

# The ant host of *Razorfemora zaragozae* and some observations of their relationships under natural conditions

Juan A. DELGADO<sup>1</sup>, R. Henry L. DISNEY<sup>2</sup>, Ricardo L. PALMA<sup>3</sup>

<sup>1</sup>Departamento de Zoología, Facultad de Biología, Universidad de Murcia, Spain

<sup>2</sup>Department of Zoology, University of Cambridge, United Kingdom

<sup>3</sup>Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand

## Abstract

The biology of the scuttle fly *Razorfemora zaragozae* Disney (Diptera Phoridae) was previously unknown, but our observations in southern Spain indicate that this phorid fly is a parasitoid of the seed harvester ant *Messor barbarus* (L.) (Hymenoptera Formicidae). We report some aspects of the host location, host selection and oviposition behaviour of *Razorfemora* flies, as well as a potential defensive response of its host ant.

**Key words:** Diptera, Phoridae, *Razorfemora*, scuttle flies, parasitoid, Hymenoptera, Formicidae, *Messor*, ants, behaviour, Spain.

## Introduction

Species of the dipteran family Phoridae are the most important parasitoids of members of the ant family Formicidae (Johnson, 2001). Since the review by Disney (1994), many papers have focused on relationships between parasitoid scuttle flies and ants (e.g. Hsieh and Perfecto, 2012; Mathis and Philpott, 2012; Elizalde *et al.*, 2018). These publications contain descriptions of new and interesting biological strategies and coevolutionary relationships between both insect groups (Lachaud *et al.*, 2012), including the role of phorid parasitoids in the biocontrol of populations of ants of economic importance (Bragança *et al.*, 2017) and of invasive species (Chen and Fadamiro, 2018).

Some ant communities, especially in the Neotropics, appear to be heavily parasitised by phorids: army ants of the genus *Eciton* Latreille (Brown and Feener, 1998), leaf-cutting ants of the genera *Atta* F. and *Acromyrmex* Mayr (Elizalde and Folgarait, 2012) and fire ants of the genus *Solenopsis* Westwood (Porter, 1998). However, data from other type of ant communities or from other geographic regions are scarce (Disney, 1994). In the large arid and semiarid areas of the planet, harvester ants (seed consumers) are of great ecological significance (Hölldobler and Wilson, 1990; Johnson, 2001). Regarding harvester ants, Disney (1994) cited some examples of the genus *Pheidole* Westwood, but Johnson (2001) stated that there were no records of phorid parasitoids associated with species of the two most important genera inhabiting arid and semiarid areas: *Messor* Forel and *Pogonomyrmex* Mayr. Although Johnson (2001) referred to the species of *Messor* from North America - now placed in the genus *Veromessor* Forel - data are still lacking about species of *Messor* in the Palearctic Region.

In a semiarid area of southeastern Spain, at the beginning of the northern 2018 summer, we found a species of phorid fly interacting with trunk trails of the harvester ant *Messor barbarus* (L.), which appeared to be a host-parasitoid association. The phorid fly was identified by RHLD as *Razorfemora zaragozae* Disney (Disney, 2006). The genus *Razorfemora* Disney was erected for

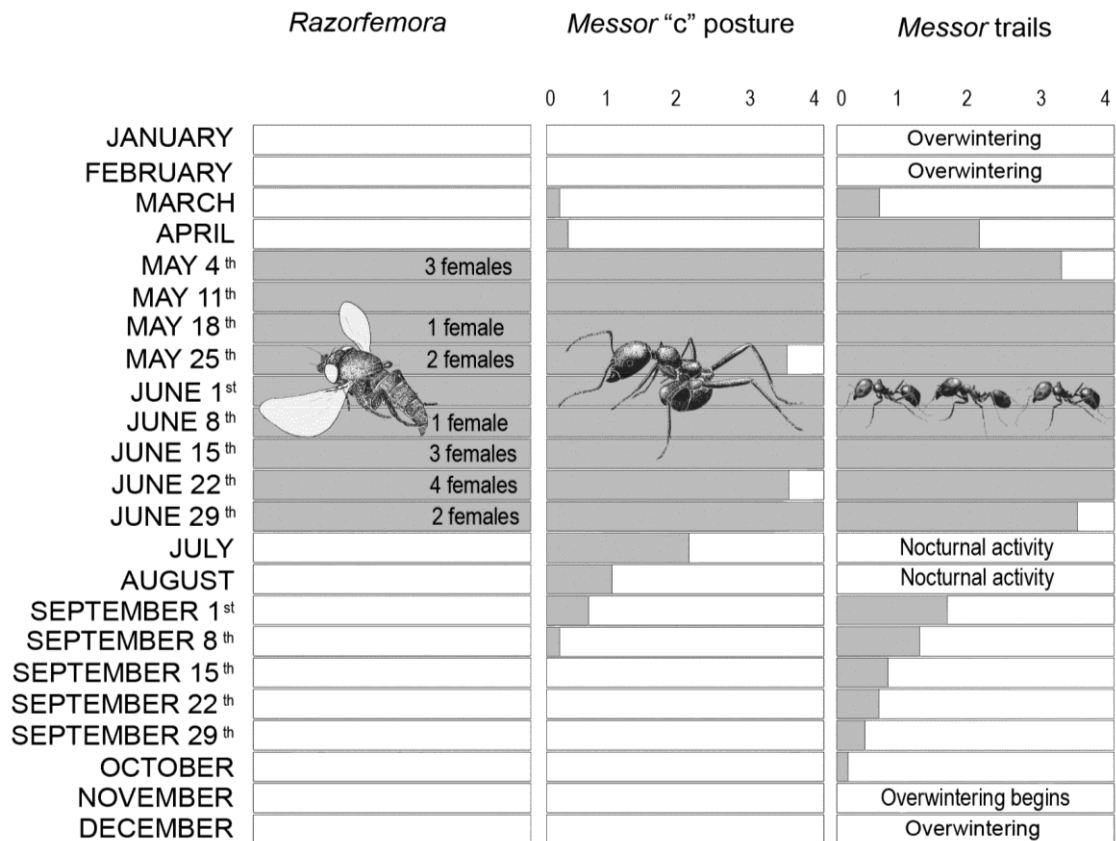
the species *Razorfemora nussbaumi* Disney from Israel, described from a single male (Disney, 1990). Disney (1994) identified a female from Spain as *R. nussbaumi* but, when a large series of *R. nussbaumi*, including both sexes, became available from Yemen, it was evident that the single female from Spain belonged to a second species (Disney, 2006). Since their description, nothing about the biology of the two species of *Razorfemora* has been published.

