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# Quality parameters and adaptation of *Muscidifuraxraptorellus* (Hymenoptera: Pteromalidae) against dipteran pests harmful to livestock and cultivated plants

L. DE PEDRO ET AL.

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[AQ0]

[AQ3]  Luis de Pedro<sup>a,bc</sup>, José Tormos<sup>a</sup>, Ángela María Guzmán<sup>d</sup>, Bernat Peris<sup>d</sup> and Francisco Beitia<sup>b</sup>

<sup>a</sup>. Universidad de Salamanca, Facultad de Biología, Unidad de Zoología, Salamanca, Spain;

<sup>b</sup>. Instituto Valenciano de Investigaciones Agrarias, Unidad Asociada de Entomología IVIA/CIB-CSIC. Apartado Oficial, Montcada, Valencia, Spain;

[AQ1]

<sup>c</sup>. Present address: Departamento de Protección de Cultivos, Control Biológico y Servicios Ecosistémicos, Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Las Torres (Murcia), Spain;

<sup>d</sup>. Departamento de Ciencia Animal, E.T.S de Ingeniería Agronómica y del Medio Natural, Universidad Politécnica de Valencia. Apartado Oficial, Valencia, Spain

**CONTACT** Luis de Pedro [ldepedro@usal.es](mailto:ldepedro@usal.es) Área de Zoología, Facultad de Biología, Universidad de Salamanca, 37007 Salamanca, Spain

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## ABSTRACT

*Muscidifuraxraptorellus* is a parasitoid which is commonly used in the biological control of harmful dipterans in stables. In this study, the adaptation of *M. raptorellus* to different species of dipteran hosts—detrimental to both agriculture (Diptera: Tephritidae) and livestock (Diptera: Muscidae)—as well as several quality parameters of this commercial parasitoid was assessed under laboratory conditions. Our results showed a higher parasitism rate of *M. raptorellus* on muscid than on tephritid hosts and a significant effect of the natal host on *M. raptorellus* female longevity and fertility; however, despite this and the innate preference for muscid hosts, *M. raptorellus* showed similar fecundity, fertility and sex ratios when reared on pupae of *Ceratitiscapitata* and on muscid pupae. Furthermore, both fertility and the sex ratio of commercially available *M. raptorellus* individuals showed values within an acceptable range of quality. Our results suggest that *M. raptorellus* could be recommended as a natural enemy in biological control programmes against dipteran pests harmful both to livestock and cultivated plants.

**Keywords:** *Muscidifuraxraptorellus*; dipteran pest; parasitism; quality parameters; biological control

## 1. Introduction

Currently, external parasites are considered one of the major threats to livestock activity. The high population levels shown by many of these organisms may lead to a decrease in animal production. Among the external parasites that are known to affect livestock, we should highlight Cyclorrhaphous Diptera, since they can reach very high densities in warm periods of the year, causing discomfort and stress to animals and workers and even carrying bacteria and viru-

ses that may result in decreased productivity and performance in farm animals (López and Molina, 2005; Chakrabarti et al., 2008; Wanaratana et al., 2013). Some of these dipteran species, such as the muscids *Stomoxys calcitrans* (Linnaeus) (stable fly), *Muscadomestica* (Linnaeus) (house fly) and *Muscina stabulans* (Linnaeus) (false stable fly), are reported to be especially harmful to livestock farming (Marchiori and Silva, 2001).

Traditionally, farmers have used insecticides in the fight against these insect species. However, flies have become resistant to many of these products; this fact, together with the risk posed by the insecticides in animal products and manure, has led to the implementation of biological control through parasitoids (Skovgård, 2006; Birkemoe et al., 2009). Most of these biocontrol agents parasitize dipteran pupae, being included within the family Pteromalidae and, more specifically, within genera such as *Pachycrepoideus* Ashmead, *Spalangia* Latreille or *Muscidifurax* Girault & Sanders (Ortiz and Torres, 1983; Zamora, 1996). In particular, three species of *Muscidifurax* are currently considered as especially relevant from the point of view of biological control: *Muscidifurax raptor* (Girault and Sanders), *M. raptor* (Kogan and Legner) and *M. raptorellus* (Kogan and Legner).

