

New host record for *Psyllaephagus pulchellus* (Mercet, 1921) (Hymenoptera, Encyrtidae) as a parasitoid of *Euphyllura olivina* (Costa, 1839) (Hemiptera, Liviidae), in Spain

Nuevo registro de *Psyllaephagus pulchellus* (Mercet, 1921) (Hymenoptera, Encyrtidae) como parasitoide de *Euphyllura olivina* (Costa, 1839) (Hemiptera, Liviidae), en España

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ABSTRACT

The “olive psyllid”, *Euphyllura olivina* (Costa) (Hemiptera, Liviidae), is for the first time reported as a host of *Psyllaephagus pulchellus* (Mercet) (Hymenoptera, Encyrtidae) in Spain. This is also the first host record for this nymphal parasitoid. To facilitate its recognition, brief taxonomic notes and illustrations of both sexes of *P. pulchellus* are provided. Prior to this work, the male of this species was previously unknown, as were data on the bionomics of *P. pulchellus*, which are reported here from studies conducted under quarantine laboratory conditions.

Key words: Olive, biological control, new host, *Euphyllura olivina*, *Psyllaephagus pulchellus*, Liviidae, Encyrtidae.

RESUMEN

Se menciona por primera vez al “algodoncillo del olivo”: *Euphyllura olivina* (Costa) (Hemiptera, Liviidae) como hospedador de *Psyllaephagus pulchellus* (Mercet) (Hymenoptera, Encyrtidae) en España. Éste es también el primer registro de huésped fiable para la ninfa del parasitoide. Para facilitar su reconocimiento, se proporcionan breves notas taxonómicas e ilustraciones de *P. pulchellus* para ambos sexos. Con anterioridad a este estudio, el macho de esta especie era desconocido, al igual que los datos sobre la bionomía de *P. pulchellus* bajo condiciones de cuarentena en laboratorio.

Palabras clave: Olivo, control biológico, nuevo hospedador, *Euphyllura olivina*, *Psyllaephagus pulchellus*, Liviidae, Encyrtidae.

INTRODUCTION

The *Euphyllura olivina* (Costa, 1839) (Hemiptera, Liviidae), can be a serious pest of olive trees in its native range, the Mediterranean Basin (JARDAK, 1984, TZANAKAKIS, 2006). First reported in California, USA in 2007, the “olive psyllid” has spread throughout the southern region of this state. *Psyllaephagus euphyllurae* (Masi, 1911) (Hymenoptera, Encyrtidae) is the dominant primary parasitoid reared from “olive psyllid” in southern Europe (AVERSENQ, 2005, TRIAPITSYN *et al.*, 2014). It is currently under testing to establish its host range and specificity, information required by the federal and state regulatory agencies in the USA in order to obtain a field release permit for use in a classical biological control program targeting *E. olivina*. During collections for *P. euphyllurae* in Spain in 2014, a second parasitoid, *Psyllaephagus pulchellus* (Mercet, 1921), was found parasitizing “olive psyllid”, though in low numbers. It was originally placed in the genus *Metaprionomitus* Mercet, 1921 (MERCET, 1921), but was later reclassified to *Psyllaephagus* Ashmead, 1900 when *Metaprionomitus* was transferred to *Psyllaephagus* (TRJAPITZIN 1967). The previous host record indicated *P. pulchellus* was a parasitoid of *Anapulvinaria pistaciae* (Bodenheimer, 1926) (Hemiptera, Coccidae) (BOUČEK, 1977), which was clearly incorrect as *Psyllaephagus* spp. are parasitoids of Psylloidea. We report here on field collections, and behavior, and parasitoid development under quarantine conditions for *P. pulchellus* reared from *E. olivina* in Spain.

