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Life History and Biological Control Potential of *Snellenius manilae* Ashmead (Hymenoptera: Braconidae), a Parasitoid of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)

Abigaile Mia V. Javier[1,*] and Flor A. Ceballo[2,*]

[1]Agriculture Research Section, Atomic Research Division, Philippine Nuclear Research Institute – Department of Science and Technology (PNRI-DOST), Commonwealth Avenue, Diliman, Quezon City 1101, Philippines
[2]Institute of Weed Science, Entomology and Plant Pathology, College of Agriculture and Food Sciences, University of the Philippines, Los Baños (IWEP, CAFS, UPLB), College, Laguna 4031, Philippines
[*]Authors for correspondence; e-mail: avjavier@pnri.dost.gov.ph, Tel.: +63 02 929-6011 to 19 loc 274; faceballo@up.edu.ph, Tel.: +63 49 536-1315

Biological attributes relevant to the reproduction, survival and development of *Snellenius manilae*, a larval parasitoid of the common cutworm (*Spodoptera litura*), were studied. The mode of reproduction of the laboratory population of *S. manilae* was arrhenotoky. The pattern of egg maturation and deposition in *S. manilae* did not fit the strict dichotomy (synovigenic versus pro-ovigenic) but followed the Type 2 index wherein initial egg loads are lower and egg deposition increases on the first few days of life before declining rapidly. The highest number of mature ovarian eggs was recorded on day 3 and then gradually decreased on each successive day until the wasp died on day 6. The ovipositing female did not discriminate already parasitized larvae. Duration of ovipositor insertion was very short (1–2 s) and was made by the female across the cutworm body with the highest number made within the head region. *S. manilae* is a koinobiont parasitoid and oviposits across the first three larval instars but progeny production and parasitism were highest in the first instar cutworm larva for both no-choice and choice method. *S. manilae* underwent three larval instars. The total developmental period of the parasitoid from egg deposition to pupation was 10.5 ± 0.08 d, pupation to adult emergence was 4.2 ± 0.10 d, while the adult lived for 6.1 ± 0.07 d with honey as food.

Key Words: arrhenotoky, egg maturation, koinobiont, ovigeny, parasitism, *Snellenius manilae*, *Spodoptera litura*, synovigeny

INTRODUCTION

Biological control is the application of ecological principles to enhance insect pest management through the manipulation of beneficial species or natural enemies (Doutt and De Bach 1964). These beneficial species, whether parasitoids, predators or pathogens, are introduced to reduce pest populations to lower levels than would occur in their absence (De Bach 1964).

The establishment of a biological control agent is a prerequisite to successful biological control (Hoy 1985), but fulfilment of the prerequisite alone does not guarantee effective control (Islam et al. 1997). Since the control of the pest may be only partial, augmentative procedures may have to be set up to enhance the performance of the biological control agent. If augmentation is required, an understanding of the entire sequence, from release of the natural enemy through establishment to the ecological and environmental constraints acting on the host-parasitoid or predator-prey interaction, is needed if the chances of success are to be improved. Relevant information may be available, or may have to be acquired through appropriate research, and relevant research regarding each component is essential in the formulation of a natural enemy conservation program if it is to be successfully incorporated into an IPM system.

For parasitoids to be successful as biological control agents, a high fecundity is regarded as compulsory. Such species are believed capable of reacting immediately to changes in pest population levels by reproducing rapidly to hold back the pest population (Huffaker et al. 1977; Waage 1990; Ehler 1995). The achievement of highest fecundity is connected to a number of external factors and internal features, including environmental pressures such as temperature, host density, and parasitoid physiological status (Fernando and Walter 1999; Ceballo and Walter 2004). For instance, the rate of egg maturation by synovigenic species is affected by the nutritional status of the female and the availability of suitable hosts (Ceballo

and Walter 2004; Davies et al. 2005; Barnacha 2009; Ramal and Ceballo 2011). Such stimuli influence patterns and levels of parasitism in nature (Sadeghi and Gilbert 2000).

In many ways, the species-related attributes of the parasitoid also influence the patterns of host mortality produced. For instance, many parasitoid species that attack the immature stages of hosts can oviposit and develop in more than one instar of their habitual host species, so they can reproduce successfully under numerous ranges of host population structures. However, the various host stages correspond to a major ecological variable. They impact a parasitoid's speed of attack, the succeeding survival and sex ratio of its progeny. Survival of the growing stages of parasitoids may also depend on whether or not they can avoid being encapsulated and killed as eggs or larvae (Van Driesche et al. 1986).

