

Effects on Microhymenopteran Progeny of Different Host Exposure Periods (*Chrysomya megacephala*, Calliphoridae) to the Parasitoid Wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae)

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ABSTRACT

To test the assumption that exposure of the host to parasitoid for long periods could provoke superparasitism, the aim of this work was to test the consequences on the immature development time, productivity of parasitoids per pupa, sex ratio and rate of parasitism of *Nasonia vitripennis* bred in *Chrysomya megacephala* pupae. Each individual pupa was placed in a glass tube with one parasitoid female for 24, 48, 72 and 96 h period of exposure, under controlled laboratory conditions. Twenty replicates of each period were performed. ANOVA with a 5% significance level was applied. The 72 h exposure caused the immature development time to increase. The mean parasitoids emergence per pupa did not vary significantly with the time of exposure. There were a significantly higher number of females than males and a trend in sex ratio deviation towards the females in all of the treatments. An increase in the number of unviable pupae rates were observed with increased exposure time.

Key words: Biological control, calliphorids, immature development, sex ratio, superparasitism

INTRODUCTION

The parasitic wasp *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae) is an ectoparasitoid of dipteran pupae, such as blowflies, flesh flies and muscid flies. It is considered to be a polifagous species, due to its

capacity to parasitize a large number of host species. Whiting (1967) reported the existence of 68 species of cyclorraphan muscoids that were parasitized by *N. vitripennis* and, more recently, a number of new hosts have also been reported (Marchiori, 2004).

This species is widely distributed geographically

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and is found in all the continents (Rueda and Axtell, 1985). In Brazil, the first report of this species dates back to 1985, and describes the parasitism of *Chrysomya* sp. (Calliphoridae) pupae (Madeira and Neves, 1985).

In their natural environment, the calliphorid flies use discrete and ephemeral substrates for feeding and laying their eggs or larvae and thus may be vectors of many diseases (Maldonado and Centeno, 2003; Oliveira et al., 2003; Carvalho and Von Zuben, 2006). These flies can also act as egg vectors for *Dermatobia hominis* (Linnaeus Jr., 1781) (Marinho et al., 2003). In addition, the larvae of these dipterans can cause myiasis in humans and animals (Greenberg, 1973; Guimarães et al., 1983; Furlanetto et al., 1984; Guimarães and Papavero, 1999; Sukontason et al., 2005).

In Brazil, muscoid dipteran control has been performed almost exclusively with the use of insecticides. However, the indiscriminate use of these products may cause severe environmental damage, since they are toxic to living organisms and also induce the development of resistant insects. Therefore, studies relating to the biology of these organisms and their parasitoid-host relationships are important, as they may enable the development of biological control techniques.

Successful parasitism by insect parasitoids is usually divided into hierarchical requirements, consisting of habitat location, host acceptance, and evaluation and physiological regulation of the host (Brodeur and Boivin 2004). The discrimination between a low and high quality host is performed by collection of a small sample of the pupae hemolymph through the parasitoid female ovipositor (Wylie, 1958, 1965; Whiting 1967, King and Ellison, 2006). The females that neglect the host quality and ovipose in an old or cryoconserved hosts (King and Skinner, 1991; Milward-de-Azevedo and Cardoso, 1996) and already parasitized puparium, usually suffer a reduction in the quantity and quality of their progeny. The oviposition in parasitized puparium can be due to the occurrence of superparasitism, that refers both to one female ovipositing repeatedly in a single host and to more than one female ovipositing in a single host (Wylie, 1963, 1965; Van Dijken and Waage, 1987).

The aim of this study was to investigate the effects and consequences on the progeny of *N. vitripennis* after exposing one pupa of *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) to one nulliparous female of *N.*

vitripennis. The periods of exposure that were tested were 24, 48, 72 and 96 h. In order to test the assumption that exposure long periods of exposure could provoke superparasitism, an attempt was made to answer the following questions: a) Were the immature development time, the parasitoid emerging from pupae, and the sex ratio of the progeny influenced by different exposure periods of the host to the parasitoid? b) Did the rate of parasitism increase with increased exposure periods to the host?