MATERIAL AND METHODS

Collection methods

Parasitized *E. olivina* nymphs were collected in Spain during the first two weeks of May in 2013 and 2014 when high numbers of psyllids were on the olive trees (C.H. PICKETT, unpublished data). All collections came from the provinces of Catalonia, Valencia and Murcia. Primarily parasitized (i.e., mummified) hosts were collected from olive trees growing in abandoned or non-commercial orchards; two collections came from commercial orchards and one of these was pesticide free. Mummified nymphs were easily distinguished from apparently healthy psyllids by a melanized exoskeleton. Some late instar nymphs that appeared to be parasitized (clear to grey in color, rather than light green) were also collected. One to three nymphs or mummies were placed in 0.5 dram glass vials plugged with cotton. Honey was added to each glass vial either as a drop to the top of the vial, or as a streak on a small piece of wax paper that was inserted into the vial. Most mummies and nymphs on 0.5 to 1.5 cm stem of olive tree were placed into vials along. Parasitized nymphs were returned under appropriate permits to the University of California, Riverside (UCR) Quarantine and Insectary Facility, where emerged parasitoids were sorted by morphospecies, identified taxonomically, and cultured on *E. olivina* infesting olive seedlings. Parasitoids that emerged from mummies collected in Spain were used in studies investigating the bionomics of *P. pulchellus*.

Material

The origins of *P. pulchellus* collected in 2014 are provided in Table 1. The two dry-mounted *P. pulchellus* specimens used for the auto-montage imaging (Figs. 1-2) and the two specimens used for the slide mounts (Figs. 3-5) originated from a site near Jumilla, Murcia. *Psyllaephagus pulchellus* from Catalonia (four females) and 13 females and eight males from Murcia were observed for daily longevity in quarantine. Female *P. pulchellus* from Catalonia and Murcia were exposed to *E. olivina* and observed for oviposition behavior (see below for details) (Table 2 and Fig. 6).

All voucher specimens from this study were preserved in 95% ethanol and deposited in the Entomology Research Museum, University of California, Riverside, California, USA (UCRC). Specimens used for slide and point-mount vouchers have the accession numbers: 4BA4 - UCRC ENT 257480, 4BA9 - UCRC ENT 147091, 4BA6 - UCRC ENT 257284, 4BA8 - UCRC ENT 72778.

Table 1. *Psyllaephagus pulchellus* collected in 2013 and 2014 from various locations in Spain.**Tabla 1.** *Psyllaephagus pulchellus* recogidos en 2013 y 2014 de diversas localidades en España.

LOCATION	COORDINATES	COLLECTION YEAR			
		2013		2014	
Catalonia: near Amposta	40.66213°N 00.58365°E	Females	- ¹	Females	0
		Males	-	Males	0
Catalonia: Sant Carles de la Ràpita	40.63309°N 00.59722°E	Females	0	Females	1
		Males	0	Males	0
Catalonia: Alcanar, near Montsià Mar	40.59252°N 00.55462°E	Females	0	Females	-
		Males	0	Males	-
Catalonia: Alcanar, near Montsià Mar	40.57948°N 00.55229°E	Females	0	Females	7
		Males	0	Males	0
Catalonia: Les Cases d'Alcanar	40.56314°N 00.53565°E	Females	0	Females	-
		Males	0	Males	-
Catalonia: Les Cases d'Alcanar	40.56043°N 00.53147°E	Females	0	Females	-
		Males	0	Males	-
Valencia: Traiguera, near San Rafael del Río	40.57850°N 00.34303°E	Females	-	Females	0
		Males	-	Males	0
Valencia: near Traiguera	40.54241°N 00.31494°E	Females	-	Females	0
		Males	-	Males	0
Valencia: Vinaròs, near Alcanar	40.53234°N 00.45688°E	Females	-	Females	0
		Males	-	Males	0
Valencia: La Jana	40.51051°N 00.23676°E	Females	-	Females	1(1) ²
		Males	-	Males	0
Valencia: La Jana	40.49635°N 00.23692°E	Females	0	Females	-
		Males	0	Males	-