Because our initial observations during 2018 coincided with the beginning of the hottest season in southern Spain, we obtained limited data. However, the discovery of what appeared to be a new host-parasitoid system between *M. barbarus* and *R. zaragozae* encouraged us to continue our field observations during 2019. Therefore, we decided to observe the temporal pattern of parasitoid activity of *R. zaragozae* towards *M. barbarus* during a complete annual cycle (figure 1). With some logistical constraints, we attempted (1) to gather data on the fly strategy to locate host colonies and select host individuals, (2) to describe the fly strategy to oviposit on a host, and (3) to analyse and interpret the putative correlation between an observed posture taken by numerous ant workers of *M. barbarus* with a defensive mechanism.

## Materials and methods

### Material examined

Three specimens collected in 2018 were preserved in ethanol and sent to RHLD for their identification, who slide mounted them in Berlese Fluid (Disney, 2001). These specimens are stored in the Department of Zoology at the University of Cambridge, UK. Several additional females are stored in alcohol in the insect collections of the Department of Zoology at the University of Murcia with the following data: 3 ♀ “SPAIN, Murcia, Near Molina de Segura, 38.086650, -1.168354, 16/6/2018, J.A. Delgado leg.”, 2 ♀ same data except: 29/6/2018, 3 ♀ same data except 4/5/2019, 1 ♀ same data except 19/5/2019, 3 ♀ same data except 25/5/2019, 1 ♀ same data except 8/6/2019 and 2 ♀ same data except 29/6/2019.



**Figure 1.** Annual cycle of five ant nests during 2019, summarizing the presence of (1) *Razorfemora* females, (2) ant workers in "c" posture and (3) ant colony activity in trunk trails (see text for further explanation of this figure).

A total of 46 dead ants were collected from the studied colonies, and 60 individuals displaying "c" posture were also collected from trunk trails, to be examined under microscope in an attempt to detect parasitoid eggs, larvae or pupae. Ants were cleared with potassium hydroxide and then stained with chlorazol black, to search for any evidence of parasitism.

During May and June 2019, about 120 isolated head capsules of *M. barbarus* workers were collected and examined in the laboratory. All were found to be completely hollow and most without mouthparts. We also searched for fly pupae around the ant nest entrances, over an area of about one meter radius, concentrating our effort on refuse piles (kitchen middens), which were collected and examined under a stereo microscope, but without finding any pupa.

### Methods

It should be noted that this research was not initially designed, but it was developed from casual field observations, which were later organised more systematically. Nevertheless, we feel that the data presented here, together with our comments, even if some maybe speculative, still provide novel information about a parasitoid-host relationship.

*M. barbarus* forms foraging groups to harvest seeds as its main food source, and is known to construct trunk trails (figure 2), which are cleared paths freed of



**Figure 2.** Trunk trail of *M. barbarus* in the study area.

obstacles, debris and vegetation, radiating a few meters from the nest entrance (Plowes *et al.*, 2013). In order to obtain a picture of the temporal variation of the ant colony and the fly attacking behaviour, we selected six different ant colonies and visited them weekly during the year 2019 (observations summarized in figure 1). Depending on the climatic conditions of the day, visits to ant nests were made between 15.00 and 22.00 hours, lasting from one to one and a half hours for all nests. Preferably, we visited the colonies just before sunset avoiding the hottest hours of the day, especially in the summer period. We tried to spend at least ten minutes per ant nest. During each visit we recorded the presence of *R. zaragozae* females, taking notes on their behaviour, but we did not collect them in every visit to reduce our impact on the host-parasitoid relationship. When we collected flies, we made sure there was only one species of scuttle fly in the study area, although our previous experience showed that *Razorfemora* was the only scuttle fly recorded here. When we found scuttle fly females attacking ants, we prolonged the observation of that ant nest from 10 to 30 minutes, thus reducing our observations of other nests during that visit.