The latter is a recognized gregarious parasitoid of muscid pupae, with a high parasitism rate on muscid and tephritid hosts and with suitable fertility and sex ratio levels for its use in biological control projects, which is currently used in America and some European countries, such as Norway and Denmark, for the control of dipterans in animal production systems in confinement (Skovgård and Jespersen, 2000). Meanwhile, in Spain, the application of these biological control strategies is in its initial phase of implementation, obtaining promising results.

The acceptable results shown by *M. raptorellus* when used as a biocontrol agent, through inundative releases, against dipteran pests of cattle (Antolin et al., 1996; Zamora, 1996; Floate et al. 2000; Lysyk, 2001; Inciso and Iannaccone 2008) as well as against tephritids such as the Mediterranean fruit fly *Ceratitiscapitata* (Edwards) (also known as the medfly, which is a highly polyphagous and multivoltine species that shows a high fecundity being currently a key pest in Spanish agriculture) on grapevine (Kapongo et al. 2007), together with the lack of studies on the quality parameters involved in laboratory rearing of this parasitoid, have led us to carry out the present study.

The overall goal was to determine the versatility of this parasitoid in order that it may be used as an agent of biological control against different dipterous pests. For this purpose, we performed several experiments with the aim of (a) determining the quality of commercially available individuals of *M. raptorellus*, by assessing their mortality (=percentage of closed puparia, i.e., pupae that do not produce a fly or parasitoids) and sex ratio; (b) establishing a laboratory rearing of *M. raptorellus* on *C. capitata* in order to assess the possible benefits of rearing this parasitoid on this fruit fly; and (c) assessing whether offspring production and sex ratio of *M. raptorellus* change depending on the natal host species, to determine its adaptability to different fly species.

## 2. Materials and methods

### 2.1. Study Centre and insects

All experiments were performed in compliance with current Spanish law. This study was carried out at the Valencian Institute of Agricultural Research (IVIA, Moncada, Valencia, Spain). Four insect species were used throughout the experiments: *Muscina stabulans*, *Muscadomestica*, *Ceratitiscapitata* (considered as dipterous pests) and *Muscidifurax raptorellus* (considered as a hymenopteran biocontrol agent), which is the main object of this study.

#### 2.1.1. Parasitoids

*Muscidifurax raptorellus* specimens were provided by Bioteknia Servicios Ambientales (Valencia, Spain; <http://www.bioteknia.es>) and came from different commercial shipments (i.e., received at different times) of the insect (parasitized pupae of an undetermined muscid species) produced by Koppert Biological Systems (Berkel en Rodenrijs, Netherlands; <https://www.koppert.nl>), which is focused on protecting ecosystems by using biological control and other methods to fight pests. Between 24 and 48 h after delivery from Bioteknia Servicios Ambientales, adult individuals of *M. raptorellus* began to emerge from the imported pupae in the facilities of the IVIA at 21–24 °C, 55%–80% relative humidity and a 16:8 h (L:D) photoperiod.

#### 2.1.2. Host pests

*Muscina stabulans* last-instar larvae were collected from mid-April to early July on the experimental goat farms of the Polytechnic University of Valencia. Immediately after collection, they were moved to the IVIA facilities, where

they were kept under the above-mentioned environmental conditions to allow pupation in 2 or 3 days (origin: experimental goat farms of the Polytechnic University of Valencia; rearing methods: collected in the farm stables; stage or age tested: pupae).

*Musca domestica* larvae were collected on the bull farm ‘Ganadería Raúl Monferrer’, located in the province of Teruel (Spain), and moved to the Polytechnic University of Valencia facilities where, together with organic matter and under environmental conditions similar to those previously mentioned for IVIA, they were allowed to develop for 4 days until pupation; 3 days after pupation, they were used for the assays (origin: bull farm ‘Ganadería Raúl Monferrer’; rearing methods: collected in the farm stables; stage or age tested: pupae).

*Ceratitis capitata* pupae were obtained from their regular rearing location in the IVIA, where this species has been semi-massively reared for more than 8 years using the method of Pérez-Hinarejos and Beitia (2008) and under the above-mentioned environmental conditions (origin: regular rearing location in the IVIA; rearing methods: mass rearing with the following rearing conditions:  $27 \pm 2$  °C,  $65 \pm 10\%$  RH and 16:8 (L:D) photoperiod; stage or age tested: pupae).

## 2.2. Experimental design

Three experiments were conducted along the present study.