Snellenius manilae is a solitary endoparasitoid attacking larvae of the *Spodoptera* species. The parasitoid has been reported parasitizing fall armyworm, *Spodoptera frugiperda* and common cutworm, *S. litura* in Japan, Taiwan and Thailand (Chiu and Chou 1976; Shepard et al. 1983; Rajapakse et al. 1985; Ando et al. 2006). It is also reported to parasitize *S. exigua* in China (Qui et al. 2012; Qui et al. 2013). In the Philippines, *S. manilae* was reported parasitizing *S. litura* in the field (Barrion and Litsinger 1987; Morallo-Rejesus and Javier 1997) and under laboratory conditions (Torreno 1990). The precise host requirements and oviposition behavior of the local population of *S. manilae* have yet to be determined.

This study was conducted to (1) establish the sexuality of the local population of the parasitoid, reportedly arrhenotokous species, (2) quantify the frequency at which the different host stages of *S. litura* are parasitized, (3) establish the most suitable host stage of *S. litura* for larval growth and survival, and (4) quantify the parasitoid reproductive potential.

MATERIALS AND METHODS

Mass Rearing of Host Insect, *S. litura*

Egg masses of *S. litura* were collected in corn, tomato, banana, gabi and legumes, and then kept inside covered trays. Newly-hatched larvae were then transferred into a freshly cleaned rearing tray (33 × 25 × 10 cm) provided with castor leaves as food. To ensure a healthy culture, larvae were regularly transferred to clean rearing cages with castor leaves as needed. Fully grown larvae that were about to pupate were transferred into an acrylic pan measuring 19 cm in diameter with an inch layer of moist soil as pupation medium. Three-day-old pupae were harvested and transferred into emergence cages (33 × 33 × 45 cm) to await adult emergence. Emergence cages at all times were provided with cotton dipped with 10% honey solution as adult food and also with fresh and clean camote tops as oviposition media for emerging adults. All leaves containing egg masses were collected and transferred into new and clean plastic containers and were allowed to hatch. Egg masses were checked daily to record the day of emergence. Larvae of the same ages were grouped to represent the larval instars that were used for host stage and host suitability assessments. About 25% of the emerging cutworm larvae were allowed to pupate in order to keep a continuing stock culture of the host test insect.

Mass Rearing of the Parasitoid, *S. manilae*

Two-day-old sentinel *S. litura* egg masses in the leaves were deployed in the field by placing the egg masses in the leaves of the plants for 4 d for field parasitization of *S. manilae*. On day 5 after deployment, *S. litura* larvae were retrieved and initially placed in plastic rearing trays with disinfected castor leaves as food and then brought back to the laboratory. First instar larvae were also collected from the field for possible *S. manilae* parasitization. Presumably parasitized larvae were sorted out and transferred individually into clean containers provided with disinfected castor leaves and kept under ambient temperature and relative humidity. The larvae were checked daily to observe the presence and development of the parasitoid.

Fully developed pupae of the parasitoid were separated from the host and transferred into an emergence cage to await emergence. All emergence cages were provided with cotton with 10% honey solution at all times as food for emerging adults. Upon emergence, about 100 two-day-old first instar cutworm larvae on castor leaves were exposed to the adult wasps for parasitization inside the emergence cage. Rearing was done using five cages in order to ensure a continuous supply of the parasitoid, *S. manilae* for succeeding evaluations.

Sexuality Assessment of *S. manilae*

Sub-samples (n = 20) of the emerging wasp population per generation were examined based on their external morphological characteristics to confirm the presence of male and female in the population and to establish their mode of reproduction. Confirmation of the individual sexes was done by dissection of the reproductive system of the wasps under the microscope. Only mating pairs were dissected to ensure sexual reproduction in the sub-samples. From the 20 sub-samples, the number of females and males per generation was recorded. This was done for 10 generations.

Rate of Maturation of Ovarian Egg

Rate of egg maturation of adult wasps was determined by

dissecting female wasps then counting the mature ovarian eggs. Examination started on the day of wasp emergence until death (n = 10). The wasps were immobilized (at −20 °C for 2 min) before dissection under saline solution. The last abdominal segment was carefully pulled out and placed onto a glass slide. A drop of red tissue marking dye was placed onto the exposed ovaries and after a few seconds washed off with a drop of saline solution. A cover slip was placed onto each specimen before examining the wasp under the dissecting microscope. The immature eggs absorbed the stain, while the mature ovarian eggs remained unstained and were then counted.

Ovipositional Patterns

Three-day-old wasps (n = 10) that had never been exposed to hosts were introduced to five individuals of first instar cutworm larvae within a circular transparent container with a diameter of 4 cm covered with a transparent lid. Each female was observed for 20 min, with the aid of a stereo microscope. Data on the number of cutworms that were stung, specific locations on the cutworm's body that were stung with the ovipositor, and the number of ovipositor insertions across the entire 20 min were taken. Special care was taken to observe the feeding activity (host feeding) of the adults.