MATERIALS AND METHODS

The *C. megacephala* colony was established from adults and larvae collected at the Rio-Zoo Foundation, which occupies an area of 138,000 m², located at the park of Quinta da Boa Vista, São Cristóvão, Rio de Janeiro State. The dipteran sampling was carried out using the traps that followed Mello et al. (2007), and contained sardines as bait. The methodology used for maintaining the stock colony was as described by Milward-de-Azevedo et al. (1995).

Capture of the parasitoids was also carried out at the Rio-Zoo Foundation. Netted cages (15 x 20 cm) were used for sampling and the net allowed the potential parasitoids to enter the cage. Inside these cages, 100 *C. megacephala* pupae varying from 0-24 h age were taken from the stock colony, and were exposed to the parasitoids. Rotting bovine meat was dispensed in another container as a source of kairomone (Cardoso and Milward-de-Azevedo, 1996). After 72 h of parasitoid exposure in the field, the potentially parasitized pupae hosts were individually collected into glass tubes (50 x 10⁻³ L), which were then sealed with cotton plugs and taken to the laboratory, where adult dipterans or parasitoids were expected to emerge. This procedure was repeated until *N. vitripennis* were obtained. These individuals were identified based on the taxonomic description of Rueda and Axtell (1985). The parasitoids that emerged were transferred to glass cages (1 L) and fed with honey droplets adhered to filter paper, and moistened cotton was used both as a source of water and to preserve humidity inside the glass cages. In order to maintain the *N. vitripennis* stock colony, *C. megacephala* pupae that were up to 24 h age were regularly offered to the female parasitoids.

The experiment was performed in controlled abiotic conditions (T= 27 °C day, 25 °C night, 60

$\pm 10\%$ relative humidity and 14 h photophase). Nulliparous *N. vitripennis* females up to 24 h age and measuring from 2 to 2.5 mm were used. These females had been previously maintained with males and fed with honey to promote mature ovaries (Wylie, 1965). Fresh *C. megacephala* pupae of up to 24 h age were used, and weighed using semi-analytical scales with a range of 0.01g in groups of ten pupae for weight padronization.

Each individual *C. megacephala* pupa was placed in a glass tube (50×10^{-3} L) with one nulliparous *N. vitripennis* female for one of four defined periods of exposure of 24, 48, 72 and 96 h under controlled laboratory conditions. The tubes were sealed with cotton plugs. Twenty replicates of each period of exposure were performed. At the end of the exposure period, the female parasitoids were discarded and the pupae were maintained isolated in their tubes, until *C. megacephala* or *N. vitripennis* emerged. The nulliparous parasitoid females belonged to the 8th generation of the stock colony and the pupae used in the treatments belonged to the 9th generation of the stock colony. Parallel to these experiments, one group of pupae was not exposed to parasitism and used as a control, in order to determine the calliphorid flies' natural mortality rate. The subjects were observed daily and maintained until the 25th day after the first adult parasitoid had emerged.

The sex ratio was determined using the method described by Silveira Neto et al. (1976), where they defined the sex ratio as: $sr = n^{\circ} \text{ female} / (n^{\circ} \text{ female} + n^{\circ} \text{ male})$.

The rate of parasitism was defined as: $pr = \text{number of puparium with emerged parasitoids} / (\text{total number of puparium exposed to parasitism}) \times 100$. ANOVA with a significance level of 5% was used, and a posteriori comparisons were made using the Tukey-HSD test (Zar, 1999) in order to test for possible differences in the duration of immature development time and the number of emerging parasitoids from each pupa between the different treatments.

RESULTS AND DISCUSSION

The immature development time, from oviposition to adult emergence varied significantly among different exposure periods (ANOVA: $F = 12.28$; $p < 0.001$). The mean maximum duration of development was observed in the 72 h exposure, and this differed significantly from all the other exposure periods (Table 1). The developmental time of the female parasitoids was also significantly different between the treatments (ANOVA: $F = 8.68$; $p < 0.001$). A shorter duration of development time was observed in the females from the treatments with exposure periods of 24 and 48 h (Table 1), in contrast, the mean developmental time of the males was not significantly different between the different treatments (ANOVA: $F = 2.23$; $p = 0.1$) (Table 1).

