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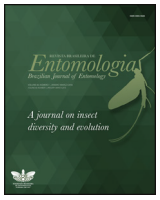

Development time and morphological characterization of immature stages of *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae) in host pupae of *Chrysomya putoria* (Wiedemann, 1830) (Diptera: Calliphoridae)

Barbara Proença[1,3*], Antonia de Castro Ribeiro[2], Valéria Cid Maia[3], Márcia Souto Couri[3], Valéria Magalhães Aguiar[4]

[1]*Universidade Federal Fluminense, Instituto de Biologia, Departamento de Biologia Geral, Niterói, RJ, Brasil.*
[2]*Instituto Oswaldo Cruz, Programa de Pós-graduação em Medicina Tropical, Rio de Janeiro, RJ, Brasil.*
[3]*Universidade Federal do Rio de Janeiro, Museu Nacional, Departamento de Entomologia, Rio de Janeiro, RJ, Brasil.*
[4]*Universidade Federal do Estado do Rio de Janeiro, Instituto Biomédico, Departamento de Microbiologia e Parasitologia, Rio de Janeiro, RJ, Brasil.*

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ABSTRACT

Nasonia vitripennis (Walker, 1836) (Hymenoptera: Pteromalidae) is a parasitoid involved in the biological control of several insects, including blow flies (Diptera, Calliphoridae) of economic importance. The aim of this study was to describe the morphological aspects of the immature stages of *N. vitripennis* and to record the postembryonic development time, using pupae of *Chrysomya putoria* (Wiedeman, 1830) as host. The experiment was conducted in a climatic chamber at 27°C/ day and 25°C/ night, with 60 ± 10% relative humidity and 14h photophase. Three experiment were conducted, each one with different host-parasitoid exposure times, of 12 hours, 16 hours and 24 hours respectively. In each experiment, the host:parasitoid relation applied was three host pupae of *C. putoria* to one parasitoid female. The experiment lasted 17 days, and it was used nine pupae per day totalizing 153 host pupae. The parasitoidism rate was 16.3% (12h), 40.5% (16h) and 94.1% (24h), respectively. The immature development duration of *N. vitripennis* with 16 and 24 hours of exposure, was as follows: egg stage (1st day), larvae (2nd to 6th day), prepupae (7th day), pupae (from 8th to 13th day) and pharate adult (14th day). The emergence of the adult parasitoids occurred on the 16th and 15th day with the exposure times of 16 and 24 hours, respectively. The exposure period of 24 hours was the ideal to observe the development time and to characterize the immature of *N. vitripennis* when compared to the 16 and 12 hours.

Introduction

Nasonia vitripennis (Walker, 1836) (Hymenoptera: Pteromalidae) has been used as a model in several studies involving genetic, ecological (King and D'Souza, 2004), behavioral (Baeder and King, 2004), evolutionary (Van den Assem and Jachmann 1982) and developmental aspects (Rivers et al., 1999). This species is worldwide distributed and can be found attacking more than 60 species of Cyclorrhapha (Diptera) and can also use other arthropods as host, including ticks (Acari) (Noyes, 2019). Rivers and Denlinger (1993) studied the action of *N. vitripennis* female venom and observed that dipterans belonging to Calliphoridae and Sarcophagidae families were the most affected hosts. This can be related to the preference by the parasitoid to this dipterous in nature (Ullyett, 1950; Cardoso and Milward-de-Azevedo, 1995).

It is important to know the time of postembryonic development of parasitoids in different host species, to assist some areas, including biological control of medical importance insects, such as *Chrysomya putoria* (Wiedemann, 1830) (Diptera: Calliphoridae), an exotic species native from Afrotropical region. This blowfly is an important mechanical vector of pathogens for humans and the larvae can cause facultative myiasis (Ferraz et al., 2011). As the immature of *C. putoria* are also decomposers of dead bodies, this species is used in forensic entomology to assist in estimating the postmortem interval (PMI) of a corpse (Leclerq and Tinant-Dubois, 1973; Anderson and Cervenka, 2002). The presence of a parasitoid in the host may influence the duration of the postembryonic developmental period or even interrupt the development of their hosts, causing an interference in the population growth and density of flies. All these aspects can alter the estimates of the PMI (Mello and Aguiar-Coelho, 2009).

*Corresponding author.
E-mail: bproenca28@gmail.com (B. Proença)

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The majority of the biological control techniques used is imported from North Temperate zone and not always adequate to the reality of the tropical countries (Neves et al., 2005). With the aim to support the implementation of biological control of economic and medical importance flies, many studies on the parasitoidism of species of *Chrysomya* and *Cochliomyia* pupae by *N. vitripennis* in laboratory have been undertaken in Brazil (Cardoso and Milward-de-Azevedo, 1996; Mello, 2007; Mello et al., 2007; Barbosa et al., 2008, 2010).

However, for a better understanding of the biology of *N. vitripennis* and its use in biological control, there is a need for a greater understanding of the development time of the parasitoid on different species of hosts at different exposure times to parasitoidism, besides knowing the morphological changes of the different immature stages of this parasitoid (Proença et al., 2022).

The aim of this study is to describe the morphological aspects of the immature stages of *N. vitripennis* on the host *C. putoria* (Wiedemann, 1830), by recording the body size and coloration changing of immature stages, the postembryonic development time of *N. vitripennis* and provide a photographic database of the immature stages of *N. vitripennis*.