Host Stage Suitability, No-choice Method

Cohorts of each larval stage were isolated in closed arenas (15 cm × 8 cm). First instar (3-day-old) was assigned and labeled L1, second instar (5-day-old) was labeled L2, and third instar (7-day-old) was labeled L3. The duration of each larval instar was based on larval development (Paris 1969). Fifteen arenas were prepared for each larval stage (L1, L2, and L3) and each arena held 10 individuals. All arenas were streaked once with honey before the wasp was introduced. One 3-day-old mated female wasp, having the highest number of mature eggs based on dissection, was introduced into each arena with the test host instar and left for 6 h before removal. The exposed cutworm instars were then maintained within the closed arenas with clean castor leaf inside at 26 ± 1 °C temperature and 65 ± 5% RH to await parasitoid emergence. The *S. manilae* adults that emerged from each host stage were counted to determine which host stage supported successful growth and development of *S. manilae*.

Host Stage Suitability, Choice Method

This setup was identical to the no-choice method, except that the three larval instars of cutworm (L1 = 10, L2 = 10, L3 =10) were placed together in each arena (n = 15, each with its own wasp). Again, 3-day-old wasps were exposed individually to each oviposition arena, now with the mixed cutworm population for parasitization.

After 6 h of exposure, the different host instars were separated and placed in a new closed arena with clean castor leaf for the S. litura larvae and a streak of honey as food for the parasitoid. The parasitoids that emerged and were produced by each female wasp were counted. Percent parasitism was determined by counting the larvae that were parasitized by S. manilae, divided by the total number of larvae that were exposed.

Statistical Analyses

Treatment means (number of mature ovarian eggs per day, the number of offspring produced from the three host stages in both the no-choice and choice methods) were compared and assessed using one-way ANOVA followed by Tukey's Honestly Significant Difference Test (HSD) at 5% level of significant difference.

RESULTS AND DISCUSSION

Sexuality and Sex Ratio of *S. manilae*

S. manilae males and females were successfully reared in the laboratory on first instar larvae of *S. litura* for over 10 generations. The wings and the legs of both males and females were morphologically similar. However, morphological differences between the males and females were observed on the antennae, thoraces and abdomens (Table 1).

Internal examination of the genitalia and closer investigation of the reproductive tract under the high power microscope showed that a newly emerged female can have mature and immature eggs and therefore ready to mate. This biological characteristic is a little bit of both a pro-ovigenic and a synovigenic parasitoid. The presence of males and females in pairs that are mating in each generation is a strong indication that the parasitoid has a sex-related parthenogenetic development or arrhenotokous mode of reproduction. Progeny are produced by mated or unmated females, but fertilized eggs produce diploid female offspring whereas unfertilized eggs produce haploid male offspring.

Mating pairs and mating activities were observed in the cage upon wasp emergence. Mating duration lasted for less than 60 seconds for all pairs (n = 26) (Table 2). The average number of males per generation was generally higher than that of the females, resulting in a male-biased population.

Rate of Egg Maturation

The ovaries of newly-emerged *S. manilae* wasps contained only a few mature eggs 5 h after emergence and the number increased steadily until day 3 (Fig. 1).

Table 1. Some morphological features of male and female *Snellenius manilae* wasps.

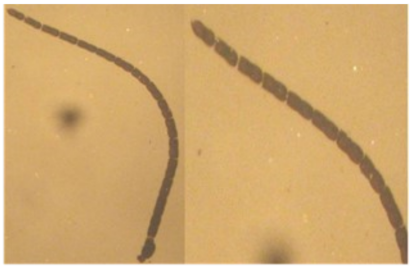
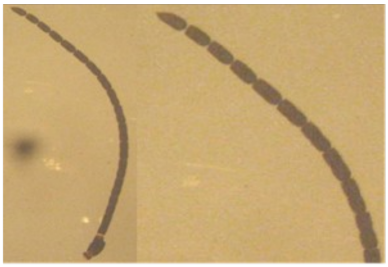
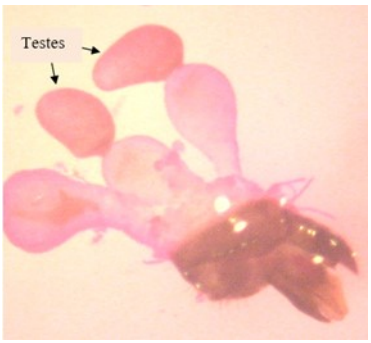
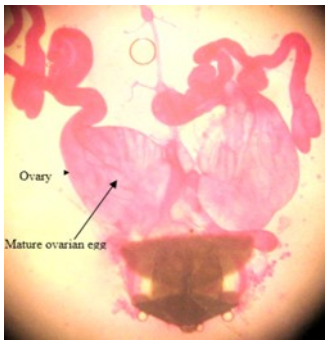
Body Part	n	Male (a)	Female (b)
Antenna	20	16 segments, last 8 segments are slightly longer with distinct peanut-like curve 	16 segments, last 8 segments are straight and slightly shorter than the males 
Thorax	20	Slightly slender	Spherical
Abdomen	20	Elongated, slender, aedeagus on terminal segment	Oval, wider, short ovipositor on terminal segment
Reproductive tract	20		