### 2.2.1. Experiment 1. Quality parameters of commercially sold *M. raptorellus*

In this experiment, our goal was to assess the quality of the individuals of *M. raptorellus* produced and commercialized by Koppert Biological Systems (hereafter commercial rearing). To this end, three assays (=replicates) were performed using three different commercial shipments of *M. raptorellus*, carried out by Bioteknia Servicios Ambientales and produced by Koppert Biological Systems. Each shipment consisted of 10 plastic bottles containing parasitized fly pupae. One hundred pupae from each bottle (establishing 10 repetitions) were collected in each assay and placed inside ventilated Petri dishes (one per repetition), which were kept in a climate cabinet (Sanyo MLR350; Sartorius, Barcelona, Spain) under controlled conditions at  $24 \pm 1$  °C,  $55 \pm 5\%$  relative humidity, and a 14:10 h (light/dark) photoperiod until adult parasitoids emerged. Closed puparia were scored, thus determining the mortality rate of *M. raptorellus*.

Additionally, in each replicate, 5 pupae were collected from each of the 10 bottles mentioned above and placed individually inside ventilated plastic tubes, being kept in the same climate cabinet until parasitoid emergence in order to determine the number and sex of the parasitoids that emerged per pupa, as well as the number of closed puparia.

### 2.2.2. Experiment 2. Establishment of a *M. raptorellus* laboratory rearing using *Ceratitiscapitata* as a host

In this experiment, we aimed to assess the possibility of establishing a *M. raptorellus* laboratory rearing on *C. capitata* (hereafter IVIA rearing). *Muscidifurax raptorellus* laboratory rearing on medflies was started on April 20, 2017, in a climatic chamber under the following conditions: 23–25 °C;  $60 \pm 5\%$  relative humidity and a 16:8 h (light/dark) photoperiod. As a rearing cage, a methacrylate box (40 × 30 × 30 cm) provided with a side and an upper muslin window for ventilation was used. This cage contained water, a Petri dish filled with sugar and another Petri dish with honey smeared on blotting paper as nutritional sources.

Two Petri dishes containing *M. raptorellus* pupae received in the first shipment were introduced into the rearing cage, where parasitoids were allowed to emerge. Immediately after emergence, two dishes containing freeze-killed pupae of *C. capitata* (Tormos et al., 2014) were offered to parasitoid adults for 48–72 h, after which these pupae, probably parasitized, were collected and replaced by new *C. capitata* freeze-killed pupae. This replacement was repeated until a large amount of parasitized *C. capitata* pupae was obtained. Then, adults emerging from all these pupae were confined to the rearing cage (after old parasitoids were removed) and used as first-generation individuals to initiate laboratory rearing. Successive generations were obtained following the above-mentioned procedure and used for subsequent assays.

Furthermore, in this experiment we assessed the gregariousness and sex ratio of *M. raptorellus* on *C. capitata*. For this purpose, 50 pupae of each of the first three generations of this parasitoid were collected randomly and placed individually inside ventilated plastic tubes into the above-mentioned climate cabinet, until their emergence (18–22 days after being parasitized). As in Experiment 1, the number and sex of the parasitoids that emerged per pupa was determined.

### 2.2.3. Experiment 3. Effects of host on *M. raptorellus* fecundity, fertility and parasitism

This experiment was conducted in order to assess the offspring production and sex ratio of *M. raptorellus* on a potential host for laboratory rearing (*C. capitata*) and the dipterous host that it parasitizes in the field (*M. stabulans*). It considered 4 different cases and was performed in the above-mentioned SANYO climate cabinet, under the same conditions mentioned for Experiment 1. Translucent plastic boxes (15 × 10 × 10 cm) were used as experimental units, each provided with an upper muslin window for ventilation and containing water and honey *ad libitum* as nutritional sources.

