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Morphological and biological characteristics of black-headed caterpillar, *Opisina arenosella* (Lepidoptera: Xyloryctidae)

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Abstract. *Nguyen H-U, Son T-T-N, Trinh T-X, Chau N-Q-K. 2025. Morphological and biological characteristics of black-headed caterpillar,* Opisina arenosella *(Lepidoptera: Xyloryctidae). Biodiversitas 26: 3021-3029*. The black-headed caterpillar, *Opisina arenosella*, has recently emerged as a significant pest affecting coconut production in the Mekong Delta, Vietnam. Research on the morphological and biological characteristics of this species was conducted at the laboratory of Tra Vinh University, Vietnam, to serve as a basis for the development of its effective management strategies. The results revealed that *O. arenosella* eggs were oval, creamy yellow in color, turning reddish brown just before hatching. The average size of the eggs was 0.72±0.13 mm x 0.42±0.10 mm. The larval color was remarkably diverse, varying from white to reddish-brown with multiple stripes on the body. They have a black or dark brown head and range in length from 1.35±0.12 mm to 15.10±2.97 mm, with widths from 0.2±0.02 mm to 1.65±0.07 mm. *Opisina arenosella* pupae were initially grayish-white and later transform into blackish-brown. The average size was 11.33±1.25 mm x 2.63±0.38 mm for female pupae and 9.30±0.99 mm x 2.07±0.30 mm for male pupae. Female adults had an average body length of 10.7±1.47 mm, light gray, while male adults were darker, averaging 8.62±0.72 mm in body length. The average life cycle of *O. arenosella* was 75.6±26.9 days, which includes 5.62±1.70 days for the egg stage, 38.7±1.89 days for larval stage, amd 8.02±1.18 days for male pupae and 8.05±0.85 days for female pupae.

Keywords: Biological characteristics, black-headed caterpillar, coconut, instar, *Opisina arenosella,* Vietnam

INTRODUCTION

Coconut (*Cocos nucifera*) is widely grown and plays an important role in the humid tropics with the ability to provide food, oil, drink, and numerous products, which are served as materials for domestic and industrial purposes (Beveridge et al. 2022). It is an important tree species and is adaptable to a wide range of habitat conditions. Over the years, coconut production has decreased due to various biotic and abiotic causes (Sudha et al. 2023). The coconut black-headed caterpillar, *Opisina arenosella* (Lepidoptera: Xyloryctidae), originated from South Asia, and has caused damage and spread in some countries in Asia. Its larvae are often concealed within galleries on the underside of leaflets, causing stunting and possible death of host plants. In addition to causing direct yield loss, the damage caused by this species also reduces the aesthetic value of the landscape (Lu et al. 2023). A study by Kumara et al. (2015) found that in coconut orchards heavily invaded by black-headed caterpillars, it took four years for the coconut trees to recover their productivity.

Furthermore, this insect causes damage to some other palm plant species (Yan et al. 2023). *Opisina arenosella* has been identified as a pest that is well adapted to a wide range of feeds. They can complete their life cycle on a wide variety of crops such as jackfruit, oil palm, cashew (Shameer et al. 2018; Yan et al. 2023), and larvae can eat pineapple, maize, and rubber leaves (Sukhirun et al. 2015). Le et al. (2023) reported that *O. arenosella* is additionally one of the serious coconut insects in Vietnam. Accordingly, *O. arenosella* was found to damage coconut trees in Ben Tre Province, Vietnam, in 2020 and has spread rapidly to coconut orchards throughout the Mekong Delta Provinces. In pest management strategies on coconut trees, chemical control methods are often difficult to apply because coconut trees are perennial plants with considerable height, and chemical pesticides can cause environmental pollution and have harmful effects on human health (Nguyen 2019).

Management strategies in various countries have clearly demonstrated the effectiveness of releasing parasitic agents to control *O. arenosella* populations (Rao et al. 2016). Among the insect pest management measures towards safety, *Brachymeria kamijoi* (Hymenoptera: Chalcididae), a parasitic wasp of black-head caterpillar pupae, has been identified as having an abundant presence in coconut orchards, capable of high parasitism rates and is a relatively effective biological control agent against *O. arenosella* in Vietnam (Le and Tran 2022). Invasive species often threaten the balance of ecosystems and cause damage to economic sectors, including agriculture (Bradshaw et al. 2016). Providing data on the biological characteristics of *O. arenosella* could be important in improving the timing of control measures and mass-rearing methods for this insect to provide hosts for its parasite production (Le et al. 2023). On that basis, from 2020 to 2021, Le et al. (2023) determined some biological characteristics of this species in the laboratory (28ºC and 12 light: 12 dark). The results from this study that the average total developmental time

from egg to adult of *O. arenosella* was 52.6-57.7 days with the larval stage having the longest development time. However, the development of many insect species can change due to the influence of many environmental factors such as temperature (Li et al. 2015; Štefková et al. 2017; Yi et al. 2019), humidity, oxygen levels, and nutritional conditions (Wang et al. 2018).