Murcia: Jumilla	38.39974°N 01.38686°W	Females	-	Females	7
		Males	-	Males	5
Murcia: Ricote, near Cañada Gil	38.16377°N 01.38952°W	Females	-	Females	0
		Males	-	Males	0
Murcia: Murcia, Huerto de Los Cipreses	37.98213°N 01.13886°W	Females	-	Females	0
		Males	-	Males	0
Murcia: near Cañada de Gallego	37.56890°N 01.47208°W	Females	-	Females	5
		Males	-	Males	0
Murcia: Murcia, near Puntas de Calnegre	37.52682°N 01.43660°W	Females	-	Females	5
		Males	-	Males	7

1. Dash indicates the location was not sampled in the given year.
2. The parentheses indicate that one specimen from that location could not be sexed.



Fig. 1. Female *P. pulchellus*, habitus (Jumilla, Murcia, Spain).

Fig. 1. Hábito general de la hembra de *P. pulchellus* (Jumilla, Murcia, España).

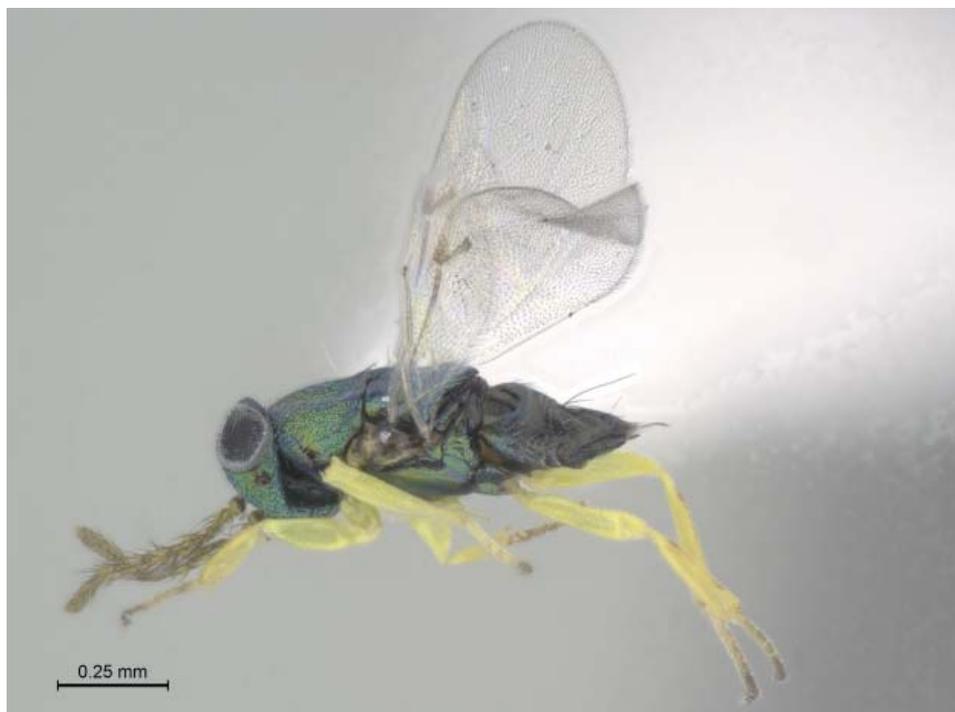


Fig. 2. Male *P. pulchellus*, habitus (Jumilla, Murcia, Spain).

Fig. 2. Hábito general del macho de *P. pulchellus* (Jumilla, Murcia, España).

Storage of P. pulchellus

From emergence until death, except during copulation and psyllid exposure, parasitoids were stored individually in unventilated 2 ml conical plastic vials (T332-6, Simport® Scientific Inc., QC J3G 4S5, Canada) in an incubation chamber at 13.5-14.8 °C with 14 light/10 dark photoperiod. Parasitoids were provided diluted clover honey, streaked vertically on the side of the vial. Honey was replenished as needed. *P. pulchellus* generally rested near the top or bottom of the vials; therefore, vials were held vertically, cap-side up, in order to minimize contact of the resting individual with honey streaks. Vials were ventilated during honey replenishment.