Table 1 - Immature developmental time (days) of *Nasonia vitripennis* bred in *Chrysomya megacephala* pupae exposed to parasitism for different periods, using one host to one female parasitoid association (T=27 °C day, 25 °C night, $60 \pm 10\%$ RH and 14 h photophase). Different letters indicate significant differences obtained through Tukey test.

Different exposure periods	Immature development time (days)								
	Female			Male			Male and Female		
	Mean	\pm sd	Range	Mean	\pm sd	Range	Mean	\pm sd	Range
24 h	14.03 _a	0.21	14 - 16	14.23 _a	0.48	14 - 16	14.06 _a	0.27	14 - 16
48 h	14.13 _{ab}	0.44	14 - 17	14.17 _a	0.38	14 - 15	14.14 _a	0.43	14 - 17
72 h	14.24 _b	0.65	14 - 17	14.20 _a	0.49	14 - 16	14.23 _b	0.63	14 - 17
96 h	14.19 _b	0.60	14 - 17	14.06 _a	0.24	14 - 15	14.06 _a	0.23	14 - 17

*sd= standard deviation, Range= minimum - maximum

A peak of parasitoid emergence was observed on the 14th day after host exposure to the parasitoid for all the treatments. The parasitoids from 72 and 96 h treatments emerged until the 17th day and the

parasitoids emergence from treatments of 24 and 48 h were only until the 15th and 16th days, respectively (Fig. 1). Barbosa (2006) observed that the duration of the immature development time of

parasitoids from pupae of *Cochliomyia macellaria* (Fabricius, 1775) (Calliphoridae) exposed for 24, 48, 72 and 96 h to female parasitoids varied from 15 to 16 days, and the developmental cycle was prolonged with increasing of the exposure period,

although this change was not statistically significant. Therefore, the results observed in the present study corroborated the data from this previous study.

