

Dear Editor,

We carefully revised the MS “Hard to choose for tiny pests: host-seeking behaviour in *Xenos vesparum* triangulins” following the suggestions of both referees:

- ✦ Introduction and Discussion have been shortened by omitting not relevant data on *Xenos vesparum* cycle;
- ✦ we added more information on host-seeking behaviour in other parasitoids;
- ✦ we clarified our experimental set-up by adding details on our apparatus and choice trials (see Methods);
- ✦ we omitted Carbon Dioxide trials – even if the role of CO<sub>2</sub> in host location may be relevant - because preliminary: limited sample size, no data about CO<sub>2</sub> levels on the wasp comb, experimental CO<sub>2</sub> emission (under 2 mL/min flow) probably too low;
- ✦ the Discussion has been re-organized according to the different topics considered (positive and negative evidences for host-location; the role of stimuli used by *Xenos* triangulins to escape from the cephalothorax; gregariousness);
- ✦ the Table has been converted to a Figure (see Fig.2).

Due to the preliminary nature of some choice trials here reported, results have been discussed with caution; therefore, we think that a Short Communication Format could be more appropriate for this paper.

With best regards

Fabio Manfredini

1 **HARD TO CHOOSE FOR TINY PESTS:**

2 **HOST-SEEKING BEHAVIOUR IN *XENOS VESPARUM* TRIUNGULINS**

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10 **ABSTRACT**

11 The 1<sup>st</sup> instar larvae of strepsipteran parasites, commonly referred as “triungulins”, are the host-  
12 seeking stage: they must locate, invade and successfully develop in the new host, in order to start  
13 their parasitic cycle. A few information are available about the behaviour of *Xenos vesparum*  
14 triungulins. They emerge in batches from the endoparasitic female infecting *Polistes dominulus*, the  
15 primary host, and reach the nest through a vector (a foraging wasp or the parasitized wasp itself).  
16 Once there, they have the possibility to penetrate into wasp immatures at different developmental  
17 stages. In this study, we performed preliminary analyses aimed to investigate which cues are  
18 important to direct triungulins movements during their brief stay on wasp nests. In laboratory  
19 conditions we selectively presented different stimuli to *Xenos* larvae: apparently, the host larva  
20 itself is attractive in an open arena, but not inside a confined space, neither epicuticular compounds  
21 of wasp larvae are able to control triungulins movements. These are more likely oriented by their  
22 gregarious behaviour, whereas light (positive phototaxy) could previously enhance their emergence  
23 via the brood canal opening in the female cephalothorax.

24  
25 **KEY WORDS:** gregariousness, host-seeking behaviour, triungulin, infective larva, Strepsiptera,  
26 koinobiont parasitoid.

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2 RUNNING TITLE: Host location by *Xenos* triungulins.

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## INTRODUCTION

1  
2 Parasites, being a significant portion of total biomass in natural ecosystems and mainly in insect  
3 societies (Hughes et al., 2008), have a great eco-ethological significance. In particular, manipulative  
4 parasites are able to redirect host relationships and to extend the range of its habitat (Lefevre et al.  
5 2009). *Xenos vesparum* is a castrator “manipulative” parasite (Hughes 2005), which has a great  
6 impact on the small annual society of the paper wasp *Polistes dominulus*, its primary host (Beani  
7 2006; Hughes 2005; Hughes et al. 2004b). In this host-parasite system, the association between the  
8 two insects is long-lasting and its success is based on the parasite tuning with host development. *X.*  
9 *vesparum* penetrates into a *P. dominulus* immature as 1<sup>st</sup> instar larva and coexists with it for weeks  
10 or months, depending on the sex of both parasite and host. The intimate association with its host  
11 forces the parasite to adapt to a changing environment: this life style is typical of insect parasitoids,  
12 a large group of insects, primarily in the orders of Hymenoptera and Diptera, whose larvae develop  
13 by feeding in / on the bodies of other arthropods (Brodeur & Boivin 2004; Strand & Pech 1995).  
14 Thus, *X. vesparum* could be reasonably linked to koinobionts parasitoids, where parasite  
15 development is finely tuned to the development of its holometabolous host, since it manages to  
16 achieve growth and metamorphosis processes (Tanaka 2006; Vinson & Iwantsch 1980).