Materials and methods

The experiment was conducted in a climatic chamber at 27°C during the day and 25°C at night, with 60 ± 10% relative humidity and 14 hours photophase, which started at 06:00 AM. Three host-parasitoid exposure times were evaluated: 12 hours, 16 hours and 24 hours. The host:parasitoid ratio applied was three host pupae of *C. putoria*, with up to 24 hours age, exposed to one parasitoid female. The insects were allocated into test tubes sealed with nylon fabric, using 51 repetitions per experiment (nine pupae per day, totalizing 17 days of the experiment). The host-parasitoid relationship was selected due to the efficiency of the parasitoid (Proença et al., 2022). The females of *N. vitripennis* were fed with honey and hydrated with water, both deposited in the test tube.

After the end of the host-parasitoid exposure time, the females of *N. vitripennis* were discarded and the freezing of nine pupae (three repetitions) a day until the 17th day after the beginning of the experiment was initiated. In the experiments using 12 and 16 hours of exposure, the pupae were frozen for about – 15º C, while in the experiment with 24 hours of exposure, the hosts were frozen only for 1 hour. Such variation in the time of freezing was taken to verify if the immature would present the morphological differences observed in the experiment conducted by Proença et al. (2022) using *Chrysomya megacephala* (Fabricius, 1794) as host, 18 and 48 hours of host-parasitoid exposure time and the freezing of the host pupae after exposure to parasitoidism.

By the end of the freezing time of each experiment, the pupae were dissected under a stereoscopic microscope and the immature and pharate adults of *N. vitripennis* were removed from the puparium of the host. The stages of the parasitoid were determined based on the development time, according with previous studies by Whiting (1967) and Werren (2000).

All parasitoid were measured from the egg to emerged adults stages, respecting its natural body curvature. The parasitoid stages were imaged with a Leica digital camera attached to a stereomicroscope (Leica, Wetzlar, Germany) and automated by a Syncroscopy® Auto-montage software.

The colored photos were useful to record data related to the changes in coloration and size of the adults and of the immature stages used in the study, because the preservation of these insects in alcohol probably would damage the color of the specimens, compromising further analysis of these data.

Results

The parasitoidism rate in the experiment of 12 hours of exposure was 16.3%, not allowing the observation of both morphological characterization and development time of the immature parasitoids. The parasitoidism rates with 16 hours and 24 hours of exposure was higher, 40.5% and 94.1%, respectively. In both experiments, it was possible to visualize the immature stages from the first to the 17th day of development after the exposure time. Different stages of the parasitod larva were observed in a single host specimen only with 16 hours of exposure.

The duration of *N. vitripennis* immature development with 16 and 24 hours of host-parasitoid exposure was as follows: egg stage (1st day), larva (2nd to 6th day), prepupa (7th day), pupa (from 8th to 13th day) and pharated adult (14th day). The emergence of the adult parasitoids was earlier in 24 hours occurring at the 15th day, while in 16 hours of exposure occurred at the 16th day. The parasitoid eggs showed ellipsoid shape and white corium, and were found over the last abdominal segment of the host. Along the development, the larval body grows in size and becomes fusiform, slightly curved, and gradually darker.

The results of the postembryonic development time, morphometry and morphological characters of *N. vitripennis*, after 16 and 24 hours of host-parasitoid exposure are presented on Tables 1 and 2. The images of the immature stages are presented in Figs 1 to 4.

Discussion

A low parasitoidism rate was observed after 12 hours (16.3%) and 16 hours (40.5%) of exposure, showing that these times were insufficient for the female wasp attack all three hosts. According to Wylie (1966),

Table 1
Morphological characteristics of *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae) immature after 16 and 24 hours of host-parasitoid exposure (one parasitoid: three hosts) at temperature of 27ª C day/25º C night, 60 ± 10% of humidity and 14 hours of photofase).

Day	Exposure time	Stage	Body color
1	16	Egg	Translucent white
1	24	Egg	Translucent white
2	16	Larva	Translucent white
2	24	Larva	Translucent white
3	16	Larva	Dark yellow
3	24	Larva	Yellow
4	16	Larva	Brown
4	24	Larva	Dark yellow
5	16	Larva	Dark brown
5	24	Larva	Brown
6	16	Larva	Black
6	24	Larva	Gray
7	16	Prepupa	Black
7	24	Prepupa	White
8	16	Pupa	Yellow
8	24	Pupa	Yellow
9	16	Pupa	Begining of eyes' pigmentation
9	24	Pupa	Begining of eyes' pigmentation
10	16	Pupa	Mesosoma darker than metasoma
10	24	Pupa	Yellow body and red eyes
11	16	Pupa	Black mesosoma and yellow metasoma
11	24	Pupa	Black head and metasoma. Yellow mesosoma
12	16	Pupae	Head darkening
12	24	Pupae	Black and yellow metasoma
13	16	Pupa	Black
13	24	Pupa	Black

Table 2
Morphometry and number of *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae) immature and adults after 16 and 24 hours of host-parasitoid exposure (one parasitoid: three hosts) at temperature of 27º C day/25º C night, 60 ± 10% of humidity and 14 hours of photofase). *range of variation of the insects' body measurement (in millimeters).