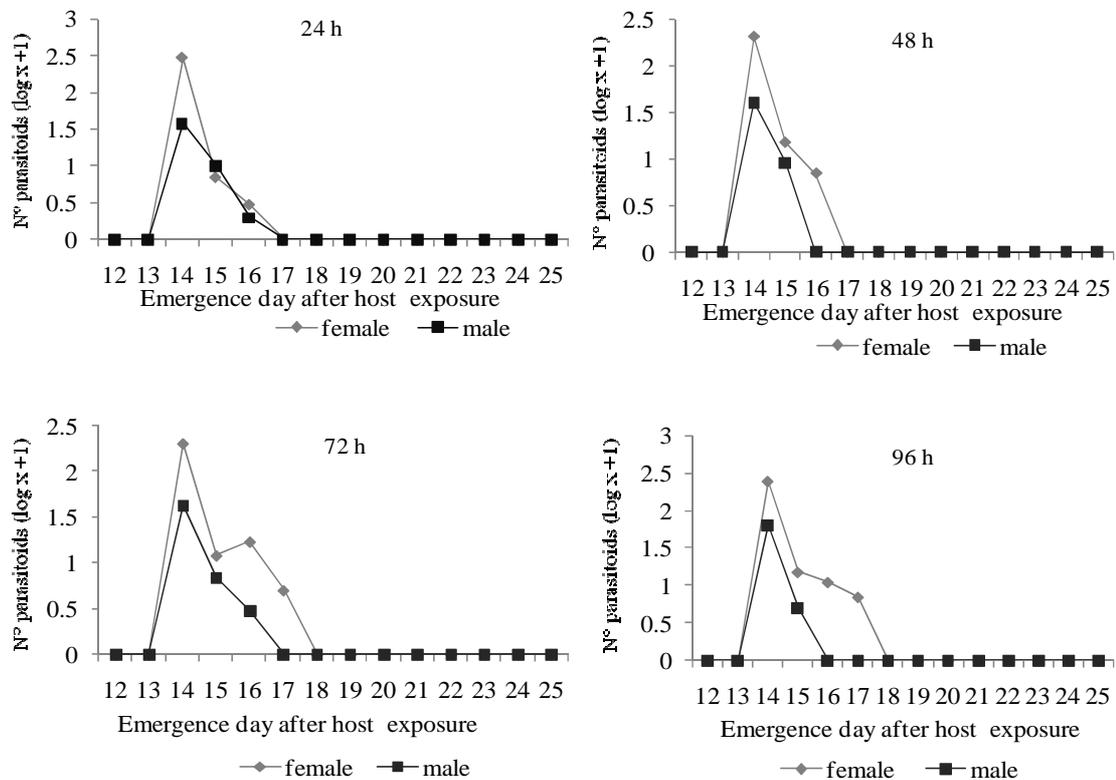


Figure 1 - Emergence of female and male of *Nasonia vitripennis* bred in *Chrysomya megacephala* pupae exposed to parasitism for different periods, using one host to one female parasitoid association (T=27 °C day, 25 °C night, 60 ± 10 % RH and 14 h photophase). *The data were transformed into the logarithm (x + 1), where x is the number of parasitoids.

The mean number of emerging parasitoids (females + males) from each pupa did not vary significantly between the treatments (ANOVA: F = 0.26; p = 0.85) (Table 2). The mean number both males and females emerged per pupa also did not vary between the treatments (ANOVA: F = 0.42; p = 0.74 and F = 1.78; p = 0.16, respectively) (Table 2). Therefore, the total number of parasitoids emerging from each pupa was not influenced by the different periods of host exposure to female parasitoids. It was expected that by using one host to one parasitoid, a trend would be apparent in the reduction of parasitoid emergence with increasing the exposure intervals, possibly due to

superparasitism, but it was not observed. Cardoso and Milward-de-Azevedo (1995) observed that the mean number parasitoids per pupa from the hosts exposed for 24 and 48 h did not vary using a 1:1 association of host to parasitoid. Therefore, the data from the present study were in accordance with the data observed by these authors. However, Barbosa (2006) used a proportion of one parasitoid to five hosts and observed a significant increase in the number of parasitoids per pupa from the hosts exposed for 72 h and a decrease in emergence when the host was exposed for 96 h. It was important to note that this author studied a distinct host species and different host-parasitoid

associations. Milward-de-Azevedo et al. (2004) studied the reproductive performance of *N. vitripennis* in cryoconserved pupae of *C. megacephala* and observed a decrease in the number of parasitoids emerged from pupae exposed for 72 h to the parasitoid. They suggested that this decrease in productivity was possibly due to intense female parasitoid exploitation of the puparium, which could cause superparasitism, resulting in an increase in the mortality rate of the immature parasitoids.

In this study, a significant reduction in the number of male was observed, when compared to the female for each treatment (Tukey Test: $p < 0.001$). Therefore, there was a trend in sex ratio towards the females (Table 2). The Fig. 2 showed a trend towards a decrease in the rate of emergence of females with increasing periods of exposure. However, the values were very similar and only varied from 0.80 to 0.86.

Many studies have suggested that the ability to control progeny sex is achieved by controlling fertilization and that males develop from unfertilized eggs and females from fertilized eggs (King, 1992). This sex allocation is, in turn, influenced by the intrinsic and extrinsic factors of the host. These factors include the presence or absence of other females on the patch, host quality (size, age, presence of toxins), quantity and distribution of hosts, and the recognition of hosts parasitized by females (Wylie, 1964; Chabora and Pimentel, 1966; King, 1987; Vinson and Iwantsch, 1980; Harvey and Gols, 1998; Husni and Honda,

2001; Mello, 2007). Under normal and favorable conditions, the proportion of females in general is larger than of males in the progeny. However, under unfavorable conditions, there is usually an increase in the proportion of males in the progeny, since they require lower quality and less resource than females to achieve maturity in the puparium (King and Hopkins, 1963).

Two major interacting factors that influence the sex ratio of parasitoids at oviposition are local mate competition and host quality. The local mate competition theory was proposed by Hamilton (1967) and predicts that the females increase the proportion of males in their progeny when multiple females are competing for one host, since they increase the chances that one of their sons will copulate with other females on the emergence local, and therefore perpetuate their genes.

Since in the present study, the parasitoid females were isolated with one host in a proportion of 1:1, there was no competition between them, thus the female parasitoids would not need to invest in increasing the male proportion of the progeny. The influence of host quality proposed by Charnov (1979, 1981) predicted that the females increased the male proportion of the progeny when they parasitized low quality hosts (Wylie, 1965, 1966, 1973; King, 1992; Harvey and Gols, 1998; Husni and Honda, 2001). The quality of a host depends on a number of physiological factors, including the presence of toxins, competing parasitoids and disease organisms and host age (Vinson and Iwantsch, 1980; Husni and Honda, 2001).