17 Together with adult males, 1<sup>st</sup> instar larvae are the sole free-living developmental stages in  
18 *X. vesparum* life cycle. They are often called “triungulins” (i.e. three-clawed) for their  
19 morphological similarity to coleopteran campodeiform larvae (Kathirithamby 1989; Kathirithamby  
20 2009; Pohl 1998), although in this species the first two pairs of legs are equipped with disc-like  
21 pulvilli, not with claws, and the last with spines (Fig. 1). They represent the host-seeking stage: they  
22 are extremely motile and resistant to environmental conditions (they may survive for 48 hours in  
23 Petri dishes without food, see Giusti et al. 2007, and independently on the humidity conditions, *pers.*  
24 *obs.*), although relative humidity has been referred as a critical longevity factor in other species  
25 (Hassan 1939, quoted in Kathirithamby 2009). Moreover, they are particularly kin in penetrating

1 into the host and establishing inside without any dramatic consequences neither for themselves nor  
2 for the host (Manfredini et al. 2007a).

3         Generally, parasitoid infective larvae are the most aggressive among immatures, capable of  
4 ensuring the parasitic success of the species by installing themselves fruitfully on the host. For this  
5 purpose, they are usually very mobile and easily exposed to physical fights with rival conspecifics  
6 (Doury et al. 1995; Giron et al. 2004); moreover, they often have a direct effect on host physiology  
7 through their bite, whereby they inject salivary glands secretions into the host, thus manipulating  
8 both cellular and humoral components of its hemolymph (Doury et al. 1997; Richards & Edwards  
9 2002). Strepsipteran triungulins possess all the morphological features of a successful infective  
10 organism (Fig. 1): antennae, eyes, mouthparts, slender legs, tarsal expansions and long caudal  
11 appendages which allow jumping movements. Almost no information are available about their host-  
12 seeking behaviour and very few is known on the invasion process (Kathirithamby 2001;  
13 Kathirithamby et al. 2003; Maeta et al. 2001). For *Xenos* triungulins this starts once they have  
14 reached a wasp nest, either by grasping to a foraging wasp (phoretic transport) or by means of a  
15 stylopized wasp which directly visits a nest (Hughes et al. 2003; Vannini et al. 2008). The  
16 “wandering” behaviour among spring colonies, recently described (Beani & Massolo, 2007) in  
17 wasps infected by *X. vesparum* females, i.e. the source of triungulins, could be interpreted as a  
18 manipulative extension of the usual wasp range by the parasite (Lefevre et al. 2009). In the field the  
19 parasite load ranges from 1 to 4.1 per adult wasp (Vannini et al. 2008) and thus it is presumably  
20 higher at infection. After the emergence of the winged male and the extrusion of the cephalothorax  
21 of the female, a neotenic permanent endoparasite, mating occurs (Beani et al. 2005). Fertilized eggs  
22 start the first steps of segmentation process, but then stop and remain quiescent during winter  
23 diapause (*pers. obs.*). The following spring, embryogenesis will terminate to give birth to hundreds  
24 of 1<sup>st</sup> instar larvae, ready for another season of infections and usually released in groups of 10, 20 or  
25 more (*pers. obs.*). Thus, *Xenos* females may over-winter inside its host, a hibernating female wasp,

1 whereas *Xenos* males die in summer, as well as their hosts, regardless of their putative caste (Beani  
2 2006).

3 For many reasons (not just morphology), *Xenos* triangulins are quite analogous to host-  
4 seeking stages of Dipteran and Coleopteran koinobiont endoparasitoids (Castelo & Lazzari 2004;  
5 Crespo & Castelo 2008; Royer et al. 1999). According to Brodeur and Boivin (2004), in parasitoids  
6 with active larvae the host seeking stage undergoes a chain of “hierarchical steps” to achieve  
7 successful parasitism: it must locate, evaluate and penetrate the appropriate host, evade or overcome  
8 the host immune response and adapt to (or regulate) the constantly changing host environment. Host  
9 seeking larvae promptly respond to different kind of cues, generally divided in three main  
10 categories (Godfray 1994): signals from host microhabitat, which are easy to detect at a distance but  
11 give little reliable information; stimuli indirectly associated with the activity of the host (for  
12 example phonotaxy, long-distance kairomones, CO<sub>2</sub> emission, etc.); stimuli from the host itself, i.e.  
13 visual and chemical cues, that are the most reliable ones, often too weak for detection over long  
14 distances, due to the strong selection pressure on hosts to avoid being recognized (for a review on  
15 parasitoids exploiting chemical information, see Fatouros et al. 2008).

