 1.27(1.20-1.37)X its length, propodeum large 0.08(0.05-0.09)X scutellum length; fore wing width 0.40(0.35-0.43)X its length, length of stigmal vein 0.13(0.11-0.17)X length of submarginal vein, marginal and postmarginal vein inconspicuous; legs yellow, length of middle tibia 1.01(0.92-1.05)X length of middle femur, length of middle tibia 1.12(1.11-1.22)X length of middle, length of hind tibia 1.19(1.12-1.27)X length of hind femur, length of hind tibial spur 0.46(0.33-0.55)X length of hind basitarsus.

Gaster of adult female. Flat on dorsum and convex on venter; dark brown coloration alternated with yellow coloration near intersegmental areas and sternites mostly of yellow coloration (Figures 2 C and D). Sculpture similar in shape to that of thorax but approximately twice as large; with seven tergites (including epipygium) and five sternites (including hypopygium). A pair of cercal plates with 4 setae that delimit the epipygium anteriorly (Figure 2D). Ovipositor strongly exserted, length of ovipositor 0.95(0.87-1.06)X length of gaster and 1.96(1.71-2.19)X length of hind tibia, length of ovipositor sheaths (3rd valvulae) 0.33(0.28-0.35)X length of ovipositor, hypopygium measuring almost half length of all gaster sternites (Figure 2 C).

Male. Length of air-dried specimens 0.50(0.40-0.70) mm. Body similar in shape to female. Antenna with scape mostly dark brown, pedicel dark brown, F1 to F5 dark brown, F6 and clava pale yellow (Figure 2B), toruli and associated secretory pores with distribution as in Figure 2 F; gaster of adult male (Figure 2 H) relatively shorter than that of adult female (Figure 2D), male genitalia long and ellipsoidal (Figure 2G).

Holotype. Adult female (♀). **Venezuela:** Maracaibo, Zulia State, (10°41′17.47″N, 71°38′14.89″W, Unidad Técnica Fitosanitaria, Facultad de Agronomía, Ciudadela Universitaria, Universidad del Zulia, Feb. 2, 2004, coll. D.T. Chirinos, reared from *C. linarosae* Kondo & Gullan, *ex* guava (*P. guajava* L.) (UZM).

Paratypes. Thirty-three adult females (♀♀), 30 adult males (♂♂). **Venezuela:** Maracaibo (12 females and 11 males, 10°41′17.47″N, 71°38′14.89″W), Mara (6 females and 5 males,10°56′25.84″N; 71°50′75.48″W; 5 females and 5 males, 10°58′06.33″N; 66°08′47.69″W) and Sucre (10 females and 9 males, 09°15′48.53″N; 71°08′11.01″W), all reared from *C. linarosae* on *P. guajava* (UZM).

Several *Metaphycus* species have been reported from countries close to Venezuela. *Metaphycus omega* Noyes, a parasitoid of whiteflies (Hemiptera: Aleyrodidae), has been reported from Brazil, Costa Rica, Ecuador, Guyana, and Trinidad.[14–16] Other *Metaphycus* species have been reported from Brazil, namely, *Metaphycus alboclavatus* Compere, *M. brasiliensis* (Compere & Annecke), *M. flavus* (Howard), and *M. discolour* (De Santis).[16] Of these, *M. marensis* is closest to *M. brasiliensis*, but differs from that species by the following features (character states of *M. brasiliensis* in parenthesis): (a) frontovertex dark brown (yellowish), (b) maxillary palps of three segments (four segments), (c) base of the first segment of the clava dark brown (white), and (d) ovipositor strongly exserted (ovipositor weakly exserted),[17] plus *M. brasiliensis* is known as a parasitoid of *Chaetococcus bambusae* (Maskell) (Hemiptera: Pseudococcidae) collected on an unknown host plant.[16,17] *M. marensis* also appears to be close to *Metaphycus entella* Noyes from Costa Rica, but differs from that species by the following combination of characters (character states of *M. entella* in parenthesis): legs yellow (dark brown), gaster with dark brown coloration alternated with yellow coloration (gaster completely dark brown) and the hypopygium 0.5X the length of gaster (hypogeum 0.8X the length of gaster).[14]

Etymology. This species is dedicated to the town of Mara, Zulia State, Venezuela, the largest producing area of guava crop in Venezuela, which was the first region where guava plants were devastated by its eriococcid host *C. linarosae* when it appeared as a pest more than two decades ago.