Day	Exposure time in hours	Stage	Sample size	Body length average in mm	Standard deviation	Coefficient of variance	Range of variation*
1	16	Egg	11	0.37	0.03	0.09	0.28-0.41
1	24	Egg	54	0.36	0.05	0.07	0.25-0.45
2	16	Larvae	05	0.45	0.04	0.09	0.39-0.50
2	24	Larvae	12	0.46	0.05	0.12	0.38-0.47
3	16	Larvae	20	0.80	0.12	0.15	0.60-0.92
3	24	Larvae	27	0.77	0.11	0.14	0.60-0.88
4	16	Larvae	16	1.08	0.16	0.15	0.80-1.27
4	24	Larvae	122	1.03	0.13	0.13	0.80-1.05
5	16	Larvae	26	2.28	0.25	0.11	1.83-2.73
5	24	Larvae	90	2.23	0.23	0.10	1.80-2.66
6	16	Larvae	45	2.40	0.39	0.16	1.24-3.06
6	24	Larvae	59	2.37	0.38	0.16	1.20-2.90
7	16	Prepupae	12	2.13	0.22	0.10	1.86-2.66
7	24	Prepupae	63	2.03	0.20	0.08	1.84-2.42
8	16	Pupae	56	2.30	0.11	0.05	2.11-2.43
8	24	Pupae	91	2.28	0.10	0.04	2.08-2.40
9	16	Pupae	30	2.29	0.13	0.06	2.07-2.49
9	24	Pupae	86	2.27	0.12	0.05	2.00- 2.45
10	16	Pupae	29	2.28	0.10	0.04	2.17-2.44
10	24	Pupae	58	2.26	0.09	0.04	2.15-2.42
11	16	Pupae	54	2.18	0.06	0.03	2.07-2.26
11	24	Pupa	37	2.15	0.06	0.03	2.00-2.25
12	16	Pupae	68	2.26	0.11	0.05	2.02-2.42
12	24	Pupae	60	2.24	0.10	0.05	2.02-2.38
13	16	Pupae	48	2.26	0.09	0.04	2.16-2.42
13	24	Pupae	49	2.26	0.07	0.03	2.14-2.40
14	16	Pharated Adult	22	2.23	0.13	0.06	2.20-2.45
14	24	Pharated Adult	74	2.21	0.11	0.04	2.10-2.38
15	16	Pharated Adult	64	2.24	0.13	0.06	2,08-2.45
15	24	Female	271	1.97	0.41	0.21	1.45-2.58
15	24	Male	60	1.93	0.25	0.12	1.48-2.30
16	16	Female	118	2.16	0.37	0.17	1.55-2.92
16	16	Male	23	1.96	0.48	0.24	1.37-2.69

one *N. vitripennis* female attacks about two hosts, in 18 hours, even if there is a major number of hosts available. This author also comments that the decrease in the parasitoidism rate can be also explained by the stress caused by the removal of the female from the colony and the transference to the test tube, the loss of olfactory attraction of the pupa during its handling as well as the inability of the female to parasitize all hosts offered in the established exposure time.

Therefore, in the presented study, it was observed that the time of 24 hours was sufficient for the female lay her eggs in all three hosts, with a 94.1% parasitoidism rate. This time was also ideal to describe the immature and the development time, because enabled the female parasitoid explore one host at time. This also contributed that each host presented similar immature stages. However, Proença et al. (2022) observed that one female of *N. vitripennis* is capable of laying eggs in all three hosts offered, in only 18 hours, but these authors used *C. megacephala* as host. These differences could be explaineb by biological differences presented by the different host species.

The different immature stages, in a single host pupa, observed in 16 hours were not expected due to the short exposure time, which could not enable various ovipositions of the parasitoid female in a single host pupa, nor the presence of immature in different stages of development. According to Slansky and Scriber (1985) the best performance of gregarious insects, meaning the survivorship rate of individual parasitoid insects, is obtained in a particular range of density of hosts with a decline of their survival occurring above or below this range, due to induction of unfavorable micro-environmental conditions for their development. Therefore, the low number of immature parasitoids per each host pupa in 16 hours would result in a heterogeneous development time of these immature. A heterogeneous developmental time was also observed in *N. vitripennis* by Barbosa et al. (2010) using *Cochliomyia macellaria* (Fabricius, 1775) (Diptera, Calliphoridae) as host.

Wylie (1964) suggested that the duration of the ontogenetic development of a parasitoid insect may be altered by environmental factors, presence or absence of superparasitism conditions, host age and quality. The quality of the host can be changed due to some factors, including the presence of toxins, competition between parasitoids andthe health of the host, which should affect the nutritional status of this one and therefore the duration of the ontogenetic development of the parasitoids (Harvey and Gols, 1998; Husni and Honda, 2001). All these factors could explain the differences between the post-embryonic development of the immature in the different times of host-parasitoid exposure used in this study.

The morphological characters as well as the development time of the immature stages of *N. vitripennis*, observed in this study, resemble those found by Werren (2000) and Proença et al. (2022) using *C. megacephala* as host. In this study, the eggs showed form