The first three cases followed the same procedure, differing only in the origin of the parasitoids and the offered host species. These cases were the following: (a) *M. stabulans* offered to parasitoids from the commercial rearing; (b) *C. capitata* offered to parasitoids from the commercial rearing; and (c) *C. capitata* offered to parasitoids from the second generation of the IVIA rearing. In each of these cases, 20 experimental units were used. One 4-day-aged *M. raptorellus* couple was introduced into half of these units and one 11-day-aged *M. raptorellus* couple was confined to the other half, after staying together in a plastic cage for 24 h to allow feeding and mating. Thus, two different treatments for female age were established. For 24 h over 14 consecutive days, a Petri dish containing 10 live pupae of the corresponding dipterous host was placed inside each experimental unit to assess the parasitic activity of females. Every day, those pupae that had been exposed the previous day were collected and placed inside ventilated Petri dishes (one per parasitism unit) that were kept in the climate cabinet. Half of the experimental units within each age treatment was used to assess female fecundity (=number of eggs laid per female and day); thus, pupae collected from the corresponding experimental units were dissected and examined under the microscope (Leica MZ8), with the help of needles and soft tweezers (Tormos et al., 2009), to find and count parasitoid eggs inside the puparium. The other half of the experimental units was used to evaluate fertility (number of descendants produced) and parasitism (percentage of pupae producing adult parasitoids + pupae that remain closed, presumably due to parasitoid activity); thus, half of the Petri dishes were allowed to evolve, under the climate cabinet conditions, until the adult emergence of the parasitoid (emergences and closed puparia were then scored).

In the fourth case, 20 experimental units were also used. One 4-day-aged commercial rearing couple from a fourth shipment received from Bioteknia Servicios Ambientales was placed inside half of the units, and one couple of the same age from the third generation of the IVIA rearing were placed inside the other half, establishing two treatments based on parasitoid origin. All couples had remained together since their emergence. For 24 h, a Petri dish containing 10 live pupae of *M. domestica* was placed inside each experimental unit to allow parasitization by females. In this case, only *M. raptorellus* fecundity was assessed, using the same procedure as in the previous cases.

### 2.3. Statistical tests and analysis

One-way ANOVA was performed to: a) to test the percentage of closed puparia (mortality) and the number of parasitoids that emerged (fertility) per pupa depending on the shipment (Experiment 1); b) to assess the number of parasitoids that emerged per pupa, establishing comparisons among generations and between the commercial shipments and the laboratory rearing (Experiment 2), and c) to test the effect of female age and the host species on *M. raptorellus* fecundity, fertility and parasitism (Experiment 3). Additionally, a Pearson's chi-squared ( $\chi^2$ ) test was used to: a) to show any significant differences in the sex ratio between and within treatments (Experiment 1) and b) to assess the sex ratio between and within treatments but, in this case, depending on the factor generation (Experiment 2).

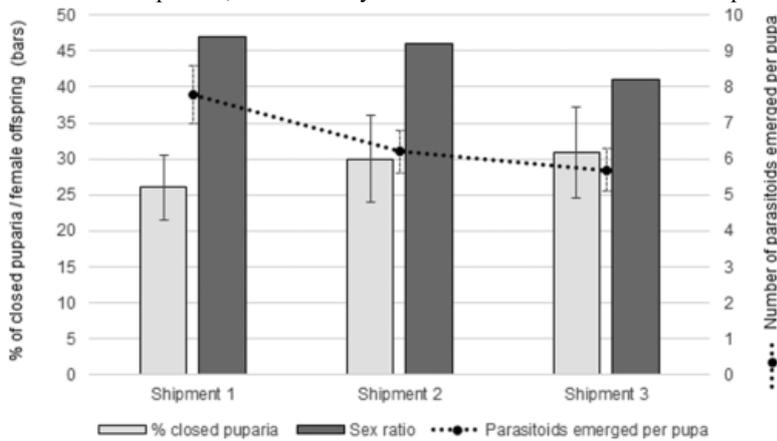
Analyses were performed using the software XLSTAT 2011 (Addinsoft) (critical  $P$  value used 0.05). All data were normally distributed. Values are reported as means  $\pm$  SE.

## 3. Results

### 3.1. Experiment 1. Quality parameters of commercially sold *M. raptorellus*

One-way ANOVA showed that for the variable % of closed puparia, with respect to factor shipment, the null hypothesis should be discarded ( $F_{2, 29} = 5.25$ ,  $P \leq 0.01$ ), although the overall percentage of closed puparia for the three shipments was about 30% (Figure 1). By contrast, one-way ANOVA did not show significant differences for the average number of parasitoids that emerged per pupa with regard to the shipment ( $F_{2, 100} = 2.47$ ,  $P = 0.08$ ) (Figure 1).

Figure 1. Percentage of closed puparia, average number of parasitoids emerged (=fertility) per pupa (both expressed as 'Mean  $\pm$  SE') and offspring sex ratio (as percentage of females produced) shown by *Muscidifurax raptorellus* from three different commercial shipments, carried out by BiotekniaServiciosAmbientales and produced by Koppert Biological Systems.