Both, ant nest entrances and trunk trails were examined. As these trails experienced a seasonal variation in foraging activity over the course of the year (figure 1), we categorized the presence and approximate number of workers in these trails, using a code number from 0 to 4, as follows: ants absent, generally due to overwintering or aestivating nocturnal activity (0); dispersed worker ants around nest but without trails (1); small trails and workers close to the colony entrance (2); long trails with a few individuals (3); crowded trunk trails with many workers (4). We also recorded the presence of workers in “c” posture (see below) along a section of one meter of trunk trail, as: no ant showing “c” posture (0); two or three ants in this posture (1); around 10% of workers in “c” posture (2); about 50% of them (3); 90% or more workers in “c” posture (4) (see figure 1).

The sizes of worker ants were not measured in an exact manner; however, we divided them into three visually, easy identifiable sizes: small minors (ranging approximately from 3 to 5 mm of body length), medium minors (from 5 to 10 mm body length) and large-headed majors (from 10 to 15 mm body length). Below, in the sections where we describe and discuss the posture “c” taken by workers of *M. barbarus*, giving quantities and percentages, we include the three size ranges of workers together.

During our preliminary observations in 2018, we noted that the scuttle flies would immediately stopped flying if there was wind. This behaviour would be expected, considering that the flies need to get very close to their target hosts. Therefore, we measured wind speed to determine the minimum speed which would stop the flies from attacking ants. Instantaneous wind speeds were measured with a pocket Benetech GM816 LCD digital anemometer. Measurements were taken directing the anemometer towards the wind and at ground level.

Vinson (1976) proposed several phases of interaction between insect parasitoids and their hosts to achieve an effective or successful parasitism. Other authors (Feener

and Brown, 1997; Mathis and Philpott, 2012) have followed Vinson’s (1976) classification to arrange their observations and we also use this classification to organise our field observations.

## Results and discussion

### Host habitat location

In the studied area, the activity of *R. zaragozae* females appeared to be concentrated around the entrance of the ant colony as well as in the first meters of the trunk trails made by the ants. A frequently observed strategy began with female flies perching or resting in the vicinity of an ant nest entrance. After several minutes, they approached the nest entrance and chose a victim leaving from or arriving to the nest. A second strategy involved females patrolling up and down the main ant trunk trails along several meters (to a maximum of 7-8 meters from the ant entrance) trying to parasitise one or several ant workers (figure 3).

*M. barbarus* uses trunk trails during a great part of its seasonal activity. Working trails appeared in late February or early March and, by early May, all colonies had them well established (figure 1). *R. zaragozae* adults seemed to coordinate their activity with the ants using the trunk trails. Probably, this is a strategy to find suitable hosts in high quantities. In addition, during this activity, ants had their mandibles occupied with a seed, hence they were considerably less able to defend themselves. According to Elizalde and Folgarait (2012), *R. zaragozae* is a species with an active host searching strategy.

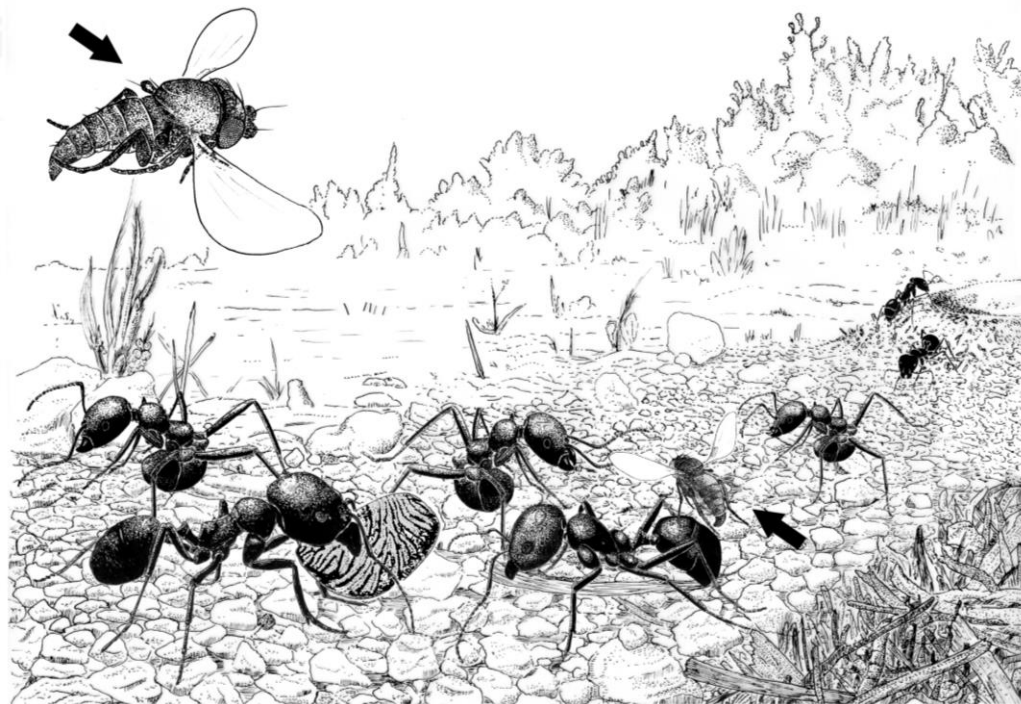
Operating around a single ant nest entrance or along a trunk trail, the number of observed fly females usually ranged from one to four. However, on 7 July 2018 we observed ten females working on a single ant colony, parasitising ants along a trunk trail of around 7 meters long. This observation represents the maximum number of females observed at a single colony.

