

Phenology of *Spalangia endius* Walker (Hymenoptera, Pteromalidae) in pupae of *Musca domestica* Linnaeus (Diptera, Muscidae) under laboratory conditions

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ABSTRACT. Phenology of *Spalangia endius* Walker (Hymenoptera, Pteromalidae) in pupae of *Musca domestica* Linnaeus (Diptera, Muscidae) under laboratory conditions. This work describes the phenology of *Spalangia endius* Walker in pupae of *Musca domestica* Linnaeus under laboratory conditions. In order to understand the developmental cycle of *Spalangia endius* under laboratory conditions, 360 *Musca domestica* pupae aged from 24 to 48 hours were exposed to 15 *S. endius* pairs for a period of 24 hours at $26 \pm 2^\circ\text{C}$. These pupae were kept in a BOD incubator at the same temperature, with a relative humidity of $\geq 70\%$, and 12 hours photophase. Fifteen hymenopteran specimens were dissected daily to evaluate their stage and development time. The phenology concluded that *S. endius* had a development cycle of 19 days with an incubation period of 24 hours. The development of the larvae of *S. endius* occurred in the subsequent eight days, during which a series of morphological alterations were observed. The pre-pupal stage occurred on the tenth day, where the movement ceased and elimination of the meconium started. The pupal stage occurred from the 11th to the 19th day, with emergence of males first, followed by female emergence approximately 24 hours later. These results allowed the evaluation of aspects of the detailed bionomics of the development of *S. endius* in order to record and program production of this parasitoid, thus optimizing its utilization as a biological control agent.

KEYWORDS. Development; Feedlot cattle; parasitoid.

RESUMO. Fenologia de *Spalangia endius* Walker (Hymenoptera, Pteromalidae) em pupas de *Musca domestica* Linnaeus (Diptera, Muscidae) em condições de laboratório. Objetivando conhecer o ciclo de desenvolvimento de *Spalangia endius* sob condições de laboratório, 360 pupas de *Musca domestica* com idade de 24 a 48 horas foram expostas a 15 casais de *S. endius* por um período de 24 horas a $26 \pm 2^\circ\text{C}$. Estas pupas foram mantidas em BOD. Com mesma temperatura, umidade relativa $\geq 70\%$ e com fotofase de 12 horas, onde diariamente dissecava-se 15 espécimes para avaliar o estágio e tempo de desenvolvimento do himenóptero. A fenologia permite concluir que *S. endius* apresenta um ciclo de desenvolvimento de 19 dias, cujo período de incubação foi de 24 horas, o desenvolvimento de larvas de *S. endius* ocorreu nos oito dias subsequentes nos quais uma série de alterações morfológicas foi observada. O estágio de pré-pupa deu-se no décimo dia onde cessa a movimentação e inicia a eliminação de mecônio. O estágio pupal ocorreu do décimo primeiro dia ao décimo nono, momento da emergência dos primeiros machos, já as fêmeas iniciaram a emergência aproximadamente 24 horas após. Estes resultados permitem avaliar aspectos da bionomia pormenorizada do desenvolvimento de *S. endius* para armazenar e programar a produção deste parasitóide, otimizando sua utilização como agente de controle biológico.

PALAVRAS CHAVES. Desenvolvimento; gado em confinamento; parasitóide.

Animal production under confined conditions favors the development of *Stomoxys calcitrans* L. and *Musca domestica* L. (Diptera, Muscidae). Both species develop in animal feces and decomposing organic material and in the case of *S. calcitrans* cause substantial economic losses due to blood-feeding, because of the transmission of pathogens and stress caused by these two species (Weinzierl & Jones 1998).

Chemical control of these flies is most often used despite the demonstrated high possibility of resistance that reduces the efficiency of these products when used over a short period of time. In addition, chemical control affects non-target species and causes environmental contamination (Greene *et al.* 1998).