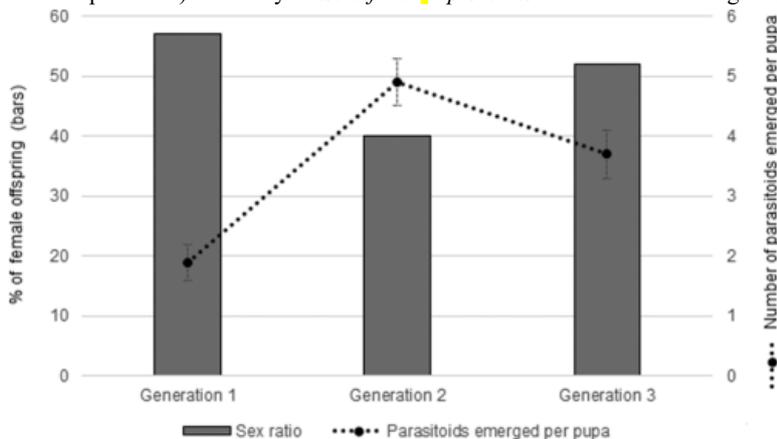


A chi-squared test showed that the sex ratio did not differ significantly among the different shipments ( $\chi^2 = 1.783$ ,  $df = 2$ ,  $P = 0.189$ ) (Figure 1). Additionally, for each shipment, we did not observe a biased sex ratio with respect to the hypothetical proportion of 1:1 (shipment 1:  $\chi^2 = 3.365$ ,  $df = 1$ ,  $P = 0.182$ ; shipment 2:  $\chi^2 = 2.483$ ,  $df = 1$ ,  $P = 0.115$ ), except in the third shipment ( $\chi^2 = 6.734$ ,  $df = 1$ ,  $P = 0.009$ ), where the sex ratio was significantly biased towards males.

### 3.2. Experiment 2. Establishment of a *M. raptorellus* laboratory rearing using *Ceratitis capitata* as a host

The proposed rearing system led to 16 generations of the parasitoid at the time this article was written, proving its effectiveness and the feasibility of using *C. capitata* as a host of *M. raptorellus*. One-way ANOVA showed that the average number of parasitoids that emerged per pupa was significantly different among the first three generations of this laboratory rearing ( $F_{2, 94} = 14.22$ ;  $P \leq 0.01$ ), being lower in the first one (Figure 2). Additionally, one-way ANOVA showed that the average number of adults that emerged per pupa was significantly different between the commercial shipments and this laboratory rearing, being higher for commercial shipments ( $F_{1, 198} = 33.49$ ,  $P \leq 0.01$ ).

Figure 2. Average number of parasitoids emerged (=fertility) per pupa (Mean  $\pm$  SE) and offspring sex ratio (as the percentage of females produced) shown by *Muscidifurax raptorellus* from the first three generations of IVIA rearing.



A chi-squared test showed significant differences in the sex ratio among the different generations ( $\chi^2 = 15.590$ ,  $df = 2$ ,  $P \leq 0.001$ ) (Figure 2). In this regard, the second generation showed a significantly male-biased sex ratio

( $\chi^2 = 14,617$ ,  $df = 1$ ,  $P \leq 0.001$ ), whilst the first and third generations showed a statistically nonsignificant female-biased sex ratio (generation 1:  $\chi^2 = 0.270$ ,  $df = 1$ ,  $P = 0.604$ ; generation 3:  $\chi^2 = 0.046$ ,  $df = 1$ ,  $P = 0.831$ ).

### 3.3. Experiment 3. Effects of host on *M. raptorellus* fecundity, fertility and parasitism

One-way ANOVA showed that the fecundity of females from commercial rearing on *M. stabulans* was significantly affected by age ( $F_{1, 18} = 5.00$ ,  $P = 0.03$ ) (Table 1). Nevertheless, the same test showed that fertility ( $F_{1, 18} = 0.27$ ,  $P = 0.60$ ) and parasitism ( $F_{1, 18} = 0.26$ ,  $P = 0.62$ ) did not vary with female age (Table 1).

Table 1. Fecundity, fertility and percentage parasitism (expressed as 'Mean  $\pm$  SE') displayed by *Muscidifurax raptorellus* females depending on host (natal and offered host species) and female age (4-day-aged vs. 11-day-aged females). For Case 1, in each row, different lowercase letter indicates a significant difference ( $p \leq 0.05$ ).