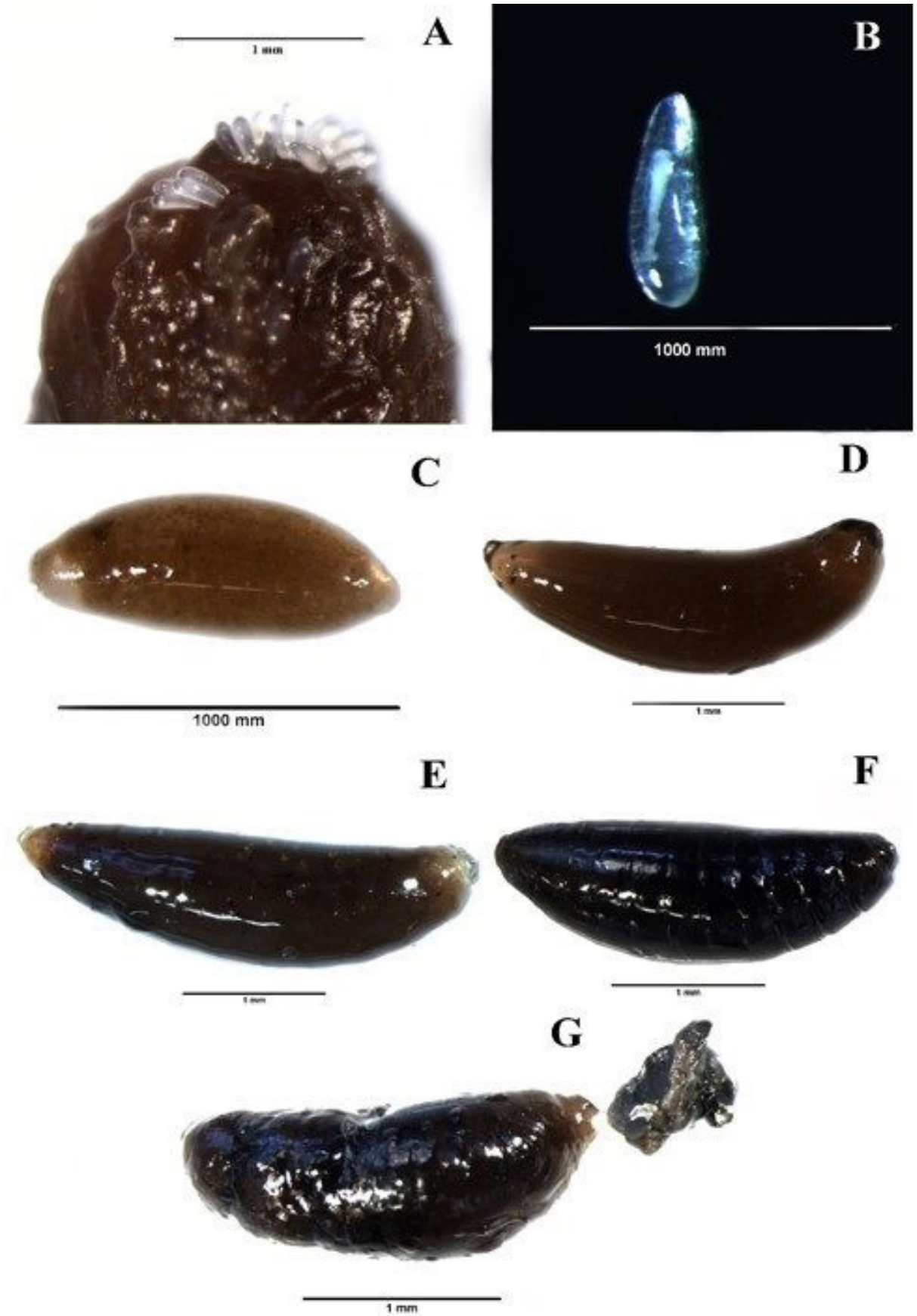

Figure 1 *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae) immature stages observed in 16 hours of exposure experiment (frozen for 17 days). Host-parasitoid exposure (one parasitoid: three hosts) at temperature of 27º C day/25º C night, 60 ± 10% of humidity and 14 hours of photofase). A: Eggs on the abdome of *Chrysomya putoria* (Wiedeman, 1830) (Diptera: Calliphoridae) on the 1st day. B: 2nd larva. C: 3rd day larva. D: 4th day larva. E: 5th Day larva. F: 6th day larva. G: Prepupa in defecation period (feces on the right side of the prepupa).