Studying the development of insects in each living condition will supplement scientific data for the application of effective management solutions. On that basis, it is necessary to determine data on the morphological and biological characteristics of *O. arenosella*. The data help to accurately define developmental stages to improve the efficiency of breeding and utilization of parasitic natural enemies for biological control of this pest.

MATERIALS AND METHODS

Period and location of study

Field surveys were conducted in farmers' coconut orchards in Tieu Can and Cang Long Districts of Tra Vinh Province, Vietnam. These are localities with many coconuts and have been damaged by black-headed caterpillars. Morphological and biological characteristics of *O. arenosella* were studied at the Entomology laboratory of Tra Vinh University, Vietnam. Research contents were conducted from January 2023 to March 2024. Specifically, field insect collection is carried out approximately every 2 months for laboratory studies.

Sources of insects

Opisina arenosella sources used in the studies were collected from 60 coconut orchards (at least 1,000 m^2) of farmers in Tra Vinh Province (Tieu Can and Cang Long Districts). Accordingly, black-headed caterpillars were collected from coconut leaflets with symptoms of infection and reared in the laboratory until they laid eggs to survey their morphological and biological characteristics.

Research procedure

Larvae and pupae of *O. arenosella* were collected in the province and reared in 5x7 cm plastic boxes (Figure 1). The boxes were kept moisturized and ventilated, with coconut leaves used as food. Under the laboratory conditions, after 12 hours of emergence, adults were allowed to pair up (one adult pair/ plastic box) and monitored every day to determine the developmental stages of black-headed caterpillars.

A total of 100 newly laid *O. arenosella* eggs within 12 hours were monitored daily from hatching to completion of their life cycle. Black-headed caterpillar larvae were bred using the individual breeding method. Newly hatched larvae were transferred to separate coconut leaf boxes and replaced periodically, once a day, to check for molting larvae through the head capsules. Based on the research methods of Grunert et al. (2015), Go et al. (2019), and Sukovata (2019), instars of *O. arenosella* larvae were determined by recording the head capsules removed after each molt. Larval length was measured from the head to the end of the abdomen while the width was measured at the middle of the body within 12h after emergence of new instar larvae using a ruler (in mm divisions) under a stereomicroscope (Meiji EMZ-8TR trinocular microscope). The process of monitoring *O. arenosella* pupae was carried out in the same way as monitoring larvae. The method of measuring pupal size was similar to that of measuring larval size. Pupation rate was calculated the percentage of larvae that transition to the pupal stage. Newly emerged *O. arenosella* adults were observed individually. Gender was determined by observing the structure of the end of the abdomen under the stereoscopic microscope. The method of measuring adult size was similar to the survey on larvae and pupae. Daily observations were implemented to determine changes in size, color, larval molt, development time, and life cycle of the black-headed caterpillar. At the same time, the number of eggs laid by each female adult and the hatching rate were further recorded along with the temperature and humidity in the laboratory. The study examined each individual from the egg stage until the adult died. For pupae and adults, additional field-collected individuals developed from larvae were further examined.

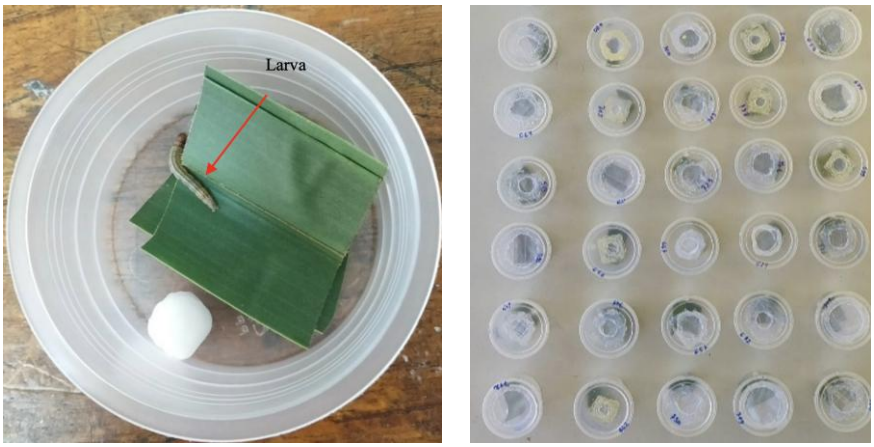

Figure 1. The black-headed caterpillar *Opisina arenosella* larvae were reared in the laboratory

RESULTS AND DISCUSSION

Morphological characteristics of *Opisina arenosella* in laboratory conditions

The survey results on the total of 100 *O. arenosella* eggs revealed that they were oval with an average size of 0.72±0.13 mm x 0.42±0.10 mm. The newly laid eggs were opaque white (Figure 2.A) while the eggshell surface was shiny, not smooth, but had a mesh-like texture. After 3-4 days, they turned into orange red (Figure 2.B). When they were in the pre-hatching stage (about 5-6 days), the larvae inside the eggshell could be seen extremely clearly with white bodies, red stripes, and black heads (Figures 2.C and 2.D). Mahadi and Ariffin (2018) also reported that the eggs were oval, approximately 0.9 mm in length and 0.4 mm in width, creamy white in color, and turned pink when close to hatching.