16 In this pioneering study we investigated which short-range cues are important to orient *X.*  
17 *vesparum* triangulins during host location on the comb: are *P. dominulus* larvae *per se* attractive for  
18 triangulins? In a series of repeated independent experiments in laboratory, we tried to recreate semi-  
19 natural choice trials, focusing on the arrival of triangulins on the nest. We registered the movements  
20 of batches of host-seeking triangulins on a neutral substrate in presence of 3<sup>rd</sup>- 4<sup>th</sup> instar wasp  
21 larvae, which were singly placed in the middle of an open arena, and successively inside a confined  
22 and screened space, in order to distinguish chemical from visual signals. In further trials, we  
23 investigated the effect of single stimuli, i.e. epicuticular compounds from wasp larvae *vs* fly larvae  
24 used as a control. Only in case of a clear-cut effect of wasp stimuli in orienting the movements of  
25 triangulins, we could apply the same technique to assess host selection, as concerns sex,  
26 developmental stage and superparasitism. Although preliminary, this study on Strepsiptera

1 represents a good model to analyse host-seeking behaviour among immatures parasitoids, due to  
2 scanty data in literature about the ecology of active 1<sup>st</sup> instar larvae (Feener & Brown 1997).

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#### METHODS

##### 5 *Study animals*

6 Hibernating clusters of *Polistes dominulus* Christ were collected in San Gimignano  
7 (Siena, Italy) at the end of the winter. They were carefully screened to exclude parasitic infections  
8 and placed in groups of 3 individuals inside 20 x 20 x 20 cm Plexiglas cages with sugar, water and  
9 *Sarcophaga* sp. larvae *ad libitum*, to allow colonies foundation. Under fixed conditions of 15L/9D  
10 and 28±2°C, *Polistes* wasps started building first nest cells around 3 weeks after collection. These  
11 animals were the starting pool for laboratory experiments, although additional colonies were  
12 collected during the summer directly from the field. In this case, nests underwent a period of  
13 quarantine to exclude any possibility of natural infection by Strepsiptera.

14 Our sources of triungulins were overwintered wasps coming from the same hibernating  
15 clusters as above and parasitized by a single (rarely 2) female of *Xenos vesparum* Rossi. These  
16 females, extruding their cephalothorax through wasp abdomen, released batches of triungulins after  
17 4 weeks at 15L/9D and 28°C. Experiments were carried out in June and July 2008, i.e. the peak of  
18 infection in wasp colonies (Hughes et al. 2003).

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##### 20 *Choice trials in laboratory*

21 For preliminary experiments of host location, we selected from different wasp colonies 3<sup>rd</sup> or  
22 4<sup>th</sup> instar larvae, i.e. the commonest stages, easier to manipulate than 1<sup>st</sup> – 2<sup>nd</sup> instars and not close to  
23 pupation as 5<sup>th</sup> instar. These developmental stages are easily recognizable for their relatively large  
24 size and not yet melanized (light brown) mouth apparatus. We carefully removed each wasp larva  
25 from its cell with forceps and placed it in the middle of a circular open arena (10 cm wide Petri  
26 dish, covered with filter paper which was changed at each trial). Then, with a thin needle, we

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1 collected batches of triungulins escaping from one *Xenos* cephalothorax (i.e. siblings) and we  
2 spreaded them on the paper (around 10 individuals per arena trial). Finally, we recorded their  
3 movements during first 20 minutes of activity with a video camera connected to a  
4 stereomicroscope. We excluded the nest as trial substrate to avoid a possible confounding source of  
5 chemical signals (for a review on wasp cuticular hydrocarbons, already present on the comb, see  
6 Gamboa 2004) and to better follow the movements of triungulins, able of walking and jumping on  
7 filter paper as well as on plastic substrates.