Adult longevity of the parasitoid

The longevity of the adult parasitoids differed significantly depending on the diet (Table 5, $P < 0.05$). Non-fed adults did not survive past the second day, while honey-fed individuals lived more than six days. The longevity of the adults of *Metaphycus* species is known to be influenced by the provided diet. Females of *M. flavus* and *M. stanleyi* Compere lived much longer when they were supplied with water plus honey, or water plus honey plus insect hosts for them to feed on.[18] A similar result was reported also for *M. luteolus* Timberlake.[19] However, although in the present study adult parasitoids were supplied with water plus honey, their longevity did not exceed ten days. The adult longevity of *M. marensis* was considerably shorter than that reported for other species of this genus.[11,18–22] Differences also were

Table 1. Longevity (in days) of adults of *Metaphycus marensis* sp. nov., under laboratory conditions (T° = 26.6°C [range: 24-28°C] and RH = 79.9% [range: 71% to 80%]).

CONDITION	FEMALES	N	MALES	N
Unfed	1.6 ± 0.1 a	82	1.4 ± 0.8 a	176
Honey-fed, with oviposition	4.7 ± 0.1 b	50	–	–
Honey-fed, without oviposition	8.0 ± 0.2 c	120	6.5 ± 0.2 b	90

T: temperature; RH: relative humidity.
Means ± standard error. Means with the same letter in the columns indicate that there were no significant differences ($P < 0.05$). Comparisons of means were made through the least squares' method; n = number of individuals evaluated.
Honey-fed individuals were fed with bee honey diluted in distilled water (1:1 v/v).

observed between honey-fed females with and without oviposition (Table 1, $P < .05$). Non-oviposited females lived on average about 8 days, whereas females that had oviposited lived on average about five days.

Fecundity of the parasitoid

The adult females of *M. marensis* laid 39.5 ± 7.0 eggs per female (range: 27-48 eggs) (n = 50) under laboratory conditions. Eggs were laid during six consecutive days without periods of pre- and postoviposition, with 65% of the eggs being laid between the second and third days. This was lower than the numbers reported for other *Metaphycus* species that range from 50 to 293 eggs per female.[11,22] The dissections carried out for conducting the observations on the egg development of *M. marensis* allowed detection of the mortality factors that occurred daily throughout the lifetime of the parasitoid, especially during the first four days. Dissections were performed every 12 h on the first two days and then every 24 h after the third day. During this study, with the exception of the mortality due to encapsulation, no eggs with morphological abnormalities were found. Furthermore, all studied eggs took about the same time from oviposition until they hatched. Therefore, in this case, the fecundity of the adult female of *M. marensis* could be considered as its fertility.

Parasitoid preference for the stage and age of the host for parasitization

M. marensis only parasitizes second-instar nymphs (2- or 4-day-old females and males) and adult females (of various ages), with a marked preference for adult females (> 85%; $P < 0.05$) (Table 2). Females of *C. linarosae* have a longevity of at least 45 days, during which period the condition of the host could vary. Therefore, 10 age ranges of *C. linarosae* were evaluated in the second experiment. Parasitoids that emerged from young second-instar nymphs (males and females) both in field and in laboratory (overlapping generations and uniform ages) were mostly solitary males (Tables 3 and 4). These results coincide with what has been reported for other *Metaphycus* species, for which males are mainly produced when the female parasitoid oviposits on small-size hosts.[11,18,23,24]

Parasitized older second-instar nymphs (4-day old) of *C. linarosae* generally moult and die as young adult females. At that age range (i.e. 4-day old second-instar nymphs), gregarious parasitization begins to increase. The number of female parasitoids per host increased significantly in older *C. linarosae* second-instar nymphs compared to young second-instar females and male nymphs. When parasitized adult females of *C. linarosae* were evaluated, the number of females increased in the parasitoid progeny, exceeding the number of males, with significantly higher values in 11- to 15-day-old adult hosts ($P < 0.05$) (Table 4).