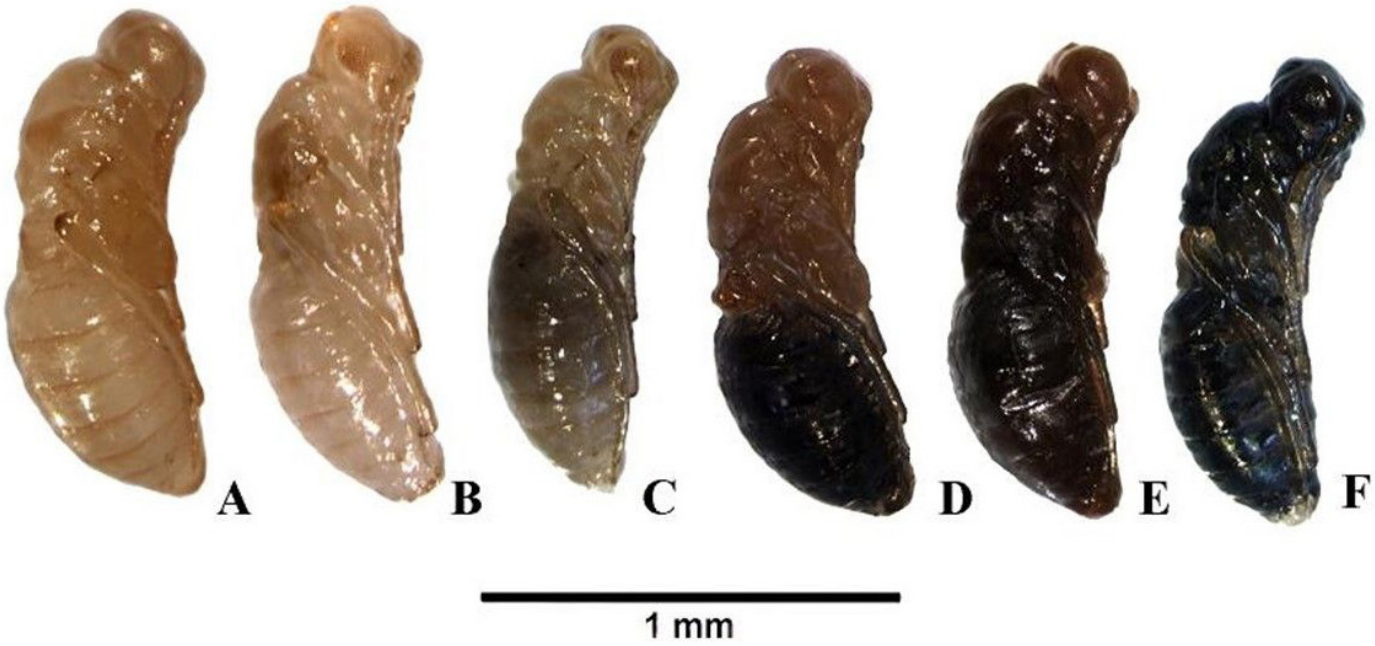

Figure 2 *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae) pupae observed in 16 hours of exposure experiment (frozen for 17 days). Host-parasitoid exposure (one parasitoid: three hosts) at temperature of 27ºC day/25ºC night, 60 ± 10% of humidity and 14 hours of photofase). A: 8th day: yellow pupa; B: 9th day - begin of eye pigmentation; C: 10th day- Begining of mesosoma pigmentation; D: 11th day- Mesosoma darker than metasoma (inversion of pigmentation); E: 12th day - head darkening; F: 13th day - black pupa.

and color similar to those presented by the later authors. The larva presented a darker color in 16 hours of exposure, than the larva observed in 24 hours of exposure. The freezing of the material may cause a change in the coloration of the larva, making its integument darker than that observed in the fresh material seen by Proença et al. (2022) or those exposed to freezing for a short period of time as seen in the 24 hours.

The larva body showed a progressive increase both in width and in length, and a gradual darkening of the integument. According to Whiting (1967), the darkening of the larval integument and the increase in length are caused by the inability of the larva to defecate during all instars, accumulating mass. These parameters, increase of the body and darkening, associated with the development time were employed to characterize and describe the five larval instars found in the present study.

It was not observed a pinkish prepupal stage as presented by Whiting (1967) and Proença et al. (2022). In 24 hours of exposure experiment, gray larvae were observed on the 6th day and 24 hours later, on the 7th day, the stage of defecation, which according to Whiting (1967) is also known as prepupal stage. Besides the defecation, body modification from larvae to pupae, associated with the development time, were used to determine the prepupal stage. The color pattern of pupae (the darkening starting from the head to the metasoma) in24 hours was similar to that reported by Werren (2000) and fresh pupae dissected by Proença et al. (2022).

In 16 hours of exposure it was observed that some individuals presented a changing on the pupae coloration, with the darkening starting from the metasoma instead of the head. This same difference in pupa's color had already been observed by Proença et al. (2022), in an experiment with the same conditions in temperature, humidity and photophase, same host-parasite ratio (1:3), using as host *C. megacephala* and exposure time of 18 hours. This difference in pupa's color can be produced by the reaction of the parasitoid to cold. Apparently, *N. vitripennis* is a species adapted to cold (psychrophilic) and their immature stages can develop at temperatures up to 10º C. Experiments showed that *N. vitripennis* is able to gain additional protection at low temperatures through the acquisition of cryoprotectant substances during its larval growth (Grassberger and Frank, 2003). Larvae of *N. vitripennis* fed on pupae of *Sarcophaga crassipalpis* Macquart, 1839 (Diptera: Sarcophagidae), a species that suffers diapause and presents high levels of glycerol, are more capable to survive at lower temperatures than those who feed in pupae of *S. crassipalpis* that does not undergo diapausing (Rivers et al., 2000).

In the present study, we observed that the immature stages of *N. vitripennis* are resistant to cold, because even after one hour exposed to a temperature of - 15º C, the immature remained alive and presented body mobility. Another interesting fact was the resistance of these insects immersed in 70% ethanol. Even after the immersion of them in this liquid for 30 minutes, the larvae remained alive and mobile, difficulting the photographic record. The parasitoid larvae stopped moving (probably died) only after an uninterrupted hour of immersion in 70% ethanol.

According to Jacobi (1939), the body length in adults of *N. vitripennis* varies from 1.0 to 3.5 mm in females and between 0.6 to 2.4 mm in males, but these two extremes are rarely found. The body length of the adults observed in this study was similar to that found by the later author, and the extremes mentioned were observed only in males that showed maximum size of 2.69 mm.

The emergence of *N. vitripennis* adults started on the 16th day in 16 hours of exposure experiment and on the 15th day in 24 hours of exposure experiment. This result is similar to those obtained in studies using the same host-parasite relationship (1:3), with *C. megacephala* as host, in which the development time of the parasitoid emergency ranged from 13 to 14 days (Proença et al., 2022) or even 16 days (Mello et al., 2007).