8 In a second set of experiments, triungulins were offered a choice in a vial between 3<sup>rd</sup> – 4<sup>th</sup>  
9 instar wasp larvae and no wasp as control: although *Polistes* nests are opened, they are often located  
10 under tiles or other shelters, thus a confined space could be appropriate to simulate a natural  
11 condition. We prepared transparent plastic tubes (1 cm wide, 7 cm long) with a small hole in the  
12 middle. The wasp larva was positioned at one extremity of the tube while the other extremity was  
13 left empty. Clusters of 20-30 triungulins were inserted into the tube with a thin needle through the  
14 small hole. A cotton membrane at 1 cm from each extremity prevented triungulins from contacting  
15 the wasp larva and a parafilm over the hole prevented triungulins from escaping. For 27  
16 independent experiments we recorded *Xenos* larvae position in respect to target and control after 1  
17 hr (time 1) and after 4 hrs (time 2). The central portion of the tube around the entry hole ( $\approx$  1 cm  
18 long) was not considered, being a neutral zone. To control for a possible effect of light, we used the  
19 same transparent tubes as above (deprived of both the wasp larva and the cotton membrane) 90°  
20 oriented to the light source and we recorded triungulins prevalence in light side vs dark side.

21 Finally, an additional set of stimuli was proposed to *Xenos* triungulins in order to observe  
22 their susceptibility to wasp cuticular compounds. Single wasp larvae were placed in a glass vial and  
23 washed in 100 $\mu$ l heptane for 30 sec to obtain a solution of larval cuticular waxes, mainly  
24 hydrocarbons (Cotoneschi et al. 2007). We created a 4 field square arena (measuring 10 cm on each  
25 side) where one field was occupied by the stimulus (a drop of hydrocarbon solution from larval  
26 wasp cuticle), the opposite one was a positive control (a drop of hydrocarbon solution from



1 immature *Sarcophaga* sp. fly, obtained following the same procedure as above) and the remaining  
2 fields were negative controls (no stimuli at all). Then we placed clusters of 20-30 triungulins in the  
3 middle of the arena and we recorded their distribution among 4 fields after 20 minutes.  
4 Experimental data were analysed with the computer program SPSS for Windows: we performed  
5 *chi-square test* and *Cohen's kappa coefficient test*.

## 7 RESULTS

8 Preliminary 20-min experiments of host location in open arenas with diffuse lightening suggested  
9 that triungulins were attracted by the presence of living wasp immatures. When we placed batches  
10 of triungulins and one wasp larva inside the arena, they usually managed to approach the target  
11 within the first 5 minutes of activity. Typically they moved towards it not directly, but describing  
12 round trajectories and lifting their body from one side to the other, alternatively. They were able to  
13 jump, by means of their caudal setae. In 5 out of 6 trials, more than 50% of triungulins approached  
14 the larva, but only a few of them began to move on the surface of the larva, whereas the others were  
15 out of our sight under the larva or disappeared from the arena at the end of the trial.

16 Further choice trials between a putative host and no wasp as control (inside a tube, i.e. a  
17 confined space) showed a rather unexpected pattern (Fig. 2). First of all, we checked for positive or  
18 negative phototaxy. Light really seemed to be an attractive factor: in all 9 trials considered, after 1  
19 hour more than 2/3 of our triungulins were positioned in the side of the tube facing the light source.  
20 For this reason, following trials were carried on by covering the tube. Among 27 trials, a significant  
21 asymmetrical distribution ( $\chi^2$  test) was observed in 21 trials, either after 1 hour or 4 hours and we  
22 observed a choice for the wasp larva in 13 cases at time 1 and in 14 cases at time 2. Despite the  
23 evidence of a trend to move towards the larva, the difference was not significant at both times ( $\chi^2 =$   
24  $1.18$  and  $2.32$ ,  $df = 1$ ,  $P_{Exact} > 0.05$ ). At all, in 15 trials in which triungulins were asymmetrically  
25 distributed, their position in respect to wasp larva and control at time 2 was not different compared  
26 to time 1, which means they tended to maintain the acquired position within the tube for longer time

1 spans: the inter-rate agreement was significant ( $K_{Cohen} = 0.609$ ,  $P = 0.022$ ). Finally, this set of trials  
2 revealed a trend towards gregariousness for *triungulins* in regard to their movements within the  
3 tube: irrespectively of their choice for wasp larva or empty tube, more than 2/3 of the members of  
4 each experimental cluster were grouped in 21 trials out of 27, both at time 1 and time 2 ( $\chi^2 = 8.33$ ,  
5  $P_{Exact} = 0.006$ ).