Table 2. Proportion of male and female offspring produced by *Snellenius manilae* per generation in the laboratory.

Gener-ation	n	No. of Mating Pairs	Mating Duration (s)	Male	Female
1	20	3	< 60	13	7
2	20	3	< 60	12	8
3	20	2	< 60	13	7
4	20	4	< 60	11	9
5	20	2	< 60	13	7
6	20	4	< 60	11	9
7	20	2	< 60	16	4
8	20	3	< 60	13	7
9	20	1	< 60	16	4
10	20	2	< 60	14	6
Total	200	26	< 60	132	68
Mean ± S.E.M.		2.6 ± 0.3		13.2 ± .6	6.8 ± 0.6

The pattern of egg maturation and deposition of *S. manilae* did not fit the strict dichotomy (synovigenic vs. pro-ovigenic) that was advocated originally by Flanders (1950).

Some parasitic wasps emerge as adults with their full complement of mature ovarian eggs and no more maturation occurs. The wasps therefore have no pre-oviposition period, no egg replenishment after egg deposition and only a short life cycle. This type of egg maturation is referred to as pro-ovigenic (Flanders 1950). However, some species of parasitic hymenopterans emerge with no mature ovarian eggs and have to spend time called pre-oviposition period, to allow their reproductive system to mature and have to mature the eggs as they live. Species having this type of egg maturation are referred to as synovigenic (Jervis and Kidd 1986).

In pro-ovigenic parasitoids, the deposited eggs may be anhydropic (yolk rich). Also, their mode of parasitism is

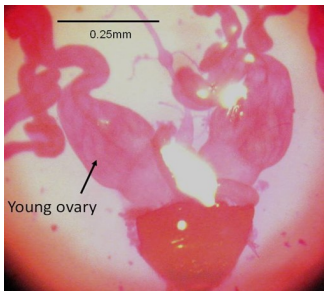


Fig. 1. Young ovaries of newly emerged *Snellenius manilae* containing a few mature eggs.

koinobiont in which host larvae continue to develop following oviposition. On the other hand, synovigenic species are those that have mainly immature eggs at emergence or at least one mature egg (Jervis et al. 2001). They have yolk-rich eggs, perform host feeding activity, and have a long life span. Parasitoids having this character have an idiobiont mode of parasitism in which the host larvae are paralyzed during oviposition.

S. manilae has characters of both pro-ovigenic and synovigenic type (Table 3). The ovaries of the newly-emerged wasps contained few mature eggs 5 h upon emergence. The females had a very short pre-oviposition period of 5 h before they were ready for mating and oviposition, thus showing a synovigenic character. Mating occurred in the population, which means that the parasitoid is arrhenotokous. This is when unfertilized eggs produce males while fertilized eggs produce females. The wasps were short-lived, lasting about 5–7 d, and did not host feed, thus showing both pro-ovigenic characters. The eggs were hydropic (yolk-deficient) whereby embryonic development is dependent on host fluid upon oviposition, manifesting a pro-ovigenic character. They also exhibited a koinobiont mode of parasitism, again showing a pro-ovigenic character.

Pro-ovigeny and synovigeny are now seen as extremes of a continuum (Jervis and Kidd 1996; Jervis et al. 2001) and it is not surprising that *S. manilae* have the characters of both extremes. A similar situation has been reported in at least two *Coccophagus* species, in *Coccidoxenoides perminutus* and in *Trichogramma evanescens* (Donaldson and Walter 1988; Walter 1988; Ceballo and Walter 2004; Ramal and Ceballo 2011). The terminology suggested by Flanders therefore seems to have little descriptive use, and its explanatory power may likewise be limited.

A recently devised ovigeny index has been suggested by Jervis et al. (2001) using the shape of the age-specific realized fecundity curve. An index of 1 (strict pro-ovigeny) denotes that all eggs are mature upon emergence while an index of 4 (extreme synovigeny) denotes that all eggs are immature upon emergence wherein initial egg load and lifetime potential fecundity are of the same quantity. *S. manilae* seems to follow the Type 2 index wherein initial egg loads are lower and egg deposition increases on the first few days of life before declining rapidly.