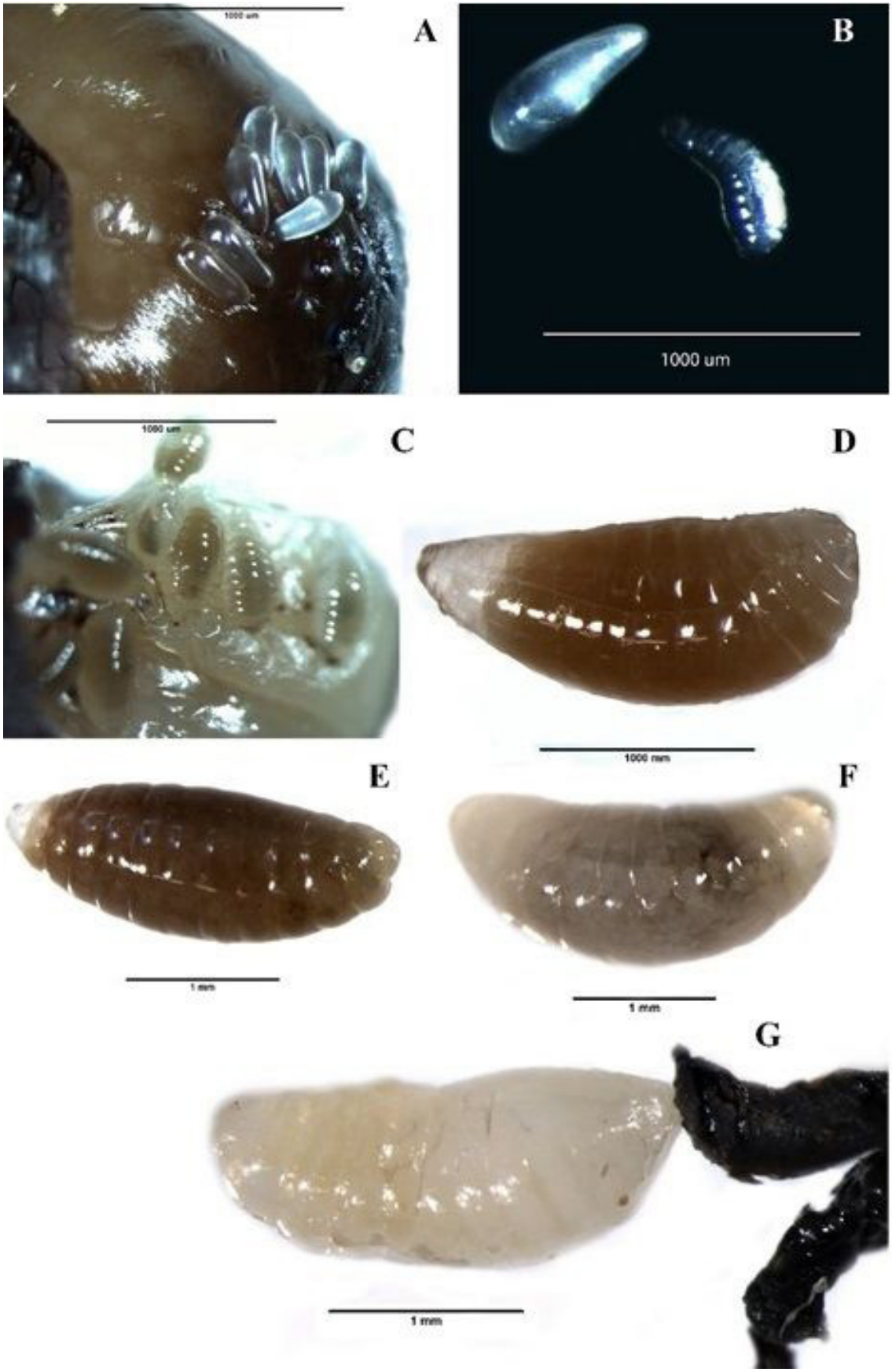

Figure 3 *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae) immature stages observed 24 hours of exposure experiment (frozen for one hour). Host-parasitoid exposure (one parasitoid: three hosts) at temperature of 27º C day/25º C night, 60 ± 10% of humidity and 14 hours of photofase). A: Eggs on the abdome of *Chrysomya putoria* (Wiedeman, 1830) (Diptera: Calliphoridae) on the first day. B: An egg and a 2nd day larva. C: 3rd day larvae. D: 4rd day larvae. E: 5th day larvae. F: 6th day larvae. G: Prepupae in defecation period (feces on the right side of the prepupae).