6 The use of single stimuli associated to the presence of the host showed that epicuticular  
7 compounds from wasp immatures did not influenced *Xenos* *triungulins*, once they were outside  
8 mother's cephalothorax. In 6 trials with diffuse lightening, *triungulins* were never clustered around  
9 spots of wasp cuticular compounds but once were significantly grouped in the opposite field with  
10 fly odorant and twice in fields without any signal ( $P_{Exact} < 0.001$ ): their overall distribution was  
11 random ( $\chi^2 = 4.96$ ,  $df = 3$ , *ns*).

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## DISCUSSION

14 Contrary to our expectations due to their short life-span, *X. vesparum* *triungulins* do not appear  
15 particularly reactive to host-associated stimuli. Nevertheless, their approaches to the wasp silhouette  
16 in an open arena suggest that they can locate the host. Their peculiar behaviour while directing  
17 themselves towards the target is noticeable: round trajectories and repeated inclinations of the body  
18 seem to respond neither to a visual nor to an acoustic stimulus (in this case we would expect a  
19 straighter displacement). On the contrary, this attitude could be interpreted as a symmetrical  
20 chemio-reception by means of antennae or further sensory organs, hypothetically placed on both  
21 sides of *triungulins* body. Anyhow, it is difficult to establish whether their movements are the motor  
22 pattern of the infection process or something casual: they jump on the wasp larva, explore it,  
23 sometimes stop there, sometimes leave and go behind later.

24 The double-choice tests in a confined space, simulating a sheltered nest, did not confirm our  
25 first expectation, i.e. that *triungulins* are able to exploit chemical cues to redirect their movements  
26 towards the host: they grouped at both extremities of the tube, independently on the

1 presence/absence of the wasp larva (see Fig. 2). Then we analysed the influence of single factors,  
2 the cuticle compounds of wasp larvae first, i.e. mainly non-volatile, long-chain hydrocarbons,  
3 which were critical for nest-mate identification in wasps (Gamboa 2004) and were recently  
4 described in *P. dominulus* larvae too (Cotoneschi et al. 2007). Unfortunately, spots of epicuticular  
5 compounds did not elicit the arrestment of triungulins on the spot, as we would expect based on  
6 similar four-well arena experiments, where chemical stimuli of bee larval food influenced the  
7 movements of *Varroa destructor* (Nazzi et al., 2004), as well as in several egg parasitoids (Fatours  
8 et al., 2008). Laboratory artefacts are unlikely, due to our simple experimental set-up, nevertheless  
9 cuticular extracts need to be concentrated in future analyses.

10         Relatively to light as directional cue, we argue that it is probably more important for  
11 promoting triungulins' exit from mother cephalothorax than for orientating their movements once  
12 outside. We already know that blowing gently at the cephalothorax evokes the emergence of  
13 batches of 1<sup>st</sup> instar larvae within a few minutes (Kathirithamby 2009), because a light CO<sub>2</sub>  
14 emission might simulate the presence of a potential host (Guerenstein & Hildebrand 2008); once  
15 activated inside the mother's body, *Xenos* larvae may exploit a light cue to find the exit of the brood  
16 canal, i.e. the cephalothorax brood opening (Beani et al. 2005). Maybe they simply react to the CO<sub>2</sub>  
17 and light stimulus by increasing their activity without taking into account any specific direction  
18 (David Giron, *pers. comm.*). Thus, "kinesis" (a non-directional stimulus-dependent change) seems  
19 to be more appropriate than "taxis" (a directional change towards gradient stimulus intensity),  
20 nevertheless in these first trials we did not measure changes in the activity of triungulins in presence  
21 or in absence of a stimulus. As a matter of fact, during laboratory infections, clusters of triungulins  
22 normally formed at the end of the abdomen of parasitized wasps (*pers. obs.*).