Table 3. Characteristics used in classifying the two patterns of egg maturation and deposition by the larval parasitoid *Snellenius manilae*.

Characters	Synovigenic Wasp	Pro-ovigenic Wasp	*S. manilae*
Mature ovarian egg upon emergence	0-1	Full complement	0-22
Pre-oviposition period required	Yes	No	Yes
Yolk rich eggs	Yes	No	No
Host feeding	Yes	No	No
Adult life span (days)	23.6-28.0	7.5-10.5	5-7
Mode of parasitism	Idiobiont	Koinobiont	Koinobiont

The ovaries of newly-emerged wasps may not contain any mature ovarian eggs from 0–6 h upon emergence but 6 h thereafter, a few mature eggs were observed. Mature ovarian eggs represent the available eggs ready for oviposition per day per female. On day 1, mature ovarian eggs on average were 18.2 ± 0.8 per female. Significantly the highest number of mature ovarian egg was recorded on day 3 ($F = 13.5$; $df = 5$; $P > 0.05$) with an average of 28.8 ± 0.7 eggs per female, and then gradually decreased on each successive day until the wasp died on day 6 (Fig. 2). There was no host feeding observed for the whole duration of the experiment, nevertheless, ovarian eggs did mature when the adults fed on honey that was provided and not on hosts' fluid.

The average number of ovarian eggs decreased gradually even when the females were not exposed to hosts for oviposition. The decrease in the number of ovarian eggs in the absence of hosts suggests that there was no further egg maturation occurring within the ovary and that there may be some resorption of mature ovarian eggs happening in response of the individual female to unavailability of hosts (Flanders 1950). However, this observation will need further tests to determine the actual cause of the decline.

Dissections made on the male reproductive tract were not successful after several attempts, thus we could not provide any data for the rate of sperm maturation. The activity needs to be pursued when an appropriate and effective dye and staining agent is available.

Ovipositional Patterns

S. manilae inserted its ovipositor indiscriminately on the host's

body (Table 4), but this was in the presence of relatively few hosts (5 per arena, exposed for 20 min). In all replicates, all three body regions including the anus were attacked. The highest number of ovipositor insertions was made within the head region with an average of 3.6 ± 1.3 insertions, and the least was made within the abdominal section with an average of 0.4 ± 0.2 insertions. The ovipositing female did not discriminate already parasitized larvae as females were observed to accept and oviposit again into previously parasitized larvae, a behavior that was repeatedly observed for a single larva by the same female. A similar observation has been reported on the egg parasitoid, *Trichogramma evanescens* (Barnacha 2009) and mealybug parasitoid, *C. perminutus* (Ceballo and Walter 2004).

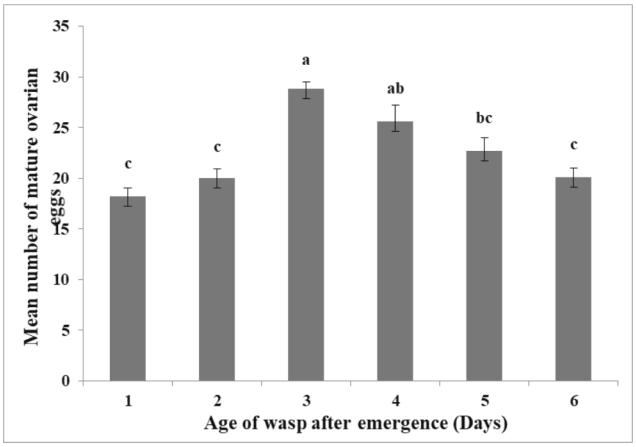


Fig. 2. Mean number of mature ovarian eggs of *Snellenius manilae* females at successive days after adult emergence. Bars having different letter are significantly different (P < 0.05).

The precise oviposition site or egg deposition is known to occur in many parasitoids, and is generally believed to represent adaptations for overcoming host immune defenses. In other instances, wasps may make use of the relatively easily penetrated parts of a host's body in order to get rid of their eggs inside (Quicke 1997). The possible reason for a relatively high number of ovipositor insertions within the head region by *S. manilae* was not investigated in this study.

Parasitic wasps are usually active for a major part of the day. Some wasps are either diurnal or nocturnal, but many partition their activity more regularly; for instance, some species emerge at a particular time of day and have a preferred time for mating or courtship (van den Assem 1976).