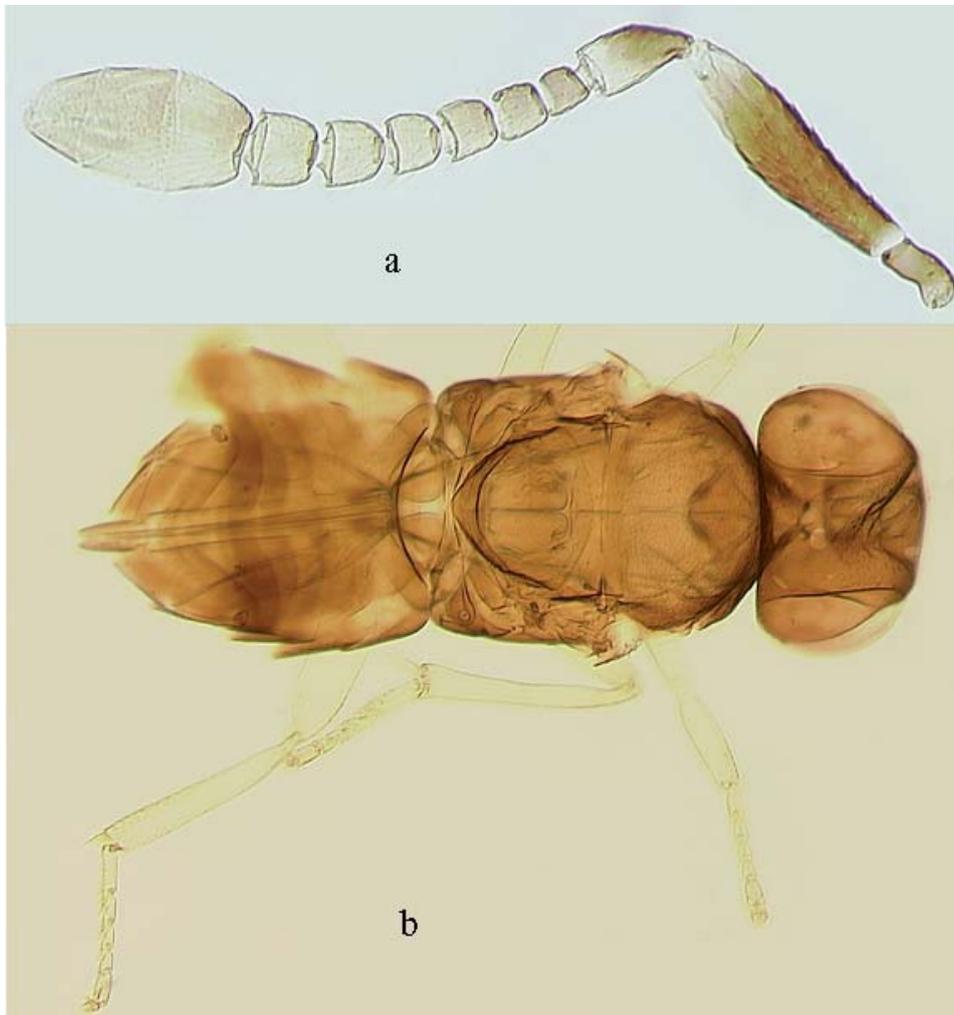


Fig. 3. Female *P. pulchellus* (Jumilla, Murcia, Spain). 3a: antenna; 3b: body.

Fig. 3. Hembra de *P. pulchellus* (Jumilla, Murcia, España). 3a: detalle de la antena; 3b: vista general.



Fig. 4. Female *P. pulchellus* (Jumilla, Murcia, Spain): forewing and hindwing

Fig. 4. Hembra de *P. pulchellus* (Jumilla, Murcia, España). Alas anteriores y posteriores.

Table 2. Longevity of *P. pulchellus* adults (days) and F1 males, emerging from *E. olivina* nymphs collected in Spain, May 2014.