The proportion of parasitoid females decreased slightly in the following age range of *C. linarosae* (16-20 days) (Table 4). This pattern has been reported also for *Aphidius urticae* Haliday (Hymenoptera: Aphelinidae) parasitizing females of *Hyalopteroides humilis* Walker (Hemiptera: Aphididae), where the percentage of parasitoid females decreased in older hosts.[25] It must be noted that *A. urticae* is a solitary endoparasitoid, whereas *M. marensis* is a facultative gregarious parasitoid. No parasitization was observed by *M. marensis* when exposed to 20-day-old adult females of *C. linarosae* (Table 4).

M. marensis prefers to parasitize 11- to 15-day-old adult females of *C. linarosae*. This contrasts with *M. helvolus* (Compere) that prefers to attack second- and third-instar nymphs of its host *Saissetia oleae* Olivier (Hemiptera: Coccidae). *M. marensis* has a similar behaviour to *M. lounsburyi* (Howard), which prefers older instar nymphs (third-instar nymphs) and adults of its host *S. oleae*.[11,26] Females of *M. alberti* (Howard), also prefer young adult hosts, although they attack hosts from first-instar nymphs to adults.[23]

It has been pointed out for some species of *Metaphycus* that the number of parasitoids that emerge is related to the size of its host, the larger the host the higher the number of emerging parasitoid individuals. Zhang et al[27] reported that larger specimens of the scale insect, *Parasaissetia nigra* Nietner, have a higher number of emerging parasitoids. In the brown soft scale, *Coccus hesperidum* L., Kapranas et al[26] observed that the number of parasitoids of the species *M. helvolus, M. luteolus, M. angustifrons* Compere, and *M. stanleyi* increased significantly when the size of the scale insect

Table 2. Percentage parasitization by *Metaphycus marensis* sp. nov. on branches with overlapping generations of *Capulinia linarosae* obtained in the laboratory and in the field at Mara and Sucre localities, where n = number of evaluated parasitized individuals.

HOST AGE	LABORATORY		MARA		SUCRE	
	%	N	%	N	%	N
First-instar ♀♂	0 c	0	0 c	0	0 c	0
Second-instar ♀	1.85 b	34	0.89 b	12	2.67 b	72
Second-instar ♂	2.89 b	54	2.68 b	36	7.55 b	204
Prepupa ♂	0 c	0	0 c	0	0 c	0
Pupa ♂	0 c	0	0 c	0	0 c	0
Adult ♀	95.25 a	1765	96.43 a	1304	89.78 a	2428

Means with the same letter in the columns do not differ significantly. Means compared through the Least Squares test ($P < 0.05$).

Table 3. Number of emerged adults of *Metaphycus marensis* sp. nov. by ages of *Capulinia linarosae* in laboratory and field conditions in Mara and Sucre localities, Zulia state.

AGE	LABORATORY				MARA				SUCRE			
	IND/HOST	♀	♂	♀:♂ RATIO	IND/HOST	♀	♂	♀:♂ RATIO	IND/HOST	♀	♂	♀:♂ RATIO
N2♀	1.00	0.11	0.89	0.12:1	1.00	0.00	1.00	0.00:1	1.00	0.12	0.88	0.13: 1
N2♂	1.00	0.07	0.93	0.07:1	1.00	0.06	0.94	0.07:1	1.00	0.17	0.83	0.20:1
A♀	2.31	1.59	0.72	2.12:1	1.53	0.93	0.60	1:55:1	1.56	0.99	0.57	1.74:1

N2: Second-instar nymph; A: adult.

Table 4. Number of parasitoids per host (individuals/host), females, males and female/male ratio of *Metaphycus marensis* sp. nov. obtained in the laboratory when exposed to different age ranges of the host (n = number of evaluated parasitized individuals).