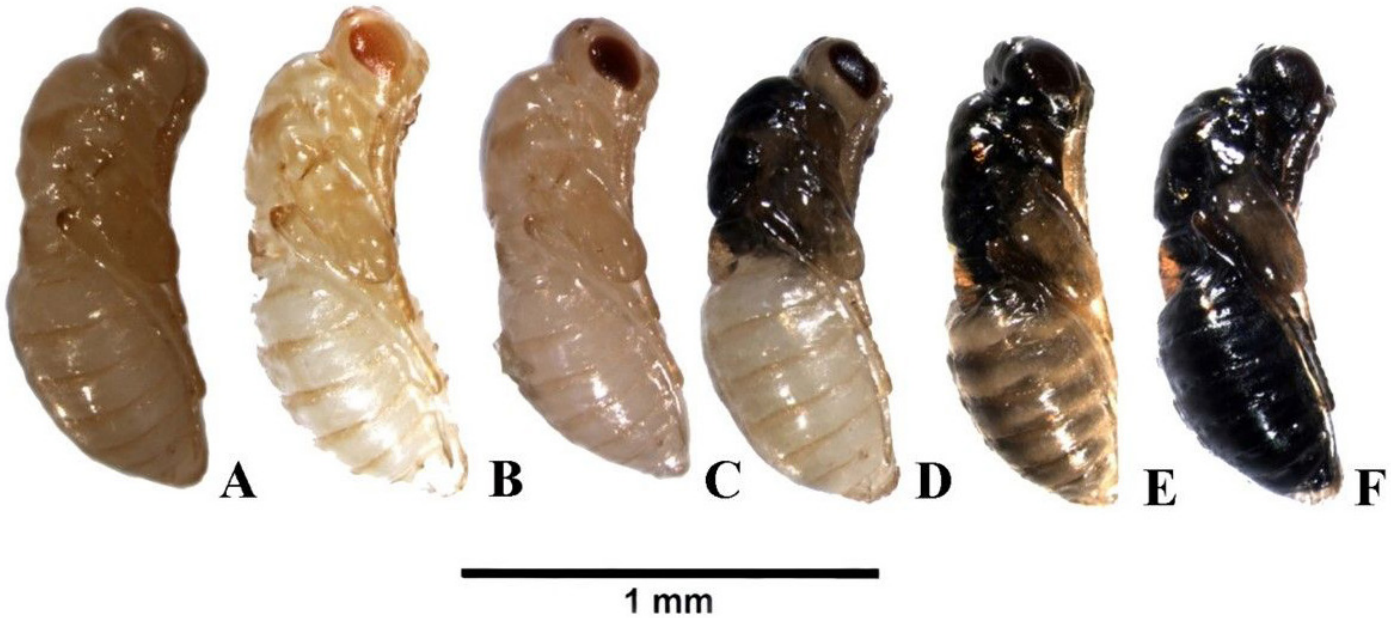

Figure 4 *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae) pupae stage observed in In 24 hours of exposure experiment (frozen for one hour). Host-parasitoid exposure (one parasitoid: three hosts) at temperature of 27ºC day/25ºC night, 60 ± 10% of humidity and 14 hours of photofase). A: 8th day- yellow pupae; B: 9th day- begin of eye pigmentation; C: 10th day- pupae with red eyes; D: 11th day- black mesosoma pupa; E: 12th day- black mesosoma and black and white metasoma; F: 13th day- black pupa.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

BP, VCM, MSC and VMA: study conception and design, analysis and writing. BP and ACR: investigation and data collection. All authors reviewed the results and approved the final version of the manuscript.

References

Anderson, G. S., Cervenka, V. J., 2002. Insects associated with the body: their use and analysis, In: Haglund, W.D., Sorg, M.H. (Eds.), Advances in Forensic Taphonomy: Method, Theory and Archaeological Perspectives. CRC Press, Florida, pp. 173–200.

Baeder, J., King, B. H., 2004. Associative learning of color by males of the parasitoid wasp *Nasonia vitripennis* (Hymenoptera: pteromalidae). J. Insect Behav. 17, 201-213. https://doi.org/10.1023/B:JOIR.0000028570.93123.dc.

Barbosa, L. S., Couri, M. S., Coelho, V. M. A., 2008. Influência do aumento do número de pupas hospedeiras de *Cochliomyia macellaria* (Diptera, Calliphoridae) no desenvolvimento do parasitóide *Nasonia vitripennis* (Hymenoptera, Pteromalidae) em laboratório. Iheringia Ser. Zool. 98, 339-344. https://doi.org/10.1590/S0073-47212008000300008.

Barbosa, L. S., Couri, M. S., Coelho, V. M. A., 2010. Desempenho do parasitóide *Nasonia vitripennis* (Walker) (Hymenoptera, Pteromalidae) utilizando como hospedeiro *Cochliomyia macellaria* (Fabricius) (Diptera, Calliphoridae), sob diferentes tempos de exposição. Rev. Bras. Entomol. 54, 125-129. https://doi.org/10.1590/S0085-56262010000100017.

Cardoso, D., Milward-de-Azevedo, E. M. V., 1995. Influência da densidade de *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) sobre a capacidade reprodutiva de fêmeas nulíparas de *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). Rev. Bras. Entomol. 39, 779-786. https://doi.org/10.1590/S0103-84782004000100032.

Cardoso, D., Milward-de-Azevedo, E. M. V., 1996. Aspectos da biologia de *Nasonia vitripennis* (Walker), (Hymenoptera: Pteromalidae) em pupas de *Chrysomya megacephala* (Fabricius) e *C. albiceps* (Wiedemann) (Diptera: Calliphoridae), sob condições de laboratório. Rev. Bras. Entomol. 40, 143-146. https://doi.org/10.1590/S0102-09352004000300024.

Ferraz, A. C. P., Dallavecchia, D. L., Silva, D. C., Carvalho, R. P., Filho, R. G. S., Aguiar-Coelho, V. M., 2011. Alternative diets for *Chrysomya putoria,* an Old World screwworm fly. J. Insect Sci. 12, 43. https://doi.org/10.1673/031.012.4301.

Grassberger, M., Frank, C., 2003. Temperature-related development of the parasitoid wasp *Nasonia vitripennis* as forensic indicator. Med. Vet. Entomol. 17, 257-262.

Harvey, J. A., Gols, G. J. Z., 1998. The influence of host quality on progeny and sex allocation in the pupal ectoparasitoid, *Muscidifurax raptorellus* (Hymenoptera: pteromalidae). Bull. Entomol. Res. 88, 299-304. https://doi.org/10.1017/S0007485300025906.