	Treatment					
	Case 1*		Case 2**		Case 3***	
	4-day	11-day	4-day	11-day	4-day	11-day
Fecundity	5.1 $\pm$ 1.1a	2.4 $\pm$ 0.4b	–	4.9 $\pm$ 1.4	4.9 $\pm$ 1.3	–
Fertility	7.6 $\pm$ 1.2a	8.8 $\pm$ 1.9a	–	2.2 $\pm$ 1.0	4.3 $\pm$ 1.3	–
% Parasitism	35.0 $\pm$ 5.2a	39.0 $\pm$ 5.9a	–	7.0 $\pm$ 2.6	15.0 $\pm$ 3.4	–

\* *M. stabulans* offered to parasitoids from the commercial rearing.

\*\* *C. capitata* offered to parasitoids from the commercial rearing.

\*\*\* *C. capitata* offered to parasitoids from the 2nd generation of the IVIA rearing.

Fecundity, fertility and parasitism of *M. raptorellus* on *C. capitata* from commercial rearing were not statistically tested since fecundity (and, consequently, fertility and parasitism) of 4-day-aged females was practically zero. The results for these variables for 11-day-aged females are shown on Table 1. Similarly, fecundity, fertility and parasitism on *C. capitata* shown by *M. raptorellus* females coming from IVIA rearing were not tested since none of them reached the 11 days of life. Table 1 shows these values for 4-day-aged females.

A global analysis of the three above-mentioned variables of *M. raptorellus* showed significant differences for fertility ( $F_{3, 36} = 4.60$ ,  $P = 0.008$ ) and parasitism ( $F_{3, 36} = 11.93$ ,  $P \leq 0.01$ ), but not for fecundity ( $F_{2,27} = 0.01$ ,  $P = 0.99$ ) among treatments. In this regard, it should be noted that fertility and parasitism in commercially reared parasitoids were higher when they parasitized *M. stabulans* vs. *C. capitata*; additionally, when *C. capitata* was offered to parasitoids, the values of these variables were higher in those previously reared on this tephritid species (Table 1).

Finally, one-way ANOVA showed that fecundity did not differ between females from commercial rearing and those from IVIA rearing ( $F_{1, 18} = 1.25$ ,  $P = 0.28$ ) when parasitizing *M. domestica* pupae (commercial rearing: range = 0–7, mean  $\pm$  SE = 2.8  $\pm$  0.9; IVIA rearing: range = 0–5, mean  $\pm$  SE = 1.6  $\pm$  0.6) (results not shown in a Table).

## 4. Discussion

In the present study, the assessment of the quality parameters of commercially sold *M. raptorellus* individuals showed that these parameters may vary according to the shipment from which the individuals come. In this regard, significant differences in the percentage of closed puparia were reported among shipments, which may affect the effective number of emerging parasitoids, as reported for other mass-reared natural enemies (Garzón-Luque and Beitia 2009). This fact could negatively affect their use in biological control programmes, hampering their establishment in crops or farms. Despite the statistical differences reported, the percentage of closed puparia was quite high (about 30%) in all cases, probably due not only to the mass rearing process itself but also to the preparation, packaging and

transport of individuals, factors which may also be responsible for the observed variability among shipments. However, the high number of emergences per pupae (due to its gregariousness) together with the offspring sex ratio reported for commercially sold *M. raptorellus* support its use in biological control programmes. Furthermore, the effectiveness of this species as a biocontrol agent may be enhanced by other phenomena (host feeding, pseudo parasitism, etc.) that may induce mortality of host pupae, as reported for other pupal parasitoids of fly pests, such as the pteromalid *Spalangia cameroni* Perkins (Pérez-Hinarejos and Beitia, 2008; de Pedro et al., 2018).

Regarding laboratory rearing of *M. raptorellus* on *C. capitata*, the significantly lower fertility per pupa observed for the first generation suggests a lack of adaptation to the host that was corrected in the following generations, which showed acceptable levels of fertility. A significant effect of natal host (muscid vs. *C. capitata*) on adult emergence per pupa has also been observed, being higher when *M. raptorellus* is reared on muscid pupae; this could be attributed to the larger size of muscid pupae, or even to other host features such as odour (King, 2002; Machtinger and Geden, 2015; Beitia et al., 2016).