HOST AGE	N	INDIVIDUALS/HOST	♀	♂	♀:♂ RATIO
N2♀ Young	36	1.00 d	0.00 e	1.00 a	0.00:1 e
N2♂ Young	42	1.00 d	0.07 e	0.93 ab	0.08:1 e
N2♀ older	67	1.86 c	0.85 d	1.01 a	0.84:1 d
A♀ 1-5 days	92	2.04 c	1.16 c	0.88 bc	1.32:1 c
A♀ 6-10 days	86	2.86 b	1.95 b	0.91 ab	2.11:1 ab
A♀ 11-15 days	113	3.65 a	2.46 a	1.09 a	2.25:1 a
A♀ 16-20 days	106	2.33 bc	1.54 bc	0.79 c	1.95:1 bc
A♀ 21-25 days	0	–	–	–	
A♀ 26-30 days	0	–	–	–	
A♀ 31-35 days	0	–	–	–	

A: adult; N2: Second-instar nymph.
Means with the same letter in the columns do not differ significantly. Means compared through the Least Squares test ($P < 0.05$).

Table 5. Number of individuals and sex of parasitoid wasps obtained for mated and unmated females of *Metaphycus marensis* sp. nov. in the laboratory.

PROGENY	PARENTS	
	MATED FEMALES (♀)	VIRGIN FEMALES (♀)
Number of females (♀)	1265	0
Number of males (♂)	588	776

host increased. These same researchers pointed out that the sex of *M. helvolus* depends on the size of the host, with males emerging from smaller hosts.

Insects of the order Hymenoptera are known to regulate their progeny's sex ratio.[28] Some factors can influence the fertilization of eggs and change the female:male ratio in their progeny.[28,29] Among the factors that affect female: male ratios are the population density of the host and the parasitoid, mortality of immature parasitoid stages, and quality, age and size of the host, among others.[18,27,29,30]

Encapsulation is one of the main mortality factors of eggs and larvae of *Metaphycus* species.[31–34] For *M. marensis*, 11- to 15-day-old females of *C. linarosae* encapsulate more eggs and larvae than other ages.[33] Kapranas et al[34] pointed out that the largest hosts tend to encapsulate a greater number of parasitoid eggs.

Sex ratio of the parasitoid

When mated females were evaluated, the progeny consisted of females and males, whereas only males were reared from virgin females (Table 5). The female to male sex ratio was 2.24: 1. *M. marensis* has an arrhenotokous reproduction, in which eggs need to be fertilized in order to produce females. In this type of reproduction, mated females give birth to females and males and unmated females only produce males. In a literature review of the general biological characteristics of species of the genus *Metaphycus*, Guerreri and Noyes[22] pointed out that in those species for which sex determination had been studied, reproduction was by arrhenotoky. The sex ratio obtained in this study is similar to that reported for *M. anneckei* Guerreri and Noyes,[22] with a 2:1 female: male ratio. In *M. melanostomatus* (Timberlake) and *M. asterolecanii* (Mercet), a 3:1 female: male ratio has been reported.[22]

Generally, more than two (2.2-2.8) parasitoids (in overlapping generations) emerged per eriococcid host in this study. In this sense, the parasitic behaviour of *M. marensis* is considered to be facultatively gregarious and biased towards a higher proportion of females, as reported for several other species of *Metaphycus*.[24,26]

Conclusion

The present study describes and illustrates a new encyrtid species, *M. marensis* Chirinos & Kondo. The biological information herein provided, including adult longevity, fecundity, host preference, and sex ratio of this new parasitoid species should become a baseline for biological control programmes of its eriococcid host, *C. linarosae*, a pest of great relevance to guava crops in Colombia and Venezuela. Future studies should be carried out to determine the duration of this parasitoid's life cycle, morphology of its larval stages, and population dynamics in the laboratory and the field.

Acknowledgements

Many thanks to Dr Penny J Gullan (The Australian National University, Canberra, Australia) for checking the English text and for useful comments. Thanks to two anonymous reviewers for their useful comments that greatly helped improve the manuscript.

Author Contributions

DTC and TK contributed to the writing of the manuscript; agreed with manuscript results and conclusions; and made critical revisions and approved final version. DTC took the photos, recorded the biological information, and conducted the main analyses. TK helped prepared the plates and translated the original Spanish text into English. Both authors reviewed and approved the final manuscript.