Due to their adaptation to a parasitic mode of life, some families of insects of the order Hymenoptera have become a group of organisms best adapted to exploiting their hosts (Cônsoi & Vilson 2009) by providing rates of parasitism

adequate for control of flies (Crespo *et al.* 1998; Skovgard & Nachman 2004; Birkemoe *et al.* 2009). Generally the success of parasitism depends on the genes transferred at oviposition or produced during the development of offspring (Penacchio & Strand 2006). The use of parasitoids approaches the goal of searching for an alternative for the problem of super populations of flies because it is a safe method, is easy to use, and is low in cost (Silveira *et al.* 1989; Carvalho *et al.* 2003).

The microhymenopteran *Spalangia endius* Walker, 1839 (Hymenoptera, Pteromalidae), is a solitary species that parasitizes pupae and can be found associated with species in the families Muscidae, Calliphoridae, Sarcophagidae and Tephritidae (Diptera) (Carvalho *et al.* 2003). They are also found in manure and decomposing organic material. The egg, larval and pupal stages of *S. endius* develop in the interior of the host pupa, where after approximately three weeks they

emerge as adult wasps (Rueda & Axtell 1985). Inundative release of *S. endius* is an efficient practice and produces suppression of populations of flies in confinement facilities of cattle, poultry and swine (Morgan *et al.* 1975; Inciso & Castro 2007).

Even though there is knowledge about a large number of species of parasitoids of flies, there is a scarcity of information about their biological characteristics, and these characteristics are fundamental to success in rearing them. Due to the effectiveness of this parasitoid and having a plan as to which stages of development occur inside the fly puparium, this work describes some aspects of the developmental phenology of *S. endius*. These characteristics are important because they permit optimum storage of the parasitoid.

Observation of the time and development of each instar can be used to decide which of them undergoes diapause and in this manner make conservation feasible and subsequently the synchrony of the emergence of the parasitoids. These aspects are important in mass rearing and inundative releases of *S. endius* as an alternative biological control in the implementation of integrated control in confined animal environments.

MATERIAL AND METHODS

A previously-established colony of *Musca domestica* and one of *Spalangia endius* were maintained in climate chambers with photophase of 12 hours, temperature of $26 \pm 2^\circ\text{C}$ and relative humidity $\geq 70\%$.

Adult *M. domestica* were maintained in screen cages and fed a diet of meat flour and refined sugar. In order to obtain the egg masses a substrate consisting of meat flour, sawdust and water was made available. The egg masses obtained were transferred to larger containers with the same substrate as above in greater quantities and deposited in a collection funnel. As the larvae completed their development they abandoned the funnel and fell into a container with moist sawdust after which they were transferred to 800 mL flasks where adult emergence occurred.

Adult *S. endius* were maintained in three-liter flasks and fed a 40% solution of honey and water. Recently formed *M. domestica* pupae were made available for oviposition, and following exposure, maintained in closed containers until emergence of the parasitoids.

During the course of the experiment 360 *M. domestica* pupae were exposed to 15 pairs of the parasitoid for oviposition. The pupae were 24 to 48 hours old and they remained available for a period of two days and maintained in a B.O.D. chamber at $26 \pm 2^\circ\text{C}$, humidity $\geq 70\%$ and photophase of 12 hours. Fifteen pupae were dissected daily and photographed, the eggs and first instars with a microscope equipped with phase contrast digital camera, and the later larger larvae and pupae with a stereomicroscope from the Department of Microbiology of the Biology Institute in order to evaluate the morphological stage and period of development of the hymenopterans.

RESULTS

The microhymenopteran *Spalangia endius* had a period of incubation of nearly 24 hours at $26 \pm 2^\circ\text{C}$, humidity $\geq 70\%$ and photophase of 12 hours. The eggs appeared transparent and elongated (Fig. 1).