Upon exposure to the host cutworm larvae, *S. manilae* wasps groomed, searched, rested, fed on honey and inserted their ovipositor presumably to lay their eggs. The relative duration of time spent by the parasitoid on these activities is presented in Figure 3. Upon exposure to hosts, wasps tend to groom and search for a suitable host. The completion of ovipositor insertion was very short (1–2 s). Additionally, they spend no more than 10–20% of their time in ovipositor insertion, although they have mature ovarian eggs available for oviposition. Peak of oviposition-related activities coincided within the mid-morning, 1000 h and mid-afternoon, 1530 h. If the peak of ovipositional activity is reflective of the actual behavior in the field, this may be in perfect synchrony with the time

Table 4. Number of ovipositor insertions of a single *Snellenius manilae* female (n = 10) exposed to first instar larvae of *Spodoptera litura* over a period of 20 min. Chi square goodness of fit was used to compare distribution of oviposition across sites.

Female Wasp	n	Oviposition Sites				Total	Mean ± S.E.M.	X^2
		Head Region	Thorax	Abdomen	Anus			
1	5	13	6	0	3	22	5.5 ± 2.8	0.54
2	5	1	0	0	0	1	0.3 ± 0.3	0.81
3	5	2	3	0	0	5	1.3 ± 0.9	0.21
4	5	1	1	0	1	3	0.8 ± 0.3	0.82
5	5	2	1	0	5	8	2.0 ± 1.3	0.01
6	5	10	2	1	2	15	3.8 ± 2.1	0.65
7	5	2	1	1	0	4	1.0 ± 0.4	0.32
8	5	1	0	1	0	2	0.5 ± 0.3	0.05
9	5	2	0	1	2	5	1.3 ± 0.5	0.21
10	5	2	2	0	0	4	1.0 ± 0.5	0.50
Total	50	36	16	4	13	73	18.3 ± 6.6	
Mean ± S.E.M.		3.6 ± 1.3	1.6 ± 0.6	0.4 ± 0.2	1.3 ± 0.5	6.9 ± 2.1	1.75 ± 0.7	
X^2		0.96	0.55	0.11	0.26			

when the first instar cutworm larvae, though nocturnal in habit, are still found within the host plants most of the time of the day exposed to parasitism. Most of their remaining time was spent on grooming themselves and resting.

Host Stage Suitability (No-Choice Method)

S. manilae larvae developed and emerged successfully from all the three larval instars (L1, L2 and L3) but the percentage of emergence varied significantly across instars (Fig. 4A). First instar larvae yielded significantly the highest number of adult parasitoids (F = 141.94; df = 2; $P > 0.05$) with an average of 6.6 ± 0.32 parasitoids per female followed by second instar larvae, which yielded an average of 1.47 ± 0.13 parasitoids per female, while the third instar larvae yielded the lowest number of parasitoids at 0.53 ± 0.13 parasitoids per female. Although *S. manilae* oviposits across a range of cutworm larval instars, its productivity relies predominantly on the first instar. Most oviposition is into L1 larvae, whether choice or no choice. Most eggs that were oviposited into first instar larvae emerged successfully into adult wasps, while none or only few survived on the third instar larvae.

The first instar larvae seem to be the most suitable host stage for parasitoid development, as they seem to be preferred by the parasitoid more than the second instar larvae. The older instars, the third instar larvae in particular, displayed vigorous defense reaction manifested by intermittent head twisting against the ovipositing parasitoid particularly during the ovipositor insertion process. In most cases, the third instar cutworm larvae resisted oviposition attempts of the parasitoid by swinging their head and thorax from side to side in an attempt to bite the parasitoid.

Some potential hosts have evolved particular strategies for avoiding parasitism. These include trashing, kicking, shaking, dropping on silken threads or simply falling off the plant, and the release of noxious liquids. Many lepidopterans, including cutworms and other caterpillars, have characteristic head-flicking and thrashing behaviors that are most certainly involved in deterring parasitoid attack (Gross 1993).

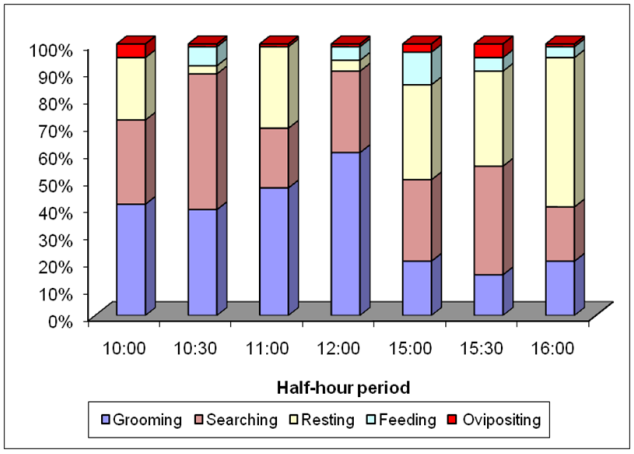


Fig. 3. Mean percentage of time spent by 3-day-old *Snellenius manilae*, fed with honey, on the five principal activities, exposed to five first instar larvae of *Spodoptera litura* as hosts.