ORCID iD

Takumasa Kondo https://orcid.org/0000-0003-3192-329X

REFERENCES

1. Kondo T, Gullan PJ, Cook LG. A review of de genus Capulinia Signoret (Hemiptera: Coccoidea: Eriococcidae) with description of the two new species. *Zootaxa*. 2016;4111:471–491.
2. Cermeli M, Geraud-Pouey F., Capulinia sp. cercana a jaboticabae von Ihering (Homoptera: Coccoidea: Eriococcidae) nueva plaga del guayabo en Venezuela. *Agron Trop*. 1997;47:115–123.
3. Chirinos DT, Romay G, Fernández CE, Castro R. Capulinia linarosae Kondo y Gullan, 2016: Historia y situación actual como plaga del guayabo, *Psidium guajava* L. en Venezuela. *Rev Fac Agron (LUZ)*. 2017;34:397–427.
4. Ramos A. *Lineamientos generales para el manejo integrado de la mota blanca de la guayaba, Capulinia linarosae Kondo y Gullan (Hemiptera: Eriococcidae)*. Bogotá, Colombia: Produmedios Edit; 2018.
5. Chirinos-Torres L, Geraud-Pouey F, Chirinos DT, et al.Efecto de insecticidas sobre la mota blanca del guayabo, Capulinia sp. (Hemiptera: Eriococcidae) En El Municipio Mara, Estado Zulia, Venezuela. *Bol Entomol Venez*. 2000;15:1–16.
6. Geraud-Pouey F, Chirinos DT, Aguirre R, Bravo Y, Quintero J. Evaluación de *Metaphycus* sp. (Hymenoptera: Encyrtidae) como agente de control natural de Capulinia sp cercana a jaboticabae von Ihering (Hemiptera: Eriococcidae). *Entomotropica*. 2001;16:165–171.
7. Natural History Museum. Metaphycus Mercet, 1917. http://www.nhm.ac.uk/our-science/data/chalcidoids/database. Up-dated 2019.
8. Romay G, Fernandez C, Castro R, Chirinos DT. Diversidad de enemigos naturales asociados con Capulinia linarosae Kondo y Gullan, 2016. *Misionero Del Agro*. 2017;13:78–88.
9. Kapranas A, Tena A. Encyrtid parasitoids of soft scale insects: biology, behavior, and their use in biological control. *Annu Rev Entomol*. 2015;60:195–211.
10. Robayo EJ, Chong JH, Schultz PB. Difference in the abundance of scale insect parasitoids among four cardinal directions. *Fla Entomol*. 2016;99:813–815.
11. Flanders SE. Metaphycus Helvolus, an Encyrtid parasite of the Black Scale. *J Econ Entomol*. 1942;35:690–698.
12. Noyes JS. *Encyrtidae (Insecta: Hymenoptera)*. Fauna of New Zealand 13. Wellington, New Zealand: Science Information Publishing Centre; 1988.
13. Noyes JS. Collecting and preserving chalcid wasp (Hymenoptera: Chalcidoidea). *J Natural History*. 1982;16:315–334.
14. Noyes JS. *Encyrtidae Chalcidoidea 2*. Metaphycus and Related Genera, Parasitoids of the Scale Insect and Whiteflies (Aleyrodidae). Ann Arbor, MI: Memoirs of American Entomological Institute; 2004.
15. Myartseva SN, Ruiz-Cancino E. Una nueva especie de Metaphycus Mercet (Hymenoptera: Encyrtidae) de México y clave de especies del género que parasitan mosquitas blancas (Hemiptera: Aleyrodidae) en la región Neotropical. *Act Zool Mex*. 2010;26:17–24.
16. Ramos ASJ, Santos de Lemos RN, Costa VA, et al. Hymenopteran parasitoids associated with scale insects (Hemiptera: Coccoidea) in tropical fruit trees in the eastern Amazon, Brazil. *Fl Entomol*. 2019;101:273–278.
17. Compere H, Annecke DP. Descriptons of parasitc Hymenoptera and comments (Hymenopt.: Aphelinidae, Encyrtdae, Eulophidae). *J Entomol Soc South Africa*. 1961;24:17–71.