Husni, Y. K., Honda, H., 2001. Effects of host pupal age on host preference and host suitability in *Brachymeria lasus* (Walker) (Hymenoptera: chalcidoidae). Appl. Entomol. Zool. 36, 97-102. https://doi.org/10.1303/AEZ.2001.97.

Jacobi, E. F., 1939. Über Lebensweise, Auffinden des Wirtes und Regulierung der Individuenzahl von *Mormoniella vitripennis* (Walker). Arch. Neerl. Zool. 3, 197-282. https://doi.org/10.1163/036551639X00336.

King, B. H., D'Souza, J. A., 2004. Effects of constrained females on offspring sex ratios of *Nasonia vitripennis* in relation to local

mate competition theory. Can. J. Zool. 82, 1969-1974. https://doi.org/10.1139/Z05-006.

Leclerq, M., Tinant-Dubois, J., 1973. Entomologie et médicine legale. Observations inédites. Bull. Med. Leg. Toxicol. 16, 251.

Mello, R. S., 2007. Efeito da densidade de *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae) e do hospedeiro *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) sobre os aspectos biológicos do microhimenóptero. Master of Science Thesis, Universidade Federal Rural do Rio de Janeiro.

Mello, R. S., Sabagh, L. T., Aguiar-Coelho, V. M., 2007. Exposição de diferentes quantidades de pupas de Chrysomya megacephala (Diptera: Calliphoridae) ao microhimenopteros Nasonia vitripennis (Hymenoptera: Pteromalidae): Efeitos biológicos sobre a progénie do parasitóide. In: VIII Congresso de Ecologia do Brasil, 2007, Caxambu. Anais. Caxambu: Sociedade de Ecologia do Brasil, pp. 1-2.

Mello, R. S., Aguiar-Coelho, V. M., 2009. Durations of immature stage development period of *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) under laboratory conditions: implications for forensic entomology. Parasitol. Res. 104, 411-418. https://doi.org/10.1007/s00436-008-1213-z.

Neves, D. P., Melo, A. L., Genaro, O., Linard, P. M., 2005. Parasitologia Humana, 4th ed. Atheneu, São Paulo.

Noyes, J. S., 2019. Universal Chalcidoidea Database. World Wide Web electronic publication. Available in: http://www.nhm.ac.uk/chalcidoids (accessed 20 March 2024).

Proença, B., Ribeiro, A. de C., Aguiar, V. M., 2022. Tempo de desenvolvimento pós-embrionário e caracterização morfológica das fases imaturas de *Nasonia vitripennis* (Walker, 1836) (Hymenoptera: Pteromalidae) em pupas de *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae). In: Mota, D.A., da Silva, C.D.D. (Eds.), Produção científica em ciências biológicas. Atena Editora, Paraná, pp. 188-198. https://doi.org/10.22533/at.ed.219223003

Rivers, D. B., Denlinger, D. L., 1993. Toxicity of the venom of *Nasonia vitripennis* (Hymenoptera: Pteromalidae) towards fly host, nontarged insetcs, different developmental stages and culture insects cells. Toxicon 31, 755-765. https://doi.org/10.1016/0041-0101(93)90381-r.

Rivers, D. B., Genco, M., Sanchez, R. A., 1999. In vitro analysis of venom from the wasp *Nasonia vitripennis*: susceptibility of different cell lines and venom-induced changes in plasma membrane permeability. In Vitro Cell. Dev. Biol. Anim. 35, 102-110. https://doi.org/10.1007/s11626-999-0009-5.

Rivers, D. B., Richard, E. L., Denlinger, D. L., 2000. Cold hardiness of the fly pupal parasitoid *Nasonia vitripennis* is enhanced by its host *Sarcophaga crassipalpis.* J. Insect Physiol. 46, 99-106. https://doi.org/10.1016/S0022-1910(99)00106-7.

Slansky, J. R. F., Scriber, M., 1985. Food consumption and utilization. In: Kerkut, G.A., Gilbert, L.I. (Eds.), Comprehensive insect physiology, biochemistry and pharmacology, vol. 3. Pergamon, Oxford, pp. 87-163.

Ullyett, G. C., 1950. Pupation habits of sheep blow flies in relation to parasitism by *Mormoniella vitripennis* (Walker) (Hymenoptera: pteromalidae). Bull. Entomol. Res. 40, 533-537.

Van den Assem, J., Jachmann, F., 1982. The coevolution of receptivity signalling and body-size dimorphism in the Chalcidoidea. Behaviour 80, 96-105. https://doi.org/10.1163/156853982X00463.

Werren, J. H., 2000. *Nasonia*: An ideal organism for research and teaching. Available in: http://www.rochester.edu/College/BIO/labs/WerrenLab/nasonia/undergrad.html (accessed 20 May 2023).

Whiting, A. R., 1967. The biology of the parasitic wasp *Mormoniella vitripennis* (=*Nasonia vitripennis*) (Walker). Q. Ver. Biol. 42, 333-406. https://doi.org/10.1086/405402.

Wylie, H. G., 1964. Effect of host age on rate of development of *Nasonia vitripennis* (Walk.) (Hymenoptera, Pteromalidae). Can. Entomol. 96, 1023-1027. https://doi.org/10.4039/Ent961023-7.

Wylie, H. G., 1966. Some effects of female parasite size on reproduction of *Nasonia vitripennis* (Walk.) (Hymenoptera: pteromalidae). Can. Entomol. 98, 196-198. https://doi.org/10.4039/Ent98196-2.