Host Stage Suitability (Choice Method)

The emergence pattern in the choice test was almost similar to that recorded in the no-choice test. The first instar larvae yielded significantly the highest number of *S. manilae* wasps (F = 16.87; df = 2; P > 0.05) with an average of 3.53 ± 0.13 wasps developing successfully into full adult parasitoids. The second instar larvae yielded an average of 2.6 ± 0.21 adult wasps and the lowest emergence was 0.33 ± 0.13 adults produced from the third instar cutworm larvae (Fig. 4B).

S. manilae is considered a koinobiont parasitoid whose hosts carry on their development for at least a while after parasitization, while hosts that do not develop further after they have been parasitized are referred to as idiobionts (Askew and Shaw 1986). Koinobionts typically

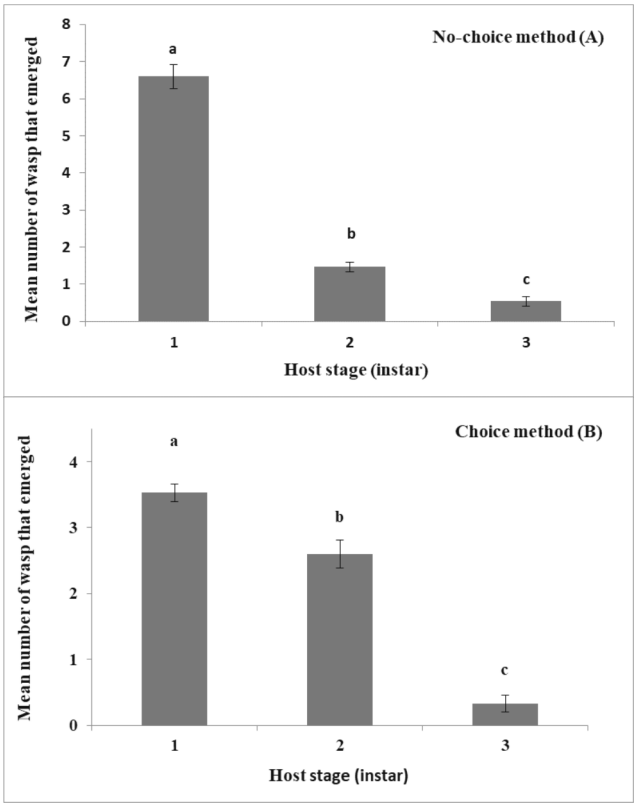


Fig. 4. Number of *Snellenius manilae* adults that successfully developed and emerged from the three different larval instars of *Spodoptera litura* in No-Choice (A), and Choice methods (B). Bars having different letter are significantly different f(P < 0.5)

attack larvae, often early instars, or eggs in the case of egg-larval and egg-pupal parasitoids. Koinobiont parasitoid species have usually had to adapt physiologically to host defenses. The parasitoid larval development is rather slow if not delayed compared with the idiobiont species which has rapid larval development. Adult life span is short, host feeding is uncommon, and hosts that are usually attacked are often smaller than the wasp itself. Most koinobionts, including *S. manilae*, have their hosts exposed and their host may not exhibit temporary paralysis.

Host Behavior and Parasitoid Developmental Duration

Full-grown larvae of the parasitoid prior to pupation came out of the host cutworm body (Fig. 5A) and continued to develop into pupae within a cocoon but attached to the dying host (Fig. 5B). Under normal circumstances, healthy and unparasitized cutworm larvae pupate under the soil. However, majority of the parasitized larvae with the developing *S. manilae* pupae were observed to move from the bottom part of the rearing cage to the upper portion, the lid of the cage. All the parasitized larvae preferred to stay at the upper surface of the rearing container which eventually became their final site for pupation. This behavioral change may have been imposed on the parasitized larvae by the developing *S. manilae*, a behavioral change similarly reported on parasitized citrus mealybugs (Ceballo and Walter 2004). The possible reason for *S. manilae* to shift from being an endoparasitoid while at the immature stage into an ectoparasitoid prior to the pupal stage was not investigated.

Fig. 5. *Snellenius manilae* last instar larva (A), and pupa (B) attached at the caudal end of second instar *Spodoptera litura*.

The total developmental period from oviposition to pupa (n = 197) was 10.5 ± 0.08 d (Table 5). The total developmental period of the parasitoid from pupa to adult emergence (n = 45) was 4.2 ± 0.10 d (Table 6).