23         It is possible that triungulins perceive the presence of the host but do not take into account  
24 any directional information, due to their direct release on the comb by a vector, a foraging wasp as  
25 well as a stylopized wasp releasing triungulins on different colonies (Beani & Massolo, 2007). If we  
26 consider that *Polistes* nests are already crowded of wasps at early summer, when most of the

1 strepsipteran infections occur, triungulins must have a huge choice and little time to decide. Wasp  
2 larvae are very reactive, provided with mandible, and adults collaborate with them in trying to  
3 remove the annoying tiny pests from the nest. These reactions belong to the first step of insects  
4 defence process against parasitoid invasions, better known as “behavioural defence” (Bailey & Zuk  
5 2008; Schmid-Hempel & Ebert 2003). In insect colonies, behavioural defence represents a key  
6 element of the social immune system, whereby single individuals cooperate to obtain avoidance,  
7 control or elimination of parasitic infections (Cremer et al. 2007). The annual colonies of paper  
8 wasps may be considered as a small “homeostatic fortresses” (Hughes et. al. 2008), where  
9 individuals control the disease due to both their hygienic behaviour and early desertion of the nest  
10 by parasitized inactive adults (Hughes et al, 2004 b).

11       The dynamic of the process is here complicated by the fact that both *Xenos* larvae and  
12 *Polistes* hosts are gregarious (Fig. 2). Regardless of the modality of transportation on wasp nest,  
13 data on parasite prevalence in naturally infected colonies (Hughes et al. 2003; Vannini et al., 2008)  
14 let us suppose that many triungulins (maybe decades) are contemporary present on the same nest,  
15 searching for a suitable *P. dominulus* larva where to penetrate. Gregariousness – here the tendency  
16 to aggregate in confined as well as in open space – promotes the displacement of batches of  
17 infective larvae through the brood canal and could have evolved to increase parasitism success  
18 when conditions are favourable. Peaks of nest infections on the field happen during the hottest  
19 hours of the day, when most foragers are outside the nest (Ortolani & Cervo 2009): this moment  
20 corresponds also to high metabolic activity of wasp larvae (thus high production of CO<sub>2</sub>) and  
21 intense light. We cannot exclude any cooperative action between sibling parasites during the  
22 infection process (Costa & Fitzgerald, 1996): a decreased risk of predation is likely to occur in  
23 group. On the other hand, movements in group could reduce host-seeking efficiency, introducing  
24 possible mutual interference among triungulins (Royer et al. 1999); however, no competition has  
25 been observed among triungulins, as confirmed by successful superparasitism by sibling and non-  
26 sibling individuals (Vannini et al., 2008).

1 For many aspects, the behavioural patterns of *Xenos* infective larvae remind us of *Meloe*  
2 *triungulins*, even though they belong to different insect orders (Strepsiptera and Coleoptera,  
3 respectively). Also beetle 1<sup>st</sup> instar larvae are gregarious, in fact they cooperate in forming  
4 aggregations (they move as a unit) and in holding onto vegetation, waiting for a foraging bee that  
5 can bring them to the nest (Hafernik & Saul-Gershenz 2000). The cooperation and chemical  
6 communication exhibited by *Meloe* *triungulins* for mutual benefit is so evident (sometimes they  
7 form living bridges with the aim to attract and contact the bee) that some authors suggest that it  
8 transcends aggregation behaviour to become social behaviour (Saul-Gershenz & Millar 2006). In  
9 our case, gregariousness is not such an advanced phenomenon, but it could represent anyhow an  
10 initial step in this direction. Another similarity with beetle 1<sup>st</sup> instar larvae is the negative  
11 thermotaxy: *Xenos* *triungulins*, in fact, tend to move in the opposite direction as respect to a heat  
12 source (*pers. obs.*).