Table 2 - Sex ratio, pupae's mean weight and mean number of emerging parasitoids of *Nasonia vitripennis* bred in *Chrysomya megacephala* pupae exposed to parasitism for different periods, using one host to one parasitoid association (T=27 °C day, 25 °C night, 60 ± 10 % RH and 14 h photophase). Different letters indicate significant differences obtained through Tukey test.

Different Exposure Periods	Sex ratio	Pupae's mean weight (mg)	Number of emerging parasitoids					
			Female		Male		Male and Female	
			Mean	Range	Mean	Range	Mean	Range
24 h	0.86	58	17.7 _a	3 - 28	2.76 _a	1 - 6	20.47 _a	4 - 32
48 h	0.83	55	14.93 _a	2 - 27	4.27 _a	1 - 7	18.07 _a	2 - 31
72 h	0.82	57	16.43 _a	9 - 27	3.85 _a	1 - 7	20.00 _a	10-28
96 h	0.80	57	16.88 _a	6 - 26	4.47 _a	1 - 14	19.82 _a	6 - 30

* Range= minimum - maximum

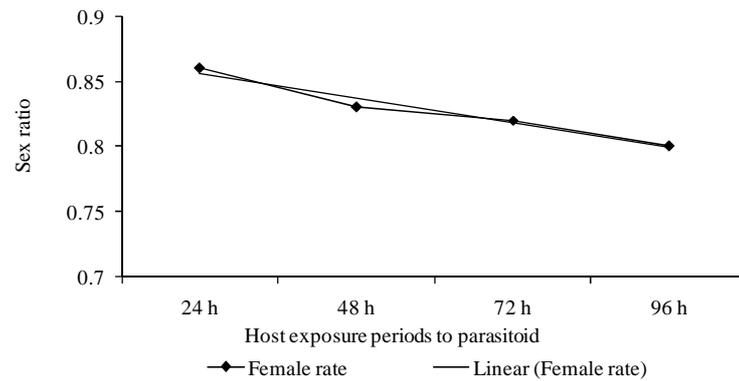


Figure 2 - Linear trend of sex ratio of *Nasonia vitripennis* emerged from *Chrysomya megacephala* pupae exposed to parasitism for different periods, using one host to a parasitoid association (T=27 °C day, 25 °C night, 60 ± 10 % RH and 14 h photophase).

The control of progeny sex may also be regulated and influenced by the age of the female parasitoid and by environmental factors such as photoperiod, temperature and relative air humidity (Velthuis et al., 1965; King, 1987). In the present study, the host quality, age of the females and environmental factors were all controlled by using pupae of approximately the same both weight and age (females of up to 24 h age) and carrying out all the treatments under the same abiotic conditions. Therefore, the only factor that could interfere in the allocation of progeny sex is possibly the period of host exposure to the parasitoid. One of the

expected consequences of this variation in exposure period would be the occurrence of the superparasitism with an increase in the proportion of the males in the progeny from pupae exposed to parasitoid females for 72 or 96 h. However, this expected result did not occur.

The rates of parasitism were similar for all of the different treatments (Fig. 3). Unviable pupae were observed in all the treatments and the emergence of dipterans was observed in only 5% of the pupae that were exposed to the parasitoid for 24 h (Fig. 3).

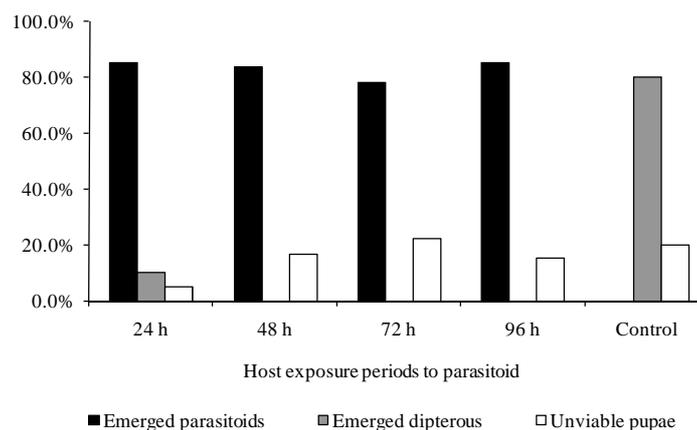


Figure 3 - Percentage of parasitized pupae (emerged parasitoids), pupae not parasitized (emerged dipterous), and unviable pupae (hosts producing neither fly nor parasitoids), using one host to a parasitoid association (T=27 °C day, 25 °C night, 60 ± 10 % RH and 14 h photophase).