### Host location

The location of a host by a phorid female seems to be associated with visual and chemical cues (Gazal *et al.*, 2009, Elizalde and Folgarait, 2012, Mathis and Philpott, 2012). The use of these cues was not evaluated in detail during this study, although some of our observations would indicate that *R. zaragozae* uses both. It would appear that the flies used vision to locate the entrance to the ant nests, the trunk trails and to attack the host. However, these are also places of high ant activity which may have high levels of pheromones. Therefore, without experimental work to eliminate one variable, we can only speculate as to which cue is most used by *R. zaragozae*.

Furthermore, we believe that these flies discriminate between workers’ sizes and postures (see below under Host discrimination), as well as between healthy and injured ants, as discussed below.

A relevant observation made on the afternoon of 16 June 2018 suggests a combined use of visual and chemical cues. Two ant colonies were fighting in an open grass area situated between their nests. In the fighting arena, there were neither ant entrances nor trunk trails, but there



**Figure 3.** A trunk trail of *M. barbarus* during the attack of two females of *R. zaragozae* (arrows). *Messor* workers are divided in three categories according to their body length ranges: small minors (approximately 3-5 mm), medium minors (5-10 mm) and large-headed majors (10-15 mm). Body length range for *Razorfemora* females: 2.2-2.3 mm.

were a great number of workers from the two colonies, including many decapitated, dead and injured ants. It could be assumed that members of the two colonies were releasing different pheromone signals during the conflict. Four *R. zaragozae* females were attracted to the fighting arena, attacking ants for about 40 minutes until sunset but, at dusk, scuttle fly activity ceased. During that period, *R. zaragozae* females always attacked active, isolated workers and never those involved in a fight with an ant enemy. Interestingly, they did not attack injured ants, although they were numerous and almost motionless. It would appear that the flies were probably attracted by the intense “scent of war” released by the ants fighting.

#### Host selection and oviposition strategy

The selection of a host by a parasitoid has been attributed to several factors, mainly the shape, size, movement and/or chemicals cues released by the host (Vinson, 1976). Host choice in *R. zaragozae* seemed to be related to host size. Our preliminary observations suggest that *R. zaragozae* preferred to attack medium size ant workers (medium minors), avoiding small minors and large-headed major workers. Also, they avoided ants in “c” posture, as we will comment below.

We monitored ant trails from several ant colonies for a number of hours and several fly attacks were observed. Females of *R. zaragozae* neither discriminated between ants returning to or leaving the nest, nor between loaded or unloaded workers. Nine observations were made in more detail, and the process was always similar: the fly selected an ant and followed it for some seconds, firstly at a distance of several centimetres, then it approached the host from its rear end, hovering over it at a distance of 2 to 4 mm.

The female fly kept approaching it selected host slowly, positioning itself parallel to the axis of the ant’s body. Once in this position, the fly directed its sclerotized terminal abdominal segments towards the ant and, suddenly, hit the ant’s gaster with the tip of its abdomen. Then, the fly retreated a few centimeters and chose a new victim, continuing its oviposition activity while there was no wind (see below), or it perched on grass or rocks nearby, close to the ground.

After an attack, the ant was displaced a small distance, ran for a few seconds, probably due to the push by the fly, and continued on the ant trail without displaying any additional reaction. In a few occasions, instead of running, the victim ant stopped for a few seconds, raised its head and antennae and then continued walking, but without adopting any static defence posture, as it has been described for species of *Solenopsis* (Wuellner *et al.*, 2002).

#### Host discrimination

We made some observations in regard to the selection of a particular ant individual - and discrimination of others - as a potential target for oviposition by the female scuttle fly. *Razorfemora zaragozae* seemed to avoid ovipositing on (1) a stationary hosts, (2) large-bodied workers exhibiting an offensive display (with erect body and open mandibles), (3) small minors, and (4) ants in “c” posture (see figure 4b-d and explanation below).

Our observations showed that, when *R. zaragozae* females approached ant workers, they appeared to assess the suitability of the potential victim. Frequently, the fly did not oviposit on the first ant selected and changed to another worker. Morrison *et al.* (1997) observed that some females of the genus *Pseudacteon* Coquillett attacked the same ant worker multiple times, which the au-



**Figure 4.** *Messor barbarus* showing different postures and working activities: **a)** Workers on a trunk trail in natural posture when *Razorfemora* females were absent; **b)** Ant workers of all sizes in “c” posture on a trunk trail during an attack of *Razorfemora*; **c)** Large-headed major ant worker at a colony entrance showing a typical “c” posture; **d)** Medium minor ant worker in “c” posture carrying a seed along a trunk trail.

thors interpreted as an indication that females were aware that some attacks did not lead to successful oviposition. In our observations, females of *R. zaragozae* never made multiple attacks on a single ant worker, although they approached several different ant workers. Without wind, females flew over and along the trunk trails, with a characteristic vertically undulating displacement, systematically patrolling the length of the trail over several meters, while approaching and moving away from their victims. These flights were repeated in both directions. Some females attacked a few ant workers, but others attacked many individuals.