The larval stage lasted for eight days, from the second to the ninth day of the total time of development (Figs. 2–5). This was a period of intense feeding and movement. Morphological alterations occurred such as the gradual loss of transparency of the larval tissues and appearance of the lateral tubercles on the sides of the body during the final days of development (Figs. 6–9).

The pre-pupal period (Fig. 10) lasted for 24 hours and was completed on the tenth day of development. It was characterized by white coloration of the body tissues and the initiation of the elimination of meconium.

The pupal stage lasted for eight days (Figs. 11–17), starting on the eleventh day and finishing on the 19th day of the total time of development. The pupa, whose appendices are not strongly adhered to the body and whose mandibles are not articulated, is of the exarate **adectic** type. The region of the pupa where the eyes are formed shows strong pigmentation from the fifth day of development of this stage, and the beginning of the sclerotization process of the tegument can be verified starting on the sixth day (Fig. 16).

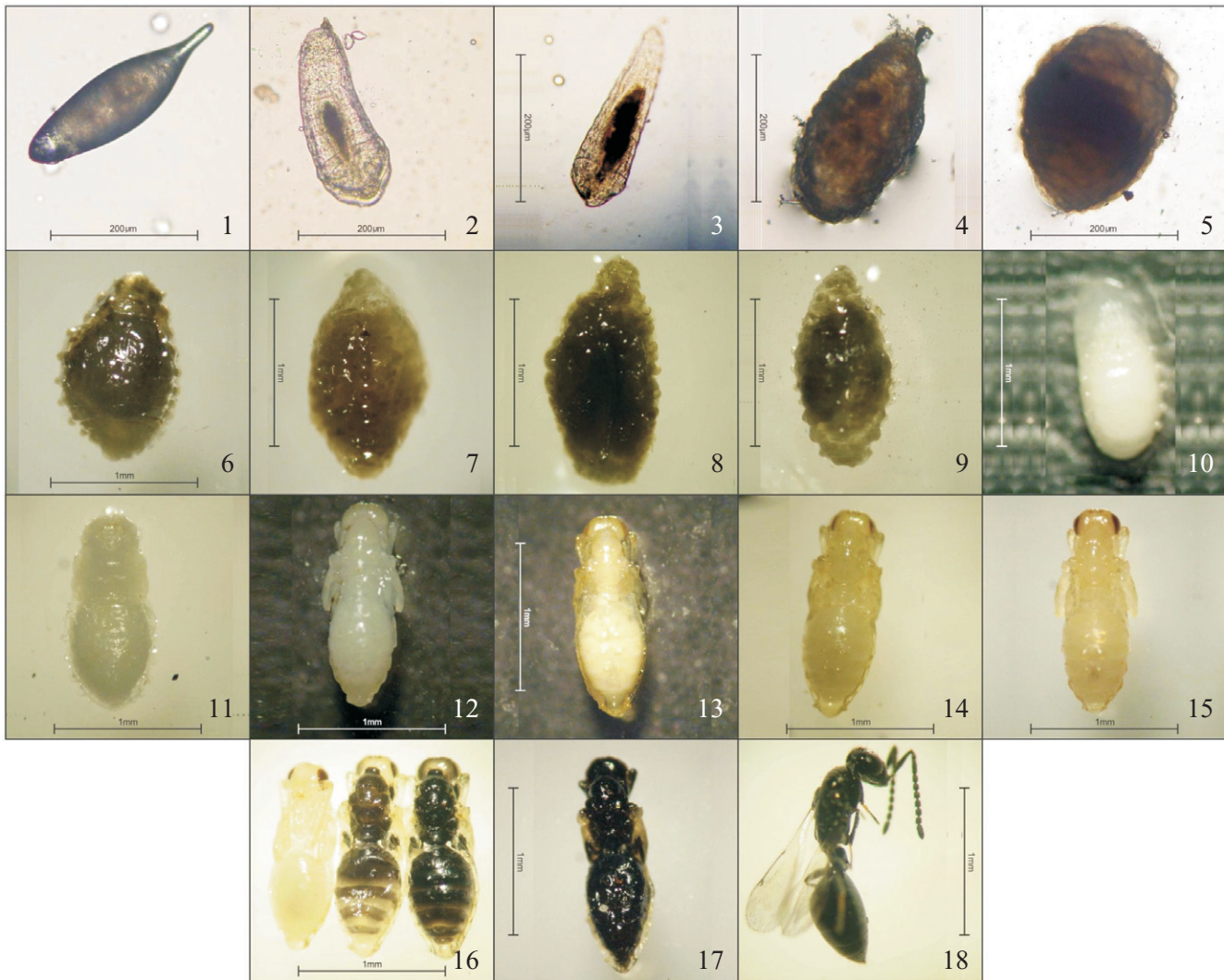
Emergence of *S. endius* begins on the 19th day of development. The first emergence of males occurs nearly 24 hours before the beginning of the female emergence. The total developmental cycle occurs in 19 days.