Cardoso and Milward-de-Azevedo (1995) compared two exposure intervals of 24 and 48 h. They observed that pupae exposed to the parasitoid for 24 h had a reduced rate of parasitism, which allowed a high percentage of *C. megacephala* to continue their development. In contrast, dipterans did not emerge from pupae exposed to nulliparous parasitoid females for 48 h. In the present study, with increased period of exposure, there was an increase in the number of unviable pupae. According to Cardoso and Milward-de-Azevedo (1995), this corroborated the assumption that superparasitism and/or an increase in the exploitation activity and feeding habits of parasitoid females occurred during the increased periods of exposure of the host to the parasitoid.

Wylie (1965) observed that superparasitism did not influence the ability of the parasitoids to abandon the puparium and, consequently, did not interfere in the rate of parasitism. However, in the present study, a significant reduction in parasitoid emergence or the rate of female parasitoid emergence was not observed with increased exposure period, which would be consequences that would be expected to occur when hosts were superparasitized. Therefore, the superparasitism might not occur during the exposure periods of 72 and 96 h using a 1:1 association of host to parasitoid.

Some studies have suggested that the poison injected by the females when they first attacked the puparium caused metabolic alterations in the host that induced the host to be rejected by others females parasitoids, thus avoiding possible superparasitism (Wylie, 1967; Whiting, 1967; Brodeur and Bovin, 2004). However, when the probability of encountering another host is lower than the probability of winning a competition with the first occupant of the host, it is always more advantageous to accept the first host encountered, even been parasitized. This way the host discrimination is not expected to evolve. Otherwise, when hosts are aggregated, the rate of host encounters increases, and so does the probability of encountering a second higher-quality host. Under such conditions, one would expect a host-seeking parasitoid to evolve the discrimination ability (Brodeur and Boivin, 2004). The ability to discriminate between the hosts confers a significant advantage to the female parasitoids, because parasitized hosts have a lower fitness value in terms of the supply of resources

for the immature parasitoids. Therefore, by avoiding the parasitized hosts when unparasitized hosts are available, females save both time and eggs.

The microhymenopterans are also capable of choosing their hosts by size and developmental stage (Wylie, 1964; Chabora and Pimental, 1966; Cardoso and Milward-de-Azevedo, 1995; Harvey and Gols, 1998; Husni and Honda, 2001). Therefore, it was reasonable to accept that in the present study, females were able to detect parasitized hosts even using different periods of exposure to parasitism, and, in doing so, avoided the successive ovipositions in a single host, consequently avoiding the superparasitism.

RESUMO

Para testar a hipótese que longos períodos de exposição do hospedeiro ao parasitóide podem ocasionar o superparasitismo, o presente estudo teve como objetivo testar as consequências sobre o tempo de desenvolvimento do imaturo, a produtividade de parasitóides, razão sexual e taxa de parasitismo de *Nasonia vitripennis* criadas em pupas de *Chrysomya megacephala*. Cada pupa foi individualizada e alocada em um tubo de ensaio com uma fêmea de *N. vitripennis* por 24, 48, 72 ou 96 h, sob condições laboratoriais controladas. Foram realizadas 20 réplicas para cada período de exposição. ANOVA com significância de 5 % foi aplicada. A exposição de 72 h causou um aumento no tempo desenvolvimento. A média de emergência de parasitóides não variou significativamente entre os tempos de exposição. Houve um número significativamente maior de fêmeas e uma tendência ao desvio da razão sexual para fêmeas em todos os tratamentos. Foi verificado um aumento no número de pupas inviáveis com o aumento do tempo de exposição.

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Martins Dias (Universidade de São Carlos, São Paulo State) confirmed our identification of the wasp *Nasonia vitripennis*.

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