Our longer observations of females flying along ant trails, showed that the number of hits on ants ranged from a few to 30, always involving several host individuals. However, we could not be sure whether all the abdominal hits by the flies against several ants achieved successful oviposition in all instances. The time the flies spent attacking ants was variable, but we noted that it is highly affected by wind velocity. Our observations showed that absence of wind is decisive for a successful oviposition. As soon as wind speed increased over 0.5 - 0.6 m/s, *R. zaragozae* ceased flight and retreated to the surrounding herbs.

Similar to flies of other dipteran families, species of Phoridae appear to lack an accessory gland that could be used to mark a host during egg-laying in order to avoid super-parasitisation. In the absence of these marks, it has been suggested that phorids can use visual cues of previous oviposition wounds on the ants (Feener and Brown,

1997; Mathis and Philpott, 2012). However, this may not be an effective strategy to avoid multiple eggs inside the same host because several parasites are frequently found inside a single host in species of some phorid genera (Mathis and Philpott, 2012). In one occasion, a female *R. zaragozae* started the approach display towards a particular ant but, after nine different attempts, did not hit it. Could this represent an example of an evaluation of previous parasitisation?

The reasons behind the avoidance of large-headed major ants by *R. zaragozae* females are still uncertain. Do flies avoid them because they identify the majors as the more aggressive and dangerous ant caste? In species of *Pseudacteon*, Wuellner *et al.* (2002) described aggressive responses from some worker ants, chasing and crushing the flies with their mandibles. We did not observe this behaviour in the field, but we did it in the laboratory. We placed approximately 30 ants inside a five-litre glass terrarium. After some hours, when ants had relaxed, a single *R. zaragozae* female was introduced into the container. Initially, the objective of the experience was to observe fly oviposition behaviour in a controlled environment. However, as soon as the fly rested on the glass wall, it was immediately captured by a major worker, which crushed the fly between its mandibles. Regarding the avoidance of small minor workers by the parasitoid, we can speculate that it is a matter of size incompatibility, as the small minors would be too small to allow the successful development of the fly.

## Host regulation

Most parasitoids manipulate their hosts to optimise successful parasitism (Orr, 1992; Henne and Johnson, 2007). Phorids can modify host behaviour inducing ants to stay inside the ant nest, which would reduce predation or deaths outside the ant nest (Henne and Johnson, 2007; Mathis and Philpott, 2012). Once the fly larva has completed its development, the parasitised ant must abandon its nest to facilitate the pupation of the fly outside. Studying *Solenopsis* ants in the laboratory, Henne and Johnson (2007) found that parasitised ants left their nest only shortly before their decapitation, and that these individuals had not left the nest until the parasitoid had virtually completed its larval development. Also, they found that mature larvae induced the ants to locate a suitable microclimate for pupation. If this is the case with *M. barbarus*, it could explain our failure to detect parasitised workers outside the nests investigated in our study area.

As mentioned in the above paragraph of methods, our search for fly pupae around the nests failed to produce any positive result. We suspect that *R. zaragozæ* does not decapitate ants, and that they pupate close to the nest entrance or even inside the nest, but we were not able to confirm these hypotheses. Also, we did not find any fly pupa or puparium after examining many head capsules of *M. barbarus* without mouthparts, which were most likely consumed by myrmecophilous ant species of the genera *Pheidole* and *Cataglyphis* Forster, which were also present in the study area. Since *Razorfemora* females aim their attacks on the abdomen of *Messor* ants, it is possible that the fly larva occupies that section of the ant body. In species of scuttle flies that decapitate their ant hosts to emerge, females seem to prefer the thorax or head for oviposition (Porter, 1998).

## Male location

Feener and Brown (1992) observed several males of *Pseudacteon* species in the vicinity of their host ant trails, most probably attracted by the possibility of finding females. However, we did not see or collect any male of *Razorfemora* around the ant trails examined in our study area.

## Ant behaviour: “c” posture in *Messor barbarus* as a response to *Razorfemora zaragozæ* attacks

Several publications (Feener, 1987; Feener and Brown, 1992; Porter *et al.*, 1995; Elizalde and Folgarait, 2012) pointed out that a part of an ant colony seems to be unavailable for parasitoid oviposition due to different defensive behaviours developed by the host ants, such as retreating underground, reducing activity or altering body posture when scuttle flies are in the area. These defensive behaviours are interpreted as coevolutionary responses by the host, induced by the attack of the parasitoid, with a variable degree of defensive value in different host-parasitoid systems. Elizalde and Folgarait (2012) have divided the host defensive responses into pre- and post-attack responses.

In species of some ant genera, such as *Solenopsis* (Wuellner *et al.*, 2002) or leaf-cutting ants of the genera *Atta* and *Acromyrmex* (Elizalde and Folgarait, 2012), several responses adopted by the ants after fly attacks

have been described. Ants of these genera become agitated, start running or take standardized postures immediately after parasitisation. Wuellner *et al.* (2002) described and labelled several ant postures related with the post-oviposition behaviour and named them as “toppled”, “u”, “n” and “c” postures. In particular, the “c” posture refers to a body shape taken by the ant, in which the gaster is pulled under its body and the forelegs are lifted off the ground (figure 4c). *Solenopsis* ants adopted the “c” posture immediately after attack, but this was not the case in *M. barbarus*, as we will comment below.