The high fecundity shown by *M. raptorellus* on *M. stabulans* may be due to the large size of the pupae of this dipterous. In this case, we report a greater number of adult emergences than eggs deposited, which may be due to the phenomenon of polyembryony, usually linked to vivi parity but also common in hymenopteran parasitoids (Tormos et al., 2014). Additionally, in this host, a significant effect of female age on egg laying is reported, with 4-day-aged *M. raptorellus* females showing higher fecundity than 11-day-aged ones; however, fertility and parasitism were similar for both age treatments, leading to a similar final efficacy in the control of the host. For its part, *M. raptorellus* from commercial rearing showed a low fecundity (practically zero when they are young) on *C. capitata*. This may be considered normal since female parasitoids, when confronted for the first time with a new and smaller host than their natal host, do not perform adequate parasitic activity (Tormos et al., 2018). However, when they are offered the same host again, they can show good adaptation (Mandeville and Mullens, 1990) and display a similar fecundity to that shown on the natal host. These results concur with those of Inciso and Iannacone (2008), who, together with this learning effect, also reported decreasing oviposition capacity with age for this and other pteromalid species, such as *Spalangiaaendius* Walker. Regarding *M. raptorellus* females from the IVIA rearing, a low longevity has been observed; specifically, in the second generation of IVIA rearing, *M. raptorellus* females did not even reach 11 days of age. This may be due to the low quality of specimens obtained from *C. capitata* pupae; in this regard, it is known that smaller hosts provide less food to parasitoid larvae and, consequently, negatively affect several biological parameters of the parasitoid adults, such as adult size and longevity (de Pedro et al., 2018). Regarding adult size, it is not unusual that parasitoid females developed in larger hosts show a larger size, resulting in a greater reproductive success (Charnov et al., 1981). Furthermore, authors' observations suggest that females of *M. raptorellus* reared on *C. capitata* seem to be smaller than those reared on *M. domestica* or from *M. stabulans*. Finally, when pupae of *M. domestica* were offered to parasitoids, no influence of natal host on fecundity was observed, suggesting that this parasitoid may not need an adaptation period to a new host if it is more suitable than the natal one. In short, from the experiment on different dipteran hosts, we conclude that *M. raptorellus* shows conditioning and learning, as well as an innate preference for larger hosts since all the considered parameters (fecundity, fertility, longevity and parasitism) showed higher values when the offered pupae were bigger. Conditioning and learning, and their effect on the host preference displayed by adult females, have been previously reported for this genus and *Nasonia* (Ohgushi, 1960), but not for other pteromalids like *Spalangia* (Tormos et al., 2018). On the other hand, the innate preference for larger hosts has also been reported for other pteromalids; for example, *M. zaraptor* is known to have a preference for pupae of *M. domestica* rather than of *Fannia canicularis* (Linnaeus) (Mandeville and Mullens, 1990).

Moreover, our findings support that, despite the abovementioned innate preference for large-sized hosts and the fact that females reared on medflies show lower longevity and lower fertility before being conditioned to this tephritid species, *M. raptorellus* displays, in general, similar fecundity, fertility and sex ratio when it is reared on freeze-killed *C. capitata* pupae to those shown when it is reared on muscid pupae.

In summary, we conclude that the biological, ecological and behavioural attributes shown by *M. raptorellus* support its use in biological control programmes, especially through inundative releases, against dipteran species detrimental both to animals and cultivated plants. Additionally, after analysing of several quality parameters, we can also recommend commercially sold *M. raptorellus* individuals for this use. Finally, it should be noted that the particular connotations of this parasitoid regarding the natal host facilitate its rearing on different hosts and its subsequent use as a biocontrol agent against different pests.

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## Disclosure statement

The authors report no conflict of interest.

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## COMMENTS

C1 Author: A space is needed in the middle of most of the species names along the text, including the title: Muscidifurax raptorellus, Spalangia cameroni, Ceratitis capitata, etc. This typographical mistake has been repeated throughout the text.;

C2 Author: Present address. A space is needed.;

C3 Author: "a parasitoid". A space is needed.;

C4 Author: Ceratitis capitata. A space is needed.;

C5 Author: Muscidifurax raptorellus. A space is needed.;

C6 Author: Musca domestica.;

C7 Author: Muscina stabulans;

C8 Author: Ceratitis capitata.;

## **AUTHOR APPROVE COMMENTS**

**Author:** I have reviewed this proof, I am satisfied with my changes and I understand I will not be able to make further corrections. Please check my comments: it is important to introduce spaces in many places of the article, especially in the species names. Thank you.