18. Bernal J, Robert LF, Morse JG. Host influences on sex ratio, longevity and egg load of two *Metaphycus* species parasitic on soft scales: implications for insectary rearing. *Ent Exp App*. 1999;92:191–204.
19. Kapranas A, Luck RF. Egg maturation, host feeding, and longevity in two *Metaphycus* parasitoids of soft scale insects. *Biol Control*. 2008: 2:147–153.
20. Noyes JS. *Metaphycus baruensis* sp. n. (Hymenoptera: Encyrtidae) parasitic in green scales, *Coccus* spp. (Hemiptera: Coccidae), on coffee in Kenya. *Bull Ent Res*. 1988;78:131–134.
21. Lampson LJ, Morse JG, Luck RF. Host selection, sex allocation and host feeding by Metaphycus helvolus (Hymenoptera: Encyrtidae) on Saissetia oleae (Homoptera: Coccidae) and its effect on parasitoid size, sex and quality. *Env Entomol*. 1996;25:283–294.
22. Guerreri E, Noyes J. Revision of European species of genus Metaphycus Mercet (Hymenoptera: Encyrtidae) parasitoids of scale insects (Homoptera: Coccoidea). *Syst Entomol*. 2000;25:147–222.
23. Stauffer S. Metaphycus alberti Hymenoptera: Encyrtidae. https://biocontrol.entomology.cornell.edu/parasitoids/metaphycus.php. Up-dated 2003.
24. Kapranas A, Hardy ICW, Morse JG, Luck RF. Parasitoid developmental mortality in the field: patterns, causes and consequences for sex ratio and virginity. *J Anim Ecol*. 2011;80:192–203. doi:10.1111/j.1365-2656.2010.01767.x.
25. Dransfield R. Aspect of host-parasitoid interactions of two aphid parasitoids, *Aphidius urticae* (Haliday) and *Aphidius uzbeckistanicus* (Luzhetski) (Hymenoptera, Aphidiidae). *Ecol Entomol*. 1979;4:307–316.
26. Kapranas A, Pacheco P, Forster LD, Morse JG, Luck RF. Precise sex ratios manifested by several encyrtid parasitoids (Hymenoptera: Encyrtidae) of brown soft scale, *Coccus hesperidum* L. (Hemiptera: Coccidae). *Behav Ecol Sociobiol*. 2008;62:901–912.
27. Zhang FP, Zhu JH, Li L, et al. Effect of host size on the oviposition selection and reproduction of *Metaphycus parasaissetiae*. *J Env Entomol*. 2017;30:1130–1134.
28. Gordh G, Legner EF, Caltagirone L. *Biology of Parasitic Hymenoptera*. San Jose, CA: Academy Press; 1999.
29. Etzel LK, Legner EF. Culture and colonization. In:Fisher TW, Huffaker CB, eds. *Handbook of Biological Control*. San Jose, CA: Academy Press; 1999: 125–176.
30. Tena A, Kapranas A, Walker GP, Garcia-Mari F, Luck RF. Larval morphology of *Metaphycus flavus* and the role of host attachment in larval cannibalism. *Bull Entomol Res*. 2011;101:365–372.
31. Blumberg D. Seasonal varations in the encapsulation of eggs of the encyrtid parasitoid *Metaphycus stanleyi* by pyriform scale, *Protopulvinaria pyriformis*. *Entomol Exp Appl*. 1991;58:231–237.
32. Kapranas A, Federici BA, Luck RF, Johnson RF. Cellular immune response of brown soft scale *Coccus hesperidum* L. (Hemiptera: Coccidae) to eggs of *Metaphycus luteolus* Timberlake (Hymenoptera: Encyrtidae). *Biol Control*. 2009;48:1–5.
33. Chirinos DT, Geraud-Pouey F, Caltagirone L. Encapsulación de *Metaphycus* sp. (Hymenoptera: Encyrtidae) por su hospedero, Capulinia sp. (Hemiptera: Eriococcidae). *Entomotropica*. 2006;21:91–103.
34. Kapranas A, Tena A, Luck RF. Dynamic virulence in a parasitoid wasp: the influence of clutch size and sequential oviposition on egg encapsulation. *Anim Behav*. 2012;83:833–838.