Elizalde and Folgarait (2012) have described similar postures for species of *Atta* and *Acromyrmex*. Wuellner *et al.* (2002) observed that ants of species of *Solenopsis* frequently froze and stayed in that posture for a while, ranging from some seconds to several hours. Eventually, they slowly relaxed and progressively returned to a normal posture and activity. Also, Wuellner *et al.* (2002) pointed out that some of the postures taken by attacked ants were previously regarded as defensive by Feener and Brown (1992). Although Wuellner *et al.* (2002) agree in that all posturing ants were unattractive to a female fly, these authors concluded that, if those postures were only taken after fly attacks, they would be completely useless for the ants as a defensive behaviour, at least for an already parasitised ant. Although Wuellner *et al.* (2002) argument is correct if applied to ant workers already parasitised, a defensive purpose as postulated by Feener and Brown (1992) cannot be ruled out because the entire colony would benefit from it, even if foraging is reduced.

Considering that the cessation of foraging activity by fly-attacked workers in species of *Solenopsis* would reduce the general efficiency of the colony, there has been interest in using this behaviour as a tool for biological control, especially as some species of *Solenopsis* have become invasive (Orr *et al.*, 1995; Chen and Porter, 2020).

In some of the host-parasitoid systems described by Elizalde and Folgarait (2012) for species of *Atta* and *Acromyrmex*, the interpretation of ant behaviour is less complex because the authors found that the ants displayed pre-attack responses, which could not be anything but defensive, thus benefiting not only the individual ant but, also, the entire colony.

In *M. barbarus*, we identified one defensive posture (figures 3, 4b-d), described as “c” posture by Wuellner *et al.* (2002). During the month following the overwintering period, when *M. barbarus* colonies resumed their activity in mid-February, we did not observe any worker in “c” posture (figure 4a). From mid-March to the end of April a few workers displayed the “c” posture, increasing in numbers as the formation of trunk trails progressed (figure 2), until April 28<sup>th</sup>, when we observed many workers adopting that posture (figures 3, 4b-d). However, the first female *R. zaragozæ* was seen on May 4<sup>th</sup>, when 90% of ants were in “c” posture (figure 1).

The number of ant workers observed in “c” posture was proportional to the number of phorid females patrolling the trails, beginning in May and reaching their maximum numbers in late June and early July (figure 1). In mid-July, when *Razorfemora* females were no longer present, most of the ant colony stop displaying the “c” posture, although some ants maintained it until mid-autumn. From

mid-July to August, the ant colonies reduced their activity (aestivation), working mainly around evening hours and at night (figure 1).

At present, we do not know if *Razorfemora* flies are active at night during the hottest period of the year. Ant colony activity exposed to the sun resumed in mid-September, with a small number of workers still persisting in their “c” posture, but they stop from late October to November, marking the end of the activity season.

It is relevant to note that, although *Razorfemora* flies selected medium minor workers as preferred targets for their attacks, all three worker castes, covering a size range of 3 to 15 mm body length, adopted the “c” posture.

In addition to our comments in the previous sections, two important questions need to be addressed:

(1) is the “c” posture taken by ant workers of *M. barbarus* defensive ?

(2) is that “c” posture a pre-attack or a post-attack response to scuttle flies presence ?

Considering what is known from previous research with ant species of various genera, such as *Solenopsis*, *Atta* and *Acromyrmex*, and our observations of the behaviour of *M. barbarus* workers (see above), we believe that the “c” posture is defensive for this species too, although this hypothesis needs to be tested with a properly designed experiment, which is beyond the scope of his study.

Regarding the second question, if the “c” posture is a pre-attack or a post-attack response, we believe that it is clearly a pre-attack response, as we show it below.

As shown in figure 1, the maximum activity of *M. barbarus* using trunk trails correlated with the presence of *R. zaragozae*, from early May to late June. During this period, female scuttle flies appeared to exploit ant behaviour to maximize their parasitic strategy, because ants were available in great numbers, walking in ordered lines and partially distracted while transporting harvested seeds. At the peak of scuttle fly attacks, over 90% of the all workers maintained the “c” posture, including while travelling along the trunk trail.

Our observations showed that a great number *Messor* workers of all sizes spontaneously adopted the “c” posture without any close physical interaction with a fly, continuing in such state for long periods while performing their normal activities (figure 4b-d). We also observed that a single phorid fly was sufficient to elicit the defensive “c” posture, even on workers that emerge from the nest entrance already in such posture.

If the “c” posture were the result of previous parasitisation by *Razorfemora*, i.e. a post-attack response, parasitised workers could not be saved, but taking such a posture could be interpreted as a warning signal by the rest of the non-parasitised workers. However, if so many as 90% of the colony workers being in “c” posture were actually infested, the colony would not be able to survive.