Table 2. Longevidad en días, de los adultos de *P. pulchellus* y de los machos de la F1, emergidos de *E. olivina* recogidos en España, en mayo de 2014.

Group	LONGEVITY (DAYS)				
	n	Mean	St. dev.	Median	Range
Female	17	90.824	31.103	101	39 - 143
Males	8	36.625	11.722	36	23 - 58
F1 Males	10	45.7	11.295	47.5	33 - 67

Exposure of P. pulchellus to E. olivina

Small olive trees between eight and eleven cm in height were grown in Cone-tainers, (SC7 Stubby Stuewe and Sons Inc., Portland, OR, USA). Foam material covered the soil in Cone-tainers in order to prevent psyllids



Fig. 5. Male *P. pulchellus* (Jumilla, Murcia, Spain): 5a, antenna; 5b, fore wing; 5c, genitalia.
Fig. 5. Macho de *P. pulchellus* (Jumilla, Murcia, España). 5a: antena; 5b: ala anterior; 5c: genitalia.



Fig. 6. *P. pulchellus* parasitism of olive psyllid.

Fig. 6. *P. pulchellus* parasitando al psílido del olivo.

and parasitoids from contacting the soil and to prevent emergence of soil borne insects such as fungus gnats. Trials exposing parasitoids to psyllid hosts were conducted in two types of cage. One cage type consisted of a 50-dram (Thornton Plastic Co., Salt Lake City, UT, USA) ventilated vial fitted with a lid, center removed. The Cone-tainer supporting the olive seedling was placed through the hole cut in the vial's lid which was then snapped onto the plastic vial. The other type of cage was constructed from two 15.24 cm x 15.24 cm x 15.24 cm acrylic risers (SW Plastics F2191, Riverside, CA, USA) taped together; one side was closed with No-See-Um® (Skeeta, Bradenton, FL) netting and the other side was fitted with a sleeve of No-See-Um® netting and glued. Prior to placement of test insects inside cages, female parasitoids were exposed to males in a 2 ml conical plastic vials for 24 hours, after which females were assumed to be mated after exposure to males, and were presented with 2nd-5th instar *E. olivina* nymphs for parasitization.

Time lapse photography was used to record parasitoid-psyllid nymph interaction behaviors. A Lumix DMC-LX7, (Panasonic Co. Kadoma, Osaka, Japan) camera, attached to a tripod, was placed outside of a sleeved cage housing the experimental arena. Images were taken of insects placed inside

the arena, aided by a 4x magnifying lens (OptiVISOR Donegan Optical Co.) attached to the acrylic side of the cage. Photography utilized varying combinations of zoom and manual focus on the densest clusters of *E. olivina* nymphs and magnification was assisted by the magnifying lens through which insects were photographed. Time-lapse imaging was performed for five different exposures, capturing each image at intervals of either two or three minutes. Total exposure time of parasitoids to *E. olivina* varied between 4 hours and 33 minutes and 21 hours. During each of five recordings, *P. pulchellus* females were exposed to 20-30 2nd-5th instar *E. olivina* nymphs. Two of these recordings involved the same female, who had been exposed to *E. olivina* once prior to initial imaging; the other three recordings involved three individual naive females. All time-lapse photography was conducted at 22.8 °C, 40-55% RH and 14L/10D photoperiod.

Exposures to hosts not subjected to time-lapse photography occurred in either the sleeved cages or the 50-dram chambers, with 40-55% RH, 14L/10D photoperiod. Two naive females were exposed to eight to ten 2nd-5th instar *E. olivina* nymphs at 32 °C in separate 50-dram chambers for four hours. Two naive females were exposed to 10 and 20-25 2nd-5th instar *E. olivina* nymphs at 22.8 °C for 5 hours 30 minutes in a 50-dram chamber and 14 hours in a sleeved cage, respectively. One female, whose first exposure to *E. olivina* was recorded using time-lapse, was exposed to 20-25 2nd-5th instar *E. olivina* nymphs at 22.8 °C in a sleeved cage for 24 hours with a male *P. pulchellus*. This female was exposed to *E. olivina* nymphs two more times at 20.5 °C to six 2nd-4th instar nymphs for 41 hours then for four hours, both at 22.8 °C in sleeved cages.