Adult Longevity of *S. manilae*

The adults (n = 45) lived for an average of 6.1 ± 0.07 d with honey as food (Table 7). Adult longevity is closely related to adult fitness and survival in the field. Adult parasitoids that live longer than others have more time to search for and locate suitable hosts. If hosts tend to be hard to find, then adult wasps with a longer life span have a better chance to search for a sufficiently large number of hosts, thus enhancing parasitoid field performance (Quicke 1997).

SUMMARY AND CONCLUSION

The laboratory-reared population of *S. manila* underwent a sex-related parthenogenetic development (arrhenotoky with a male-biased population recorded over 10 generations. The pattern of egg maturation and deposition did not fit the dichotomy (synovigenic versus pro-ovigenic) but followed the Type 2 index where initial egg loads are lower and egg deposition increases on the first few days of life before declining rapidly. The highest number of mature ovarian eggs was recorded on day 3 and then gradually decreased on each successive day until the wasp died on day 6. The ovipositing female did not discriminate already parasitized larvae in the presence of relatively few hosts; however, the highest number of ovipositor insertions was made within the head region. Ovipositor insertion was very brief (1–2 s). The peak of oviposition activities was within mid-morning, 1000 h and mid-afternoon, 1530 h. The first instar larvae yielded the highest number of adult parasitoids, followed by second and third instars in both the no-choice and choice methods. *S. manilae* is a koinobiont parasitoid allowing the parasitized larvae to carry on their development for a while (at least the larval stage) after parasitization. The total developmental period of the parasitoid from egg to pupation was 10.5 ± 0.08 d, pupation to adult emergence was 4.2 ± 0.10 d, while the adult lived for an average of 6.1 ± 0.07 d with honey as food.

The results of this study seem to indicate that in the field where there is a mixed population of the various instar larvae of *S. litura*, the parasitoid may oviposit into 1st instar, 2nd instar, and 3rd instar larvae, but it was the 1st instar larvae of *S. litura* that was the most suitable stage and produced the highest number of adult emergences.

Table 5. Developmental period (days) of the parasitoid *Snellenius manilae* from egg deposition to pupal stage reared on first instar larvae of *Spodoptera litura*.

Duration (Days)	No. of *S. manilae* Pupae Recovered I n = 200	II n = 200	III n = 200	Total	Mean
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	Days from egg to last larval stage inside host			
6	-				
7	-	-	-	-	-
8	-	-	-	-	-
9	0	33	4	37	9.3
10	21	3	47	71	23.7
11	3	3	48	54	18.0
12	17	0	0	17	5.7
13	18	0	0	18	6.0
Total	59	39	99	197	65
Mean ± S.E.M.	11.5 ± 0.17	9.2 ± 0.10	10.4 ± 0.06	10.5 ± 0.08	

Table 6. Developmental period (days) of *Snellenius manilae* from pupal stage to adult emergence in first instar larvae of *Spodoptera litura*.

Duration (Days)	No. of Adults that Emerged I n = 15	II n = 15	III n = 15	Total	Mean
1	0	0	0	0	0
2	0	0	0	0	0
3	3	1	1	5	1.7
4	10	12	8	30	10.0
5	2	2	4	8	2.7
6	0	0	2	2	0.7
Total	15	15	15	45	15
Mean ± S.E.M.	3.9 ± 0.15	4.07 ± 0.12	4.5 ± 0.22	4.2 ± 0.10	

The adult wasp that fed on honey lived for 6 d while in captivity. The normal life span of *S. manilae* adult is short, if it is to be used for augmentative release against the cutworm. Providing supplemental food sources in the field could well enhance survival of the adults which consequently could gain additional time to search for suitable hosts to parasitize. Supplemental food sources may be provided by maintaining flowering non-crop plants and flowering weeds along the margins of the field. For maximum parasitization impact, the best age for field release of 3-day-old *S. manilae* wasps must be timed when majority of the cutworm population is in the first instar larvae of development.

Table 7. Longevity of *Snellenius manilae* wasps under laboratory condition provided with honey as food.

Duration (Days)	No. of Surviving Adults I n = 15	II n = 15	III n = 15	Total	Mean
1	15	15	15	45	15.0
2	15	15	15	45	15.0
3	15	15	15	45	15.0
4	15	15	15	45	15.0
5	13	15	14	44	14.0
6	3	1	2	6	2.0
7	dead	dead	dead		
Total	15	15	15	45	
Mean ± S.E.M.	6.1 ± 0.15	6.1 ± 0.07	6.1 ± 0.12	6.1 ± 0.07	

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