Furthermore, our examination of 60 workers of all sizes in “c” posture did not detect any sign of infestation, neither eggs nor larvae inside the ants. Absence of an egg or a larva would imply a non-infestation. Nevertheless, the possibility remains that the “c” posture was adopted shortly after the attack and, even if eggs were present, we

failed to find them due to their very small size. However, Feener and Brown (1992) suggested that the number of parasitised ants in a single nest is usually low in all the ant systems studied at the time.

Some authors (Feener and Brown, 1992; Orr *et al.* 1995; Morrison *et al.*, 1997) have shown that the foraging behaviour of an ant colony was disrupted - in some cases considerably - in the presence of scuttle flies, but this scenario did not apply to *M. barbarus*. Unlike *Solenopsis* ants, where a worker stands still rising up on their legs for several minutes after having been attacked, workers of *M. barbarus* did not stop walking on the trail. In fact, almost the entire colony adopts the “c” posture during a number of weeks in summer, but still performs all its regular activities.

Finally, the most convincing evidence that the “c” posture in *M. barbarus* ants is primarily a pre-attack defensive response is the fact that only medium size minors were attacked by *Razorfemora* flies, while all other size workers also adopted the “c” posture in response to the presence of scuttle flies around the ant colony.

## Conclusion

Finding a new host-parasitoid association is always of interest, but it is especially relevant when it involves species of known ecological importance. In arid and semi-arid ecosystems, harvester ants play a critical role in the energy flow of the systems, with a significant outcome in the composition and relative abundance of annual plants and the animals which interact with them (Johnson, 2001). In this paper, we recorded the scuttle fly *R. zaragozae* as a parasitoid of the harvester ant *M. barbarus*, giving data on the biology and behaviour of these two insects, which includes one of the most ecologically important species in the Mediterranean grasslands (Detrain and Tasse, 2000).

Although a number of questions are still to be answered, our data indicates that, within the family Phoridae, *R. zaragozae* belongs to a group in which females locate potential hosts around the entrance of ant nests and, in particular, over trunk trails made by the host during periods of maximum colony activity. Also, we believe that scuttle fly females actively search and locate their hosts using visual and/or chemical cues, but we know that these females oviposit on the gaster of the ants and not on their head or thorax. Furthermore, we know that to achieve positive oviposition, female flies perform precise manoeuvres to physically reach the back of their targeted hosts, and that they stop flying when wind prevents such manoeuvres.

Host-parasitoid associations have coevolved according to what has been interpreted as an “arms race”, a scenario where each species tries to outdo the other one to survive. In this respect, we evaluate the possible adaptive value of a particular posture - described and named as “c” posture - taken by workers of *M. barbarus* and induced by the presence of parasitoid females. We propose that such posture, observed in the colonies of *M. barbarus* studied in southern Spain, is a pre-attack defensive response, i.e. taken before the parasitoid oviposits on any worker ant.

We also observed that, unlike what is known from other ant species, workers of *M. barbarus* maintain the defensive “c” posture while they forage and perform other key activities in a normal fashion, a behaviour which we interpret as an efficient strategy to overcome the negative effect of phorids and other parasitoids.

## Acknowledgements

We are very grateful to two anonymous reviewers for making constructive suggestions that have substantially improved the manuscript. RHL D’s work on Phoridae is currently funded by the Balfour-Browne Trust (University of Cambridge, United Kingdom).