13 Proximate explanations for *triungulins* behaviour are otherwise still to be defined. A  
14 parsimonious hypothesis – based on the peculiar ecology of parasite and host – could be that, once  
15 on the nest, *triungulins* are more likely to find very easily a host, especially if they do not  
16 discriminate host quality. A few choice trials between large vs small larvae, or healthy vs infected  
17 ones, suggested that *triungulins* “overwhelmingly entered the first host they encountered” (Hughes,  
18 2003). Based on our previous experiences, there is no evidence of any strict physiological barrier in  
19 host selection mechanisms by *X. vesparum*, differently from what reported in other host-parasitoid  
20 systems. In the laboratory we managed to successfully infect various developmental stages, naïve or  
21 already parasitized wasps (superparasitism by sibling and non-sibling *triungulins* is also well  
22 diffused in the field, see Vannini et al. 2008), males which are less parasitized than females in  
23 nature (Hughes et al. 2004a; Hughes et al. 2004b) and even non-primary hosts, as *P. gallicus*, in  
24 which the parasite development was interrupted (Manfredini et al. 2007b). These findings clearly  
25 show that, in our system, ecological-behavioural barriers are perhaps more important than chemical  
26 and visual signals for a perfect tuning of the parasitoid with the biology of the host.

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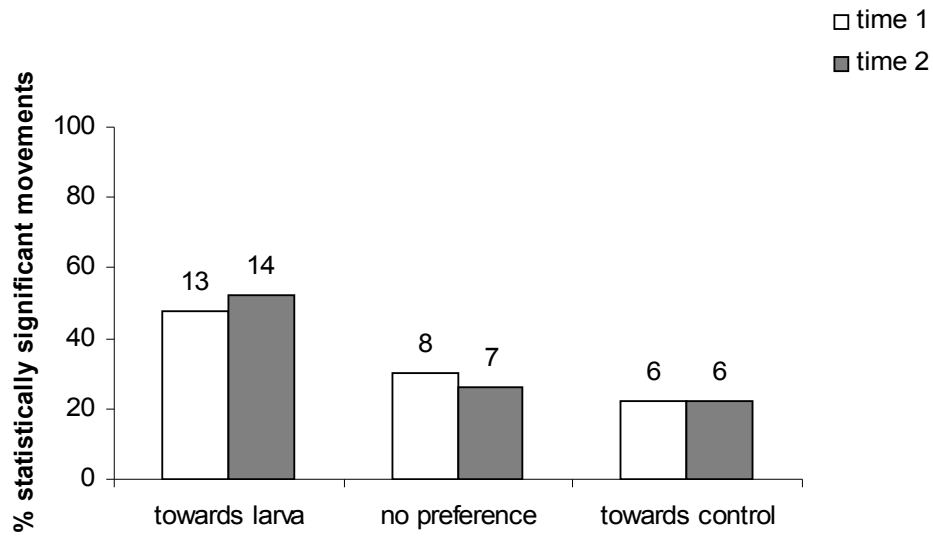
1 **FIGURE CAPTIONS**

2

3 **Figure 1** – Scanning electron micrographs of *Xenos vesparum* 1<sup>st</sup> instar larvae. (A) Dorsal view of a  
4 triungulin: note disc-pulvilli on the first two pairs of legs and spines on tarsi in the last pair  
5 (**arrowhead**). (B) Dorsal view of the cephalic segment with external photoreceptors well evident  
6 (**arrows**), whereas “Stemmata” (*sensu* Pohl 1998, meaning ocelli) are here not visible. (C) Ventral  
7 view of the cephalic segment, with mandibles (**arrow**), jaws (maxillae, **Mx**) and labium (**Lb**). (D)  
8 Detail of a disc-pulvillum on the first pair of legs. (E) Two pairs of setae on the last abdominal  
9 segments: caudal appendages (**arrowheads**) are longer and thicker.

10

11 **Figure 2** – Double-choice trials by groups of triungulins (active individuals ranging from 15 to 35  
12 for each group) moving inside a tube with one wasp larva at one extremity and no wasp as control.  
13 For both time points (**time 1** = 1 hr and **time 2** = 4 hrs) the percentage of trials is reported where  
14 triungulins were significantly grouped (*chi-square test*) towards the wasp larva or the control.  
15 Above each column is the number of observations (out of a total of 27 trials).



1

2

3 **Figure 2**