DISCUSSION

The incubation period of *Spalangia endius* differs from the period of *Nasonia vitripennis* (Walker, 1836) (Hymenoptera, Pteromalidae) due to the fact that under similar conditions the egg development period was of 36 hours (Werren 2000). The eggs had an elongated shape with fine chorium and were practically smooth. According to Thomazini & Berti-Filho (2001) the eggs of the majority of the pteromalids have these characteristics. The larvae represent the stage of accumulation of nutrients, and therefore, it is certain that the larval phase is that of growth of the imaginal tissues that form the morphological and reproductive structures (Cõnsoli & Vilson 2009).

Tubercles on the lateral part of the body of the larvae of *S. endius* appear in the last days of development (Figs. 6–9) as described by Thomazini *et al.* (2001) for *Muscidifurax uniraptor* (Kogan & Legner) (Hymenoptera, Pteromalidae).

The number of larval instars was not determined in the study because the methodology used did not guarantee this result. Nevertheless, studies concerning the Hymenoptera Order report the occurrence of three to five instars according to the species and depend on the size of the cephalic capsule. However, there are records of the number of instars of other Hymenoptera in the same family such as *S. endius* (p. ex. *N.*



Figures 1–18. Phenology of *Spalangia endius* Walker 1938, (Hymenoptera, Pteromalidae), in pupae of *Musca domestica* Linnaeus, 1758, (Diptera, Muscidae) at 26°C, relative humidity $\geq 70\%$ and photophase of 12 hours. (Fig. 1) egg; (Figs. 2–9) larval period; (Fig. 10) prepupa; (Figs. 11–17) pupal period, and (Fig. 18) adult. Scale 200 μm (Figs. 1–5) and scale 1 mm (Figs. 6–18).

vitripennis) with three larval instars, and *Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera, Pteromalidae) with five (Werren 2000; Tormos *et al.* 2009).

The development of the parasitoid occurs in the space between the puparium and the body of its host, and there are no cells or protective cocoons that are different from other hymenopterans (Gullan & Cranston 2008).

Emergence of various individuals of *S. endius* in a short space of time confirms the observation of King (2006) that in hosts with an aggregated distribution, multiple wasps emerge with a certain special and temporal synchrony. Another study demonstrated emergence of males two days before emergence of females (King 2002). In *N. vitripennis* emergence begins on the 14th day of development (Werren 2000) and in *P. vindemmiae* on the 18th day, and both had a pattern similar to that observed in our work for the sexes, although the interval was greater for emergence of *P. vindemmiae* females (Tormos *et al.* 2009).

The total developmental cycle occurred in 19 days, a period less than that observed by Rueda and Axtell (1985) at 26.7°C for the same species.

Knowledge of the phenology of *S. endius* serves as the basis for the development of future research of the detailed bionomics, such as identification of the instars in which diapause occurs, and the viability of stages which will permit the implementation of strategies of rearing and storage with the consequent viability for the use of *S. endius* as an agent of biological control.

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