## References

- BRAGANÇA M. A. L., ARRUDA F. V., SOUZA L. R. R., MARTINS H. C., DELLA LUCIA T. M. C., 2017.- Phorid flies parasitizing leaf-cutting ants: their occurrence, parasitism rates, biology and the first account of multiparasitism.- *Sociobiology*, 63 (4): 1015-1021.
- BROWN B. V., FEENER D. H. Jr., 1998.- Parasitic phorid flies (Diptera: Phoridae) associated with army ants (Hymenoptera: Formicidae: Ecitoninae, Dorylinae) and their conservation biology.- *Biotropica*, 30: 482-487.
- CHEN L., FADAMIRO H. Y., 2018.- *Pseudacteon* phorid flies: Host specificity and impacts on *Solenopsis* fire ants.- *Annual Review of Entomology*, 63 (7): 47-67.
- CHEN L., PORTER S. D., 2020.- Biology of *Pseudacteon* decapitating flies (Diptera: Phoridae) that parasitize ants of the *Solenopsis saevissima* complex (Hymenoptera: Formicidae) in South America.- *Insects*, 11 (2): 107.
- DETRAIN C., TASSE O., 2000.- Seed drops and caches by the harvester ant *Messor barbarus*: do they contribute to seed dispersal in Mediterranean grasslands?- *Naturwissenschaften*, 87: 373-376.
- DISNEY R. H. L., 1990.- Problem genera close to *Megaselia* (Diptera: Phoridae).- *British Journal of Entomology and Natural History*, 3: 11-22.
- DISNEY R. H. L., 1994.- *Scuttle flies: the Phoridae*.- Chapman & Hall, London, UK.
- DISNEY R. H. L., 2001.- The preservation of small Diptera.- *Entomologist’s Monthly Magazine*, 137: 155-159.
- DISNEY R. H. L., 2006.- Insects of Arabia scuttle flies (Diptera: Phoridae) Part I: all genera except *Megaselia*, pp. 473-521. In: *Fauna of Arabia*, vol. 22 (KRUPP F., Ed.).- Senckenbergische Naturforschende Gesellschaft, Frankfurt am Main, Germany, King Abdulaziz City for Science and Technology, Riyadh, Kingdom of Saudi Arabia.
- ELIZALDE L., FOLGARAIT P. J., 2012.- Behavioral strategies of phorid parasitoids and responses of their hosts, the leaf-cutting ants.- *Journal of Insect Science*, 12: 135.
- ELIZALDE L., PATROCK R. J. W., DISNEY R. H. L., FOLGARAIT P. J., 2018.- Spatial and temporal variation in host-parasitoid interaction webs: leaf-cutter ant hosts and their phorid parasitoids.- *Ecological Entomology*, 43: 114-125.
- FEENER D. H. Jr., 1987.- Size-selective oviposition in *Pseudacteon crawfordi* (Diptera: Phoridae), a parasite of fire ants.- *Annals of the Entomological Society of America*, 80 (2): 148-151.
- FEENER D. H. Jr., BROWN B. V., 1992.- Reduced foraging of *Solenopsis geminata* (Hymenoptera: Formicidae) in the presence of parasitic *Pseudacteon* ssp. (Diptera: Phoridae).- *Annals of the Entomological Society of America*, 85 (1): 80-84.
- FEENER D. H. Jr., BROWN B. V., 1997.- Diptera as parasitoids.- *Annual Review of Entomology*, 42: 73-97.
- GAZAL V., BAILEZ O., VIANA-BAILEZ A. M., 2009.- Mechanism of host recognition in *Neodohrniphora elongata* (Brown) (Diptera: Phoridae).- *Animal Behaviour*, 78: 1177-1182.
- HENNE D. C., JOHNSON S. J., 2007.- Zombie fire ant workers: behaviour controlled by decapitating fly parasitoids.- *Insectes Sociaux*, 54 (2): 150-153.
- HÖLLDOBLER B., WILSON E. O., 1990.- *The ants*.- Harvard University Press, Cambridge, Massachusetts, USA.
- HSIEH H-Y., PERFECTO I., 2012.- Trait-mediated effects of phorid flies on ants.- *Psyche*, 2012: 380474.
- JOHNSON R. A., 2001.- Biogeography and community structure of North American seed-harvester ants.- *Annual Review of Entomology*, 46: 1-29.
- LACHAUD J-P., LENOIR A., VOLKER W., 2012.- Ants and their parasites.- *Psyche*, 2012: 342157.
- MATHIS K. A., PHILPOTT S. M., 2012.- Current understanding and future prospect of host selection, acceptance, discrimination and regulation of phorid fly parasitoids that attack ants.- *Psyche*, 2012: 895424.
- MORRISON L. W., DALL’AGLIO-HOLVORCEM C. G., GILBERT L. E., 1997.- Oviposition behavior and development of *Pseudacteon* flies (Diptera: Phoridae), parasitoids of *Solenopsis* fire ants (Hymenoptera: Formicidae).- *Environmental Entomology*, 26 (3): 716-724.
- ORR M. R., 1992.- Parasitic flies (Diptera: Phoridae) influence foraging rhythms and caste division of labor in the leaf-cutter ant, *Atta cephalotes* (Hymenoptera: Formicidae).- *Behavioral Ecology and Sociobiology*, 30 (6): 395-402.
- ORR M. R., SEIKE S. H., BENSON W. W., GILBERT L. E., 1995.- Flies suppress fire ants.- *Nature*, 373: 292-293.
- PLOWES N. J. R., JOHNSON R. A., HÖLLDOBLER B., 2013.- Foraging behaviour in the ant genus *Messor* (Hymenoptera: Formicidae: Myrmicinae).- *Myrmecological News*, 18: 33-49.
- PORTER S. D., 1998.- Biology and behavior of *Pseudacteon* decapitating flies (Diptera: Phoridae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae).- *The Florida Entomologist*, 81 (3): 292-309.
- PORTER S. D., VANDER MEER R. K., PESQUERO M. A., CAMPIOLLO S., FOWLER H. G., 1995.- *Solenopsis* (Hymenoptera: Formicidae) fire ant reactions to attacks of *Pseudacteon* flies (Diptera: Phoridae) in southeastern Brazil.- *Annals of the Entomological Society of America*, 88 (4): 570-575.
- VINSON S. B., 1976.- Host selection by insect parasitoids.- *Annual Review of Entomology*, 21: 109-133.
- WUELLNER C. T., DALL’AGLIO-HOLVORCEM C. G., BENSON W. W., GILBERT L. E., 2002.- Phorid fly (Diptera: Phoridae) oviposition behavior and fire ant (Hymenoptera: Formicidae) reaction to attack differ according to phorid species.- *Annals of the Entomological Society of America*, 95 (2): 257-266.

**Authors’ addresses:** Juan A. DELGADO (corresponding author: jdelgado@um.es), Departamento de Zoología, Facultad de Biología, Universidad de Murcia, 30100, Murcia, Spain; R. Henry L. DISNEY (rhld2@hermes.cam.ac.uk), Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, UK; Ricardo L. PALMA (ricardop@tepapa.govt.nz), Museum of New Zealand Te Papa Tongarewa, PO Box 467, Wellington, New Zealand.

Received November 27, 2019. Accepted May 11, 2020.