Due to the nature of time-lapse photography, duration of contacts was approximated. Estimated contact time was determined by the time in between the first and last recordings, which was based upon the time-lapse recording interval between images. However, due to the innate variance of time-lapse photography caused by the recording interval, one extra minute is added to the total duration, 30 seconds for before the first picture and 30 seconds for after the last picture. Therefore, the equation for estimated contact duration is $x*(n-1)+1$; where n is the number of images recording the contact and x is the interval time of the time-lapse. For example, for a contact event, which is recorded with 2 images and the time-lapse recording at 2 minute intervals, the total estimated contact time between parasitoid and psyllid nymphs is 3 minutes.

RESULTS

Notes on taxonomy and distribution of P. pulchellus

Metaprionomitus pulchellus Mercet 1921: 266-267. Type locality: Madrid, Spain.

Psyllaephagus pulchellus (Mercet, 1921). NOYES, 1981: 178 (lectotype designation).

Material examined: Spain, Murcia, near Jumilla, 38°23'59"N 1°23'13"W, 411 m, 10.v.2014, C.H. Pickett (emerged 22.v.2014 in UCR Quarantine from *E. olivina*, J.M.L. Jones) [2 females, 2 males, 4BA4 - UCRC ENT 257480, 4BA9 - UCRC ENT 147091, 4BA6 - UCRC ENT 257284, 4BA8 - UCRC ENT 72778].

Distribution. Croatia, Greece, Montenegro, Spain, and Turkey (NOYES, 2014).

Host. *Euphyllura olivina* (new record).

Comments. The type material of this species was collected on white poplar, *Populus alba* (MERCET, 1921) in Madrid, Spain.

Taxonomic notes. To facilitate recognition of this little known species, illustrated here are the female (Figs. 1, 3, 4) and male (Figs. 2, 5); the latter sex of *P. pulchellus* was previously unknown. The body length of the critical point dried female is 0.93 mm, and that of the male is 0.89 mm.

Host association and bionomics of P. pulchellus

Of the seven naive females of *P. pulchellus* exposed to *E. olivina* nymphs, two successfully parasitized their hosts. The first female parasitized one *E. olivina* (producing one male), but died within the 20 hour exposure duration. The second female parasitized three *E. olivina* nymphs producing one male and two individuals that emerged, but were not recovered (based on exit holes in mummies). This female was exposed to *E. olivina* two additional times and successfully parasitized nymphs in both instances. Of these two subsequent exposures to *E. olivina*, the first resulted in seven males; the second resulted in one male and three other adults that escaped detection (based on exit holes in mummies). (Figs. 7 and 8). Five females did not parasitize any *E. olivina* nymphs, including one female that was exposed to *E. olivina* four times. All successful parasitism occurred in sleeve cages at 22.8 °C and all 10 recovered first generation progeny (F1) produced by two females were males.



Fig. 7. *P. pulchellus* F1 mummy casing.

Fig. 7. *P. pulchellus* F1 exuvia de la crisálida.



Fig. 8. *P. pulchellus* F1 mummy casing - emerged from gaster.

Fig. 8. *P. pulchellus* F1 exuvia de la crisálida. Se aprecia el orificio de emergencia en el gaster.

In the time-lapse recording, three of the five replicates showed *P. pulchellus* contacting *E. olivina*: one replicate recorded only one contact and the other two recorded multiple contacts. The single contact lasted more than 30 minutes and produced one male offspring. Of the two replicates with multiple contacts, one made 16 contacts that ranged from an estimated one minute to eleven minutes with a mean \pm SEM contact time of 2.38 ± 0.70 minutes. These encounters produced seven male *P. pulchellus*. The other replicate made nine contacts ranging from one to 25 minutes with mean \pm SEM contact time of 9.67 ± 3.22 minutes. This resulted in the production of four mummies (Figs. 7, 8), of which one male was recovered and three emerged but were not found. Both of these replicates involved the same female *P. pulchellus*.

Casual observations noted that *P. pulchellus* will “squeeze” through tight spaces – such as the gap between the foam soil cover and the olive stem. Depending on the individual’s size, parasitoids can become “lost/trapped” in the foam cover or under the foam soil cover in the soil. These incidences were the most probable cause of individuals not being recovered.

Longevity records indicated that females live longest and F1 males lived longer than the parent (F0) males (Fig. 9).

Of the eleven recovered F1 males, development time in the host ranged from 22 to 34 days with a mean of 26.6 ± 1.01 days and a median of 26 days.

DISCUSSION

P. pulchellus can act as a primary parasitoid of *E. olivina*. Exposure trials in quarantine demonstrated that two of seven female *P. pulchellus* successfully reproduced on *E. olivina*. According to RIEK (1962), two Australian species of *Psyllaephagus*, *P. faustus* Riek, 1962 and *P. clarus* Riek, 1962, are hyperparasitoids of other *Psyllaephagus* species which are primary parasitoids of psyllids. *Psyllaephagus faustus* was later confirmed as a hyperparasitoid of other *Psyllaephagus* species (DAHLSTEN *et al.*, 2003). However, these instances of observed hyperparasitism behavior by *P. faustus* were not distinguished between obligatory or facultative hyperparasitism. Additional investigations are required to determine if *P. pulchellus* is a facultative hyperparasitoid or an obligate primary parasitoid.

One possibility for low levels of reproduction during this study and the production of male offspring is that *E. olivina* may not be the preferred host of *P. pulchellus*. The lack of recovered females could also be due to unsuccessful copulation as no mating events were observed during the 24 hr

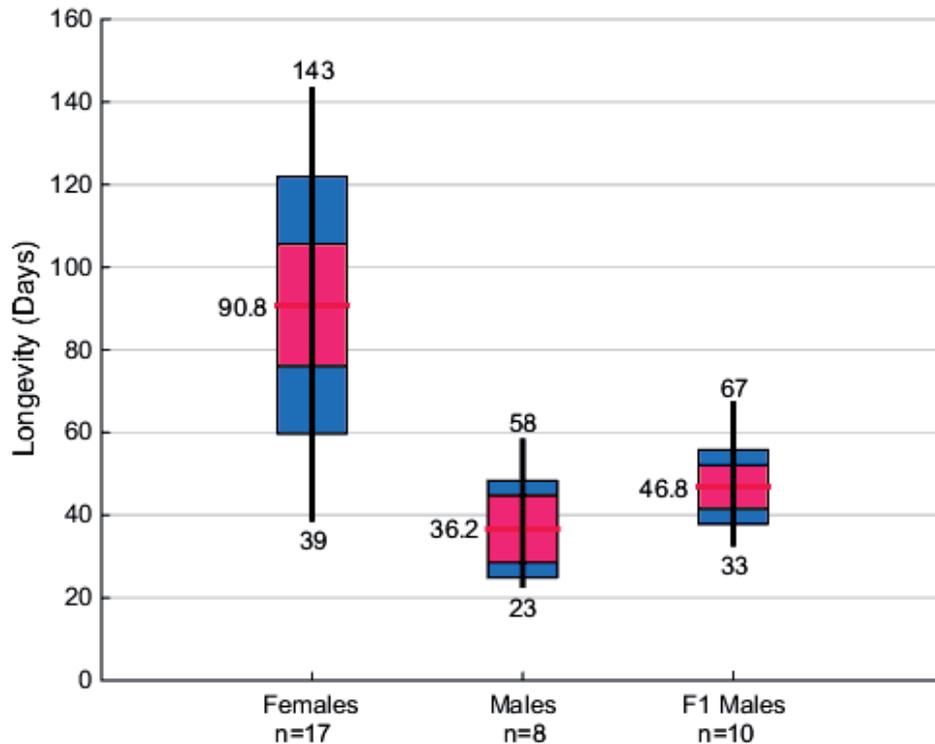


Fig. 9. Box plot of longevity of *P. pulchellus* females, males and F1 males. The center horizontal line represents the mean, the first boxes from the mean represent the 95% confidence interval, the outer box represents the standard deviation, and the vertical line represents the data range. Values shown are the mean and range of each group: n=sample size of the group.

Fig. 9. Representación de la longevidad de las hembras, machos y machos de la F1 de *P. pulchellus*. La línea horizontal central representa la media, el primer rectángulo representa el 95% del intervalo de confianza, los otros rectángulos representan la desviación estándar y la línea vertical el rango de variación de cada dato. n= número de individuos de cada grupo

exposure period. Alternatively, parent age could be a factor affecting offspring sex ratios – KANT *et al.* (2013) found that the ability of *Diaeretiella rapae* (McIntosh, 1855) which was mated once to produce females and oviposit decreased with the age of the female parasitoid. There is also the possibility of host preference affecting the sex ratio. In both *Metaphycus flavus* (Howard, 1881) and *M. stanleyi* Compere, 1940 (Hymenoptera: Encyrtidae), solitary mated females produced more females in larger hosts (BERNAL *et al.*, 1999). This is also seen in *Pachycrepoideus vindemiae* (Rondani, 1875), which is a facultative hyperparasitoid of several other parasitoids (ALPHEN & THUNISSEN, 1983). Alternatively, female parasitoids may need larger hosts to produce males, as observed in *Macrocentrus ancylivorus* (Rohwer,

1923) (FINNEY *et al.*, 1947). However, based on causal comparisons, F1 *P. pulchellus* males varied greatly in size despite mummies being similar in size. DAANE *et al.* (2005) found that *Psyllaephagus bliteus* Riek, 1962 larvae delayed their development until the host was near or in the 5th instar. Alternatively, *P. pulchellus* may be very specific to a particular psyllid instar which were not always available. In both cases, male size likely could have been dependant on host quality. Regardless, observations on the full range of host sizes encountered and used for oviposition was not possible through the time-lapse photographs, since some contacts occurred out of focus or partially out of view. Host feeding was not observed; either *P. pulchellus* does not host feed or host feeding was not recorded under time-lapse photography. The two female parasitoids that successfully reproduced, parasitized olive psyllid nymphs on the first contact - one parasitized in the two subsequent exposures and the other died during the first exposure, which suggests that *P. pulchellus* may not need to host feed to mature eggs.

Contact duration between *P. pulchellus* and psyllid nymphs varied from approximately two to ten minutes. In *Psyllaephagus*, contact duration can increase with nymph psyllid size (DAANE *et al.*, 2005; MEHRNEJAD & COPLAND, 2006). *P. bliteus* contacted fourth and fifth instar nymphs on average for around four minutes versus just over one minute on earlier instars. There was no successful parasitism from contact with fifth instar nymphs (DAANE *et al.*, 2005). *Psyllaephagus pistaciae* Ferrière, 1961 contacted fifth instar nymphs for about two minutes versus approximately one minute on earlier instar nymphs and the successful parasitism rate was not significantly different between instars (MEHRNEJAD & COPLAND, 2006). Host feeding contacts were significantly longer than oviposition contacts, by up to ten minutes in *P. bliteus* (DAANE *et al.*, 2005) and two to seven minutes in *P. yaseeni* Noyes, 1990 (PATIL *et al.*, 1993). Since the nature of host contacts (i.e., oviposition or host feeding) is unclear in this study, and only two contacts are compared (both by the same female), we cannot make any definite conclusions about the contact behavior of *P. pulchellus* with *E. olivina*. This is an area that also needs additional investigation.

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