The first instar of *O. arenosella* was at 1.35±0.12 mm (length) and 0.20±0.02 mm (width) and increased gradually with the development period. The results in Table 1 additionally indicated that the length of the larvae increased rapidly during the developmental period from the seventh to ninth instar, while the width increased relatively steadily across instars. Under the laboratory conditions at Tra Vinh University, the research results demonstrated that the maximum size of the larvae reached 15.10±2.97 mm in length and 1.65±0.07 mm in width (the eleventh instar). The larvae had 11 segments and numerous small white hairs on their bodies. The first and second instar larvae were white with distinct red horizontal stripes, which faded in later instars. From the third instar, the larva began to have red stripes along its reddish-brown body and turned into dark brown at the fourth or the fifth instar. At this stage, the food present inside the larvae could still be observed. From the sixth instar onwards, the larvae changed to brown with distinct reddish-brown stripes along their bodies. When preparing to pupate, they were reddish-brown and had dark brown stripes. Their bodies were slightly contracted, and the body segments were clearly visible (Figure 3).

Table 1. Size (mean±SD) of the developmental stages of *Opisina arenosella* reared in the laboratory of Tra Vinh University, Vietnam, 2023 (T: 28-31°C, RH: 60-75%)

Development stage	Samples	Average sizes (mm)			
		Body length	Body width	Head capsule length	Head capsule width
Eggs	100	0.72±0.13	0,42±0,10	-	-
1st instar	75	1.35±0.12	0.20±0.02	0.20±0.06	0.20±0.06
2nd instar	75	1.86±0.46	0.30±0.05	0.33±0.08	0.31±0.07
3rd instar	75	3.13±0.51	0.40±0.09	0.49±0.11	0.46±0.12
4th instar	75	4.11±0.62	0.71±0.17	0.63±0.14	0.61±0.15
5th instar	75	5.59±0.94	0.90±0.18	0.94±0.31	0.91±0.34
6th instar	73	6.92±1.63	1.03±0.18	0.98±0.31	0.95±0.35
7th instar	64	8.27±1.91	1.12±0.53	1.13±0.31	1.11±0.36
8th instar	51	11.06±2.39	1.19±0.11	1.77±0.47	1.82±0.52
9th instar	27	13.17±2.10	1.24±0.11	1.97±0.30	1.93±0.35
10th instar	7	14.14±2.64	1.43±0.10	2.19±0.27	2.23±0.73
11th instar	2	15.10±2.97	1.65±0.07	2.40±0.01	2.33±0.02
Female pupae	50	11.33±1.25	2.63±0.38	-	-
Male pupae	50	9.30±0.99	2.07±0.30	-	-

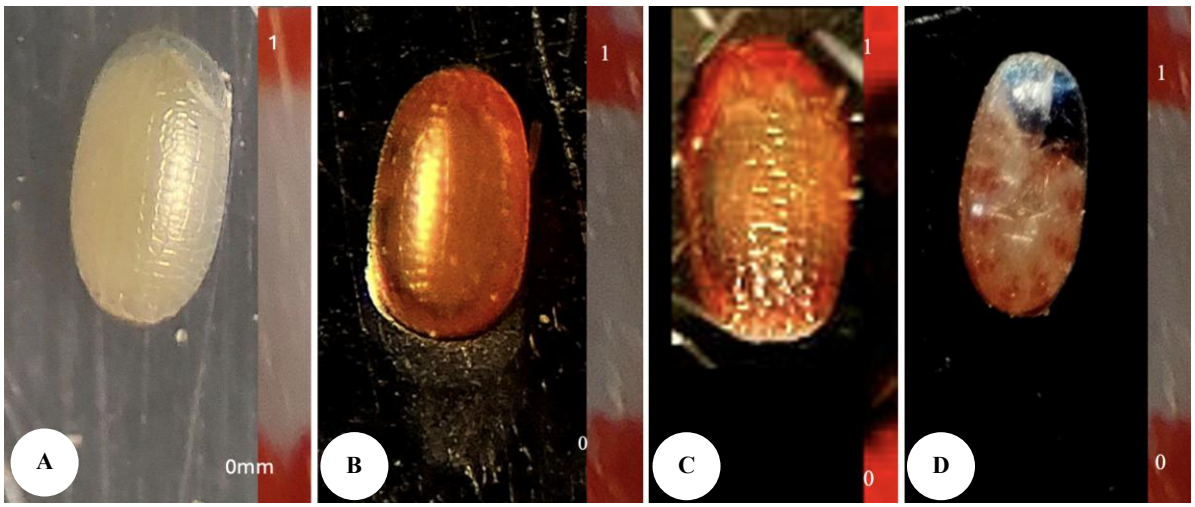

Figure 2. Morphology of *Opisina arenosella* egg. A. Newly laid, B and C. Developing, D. Pre-hatching

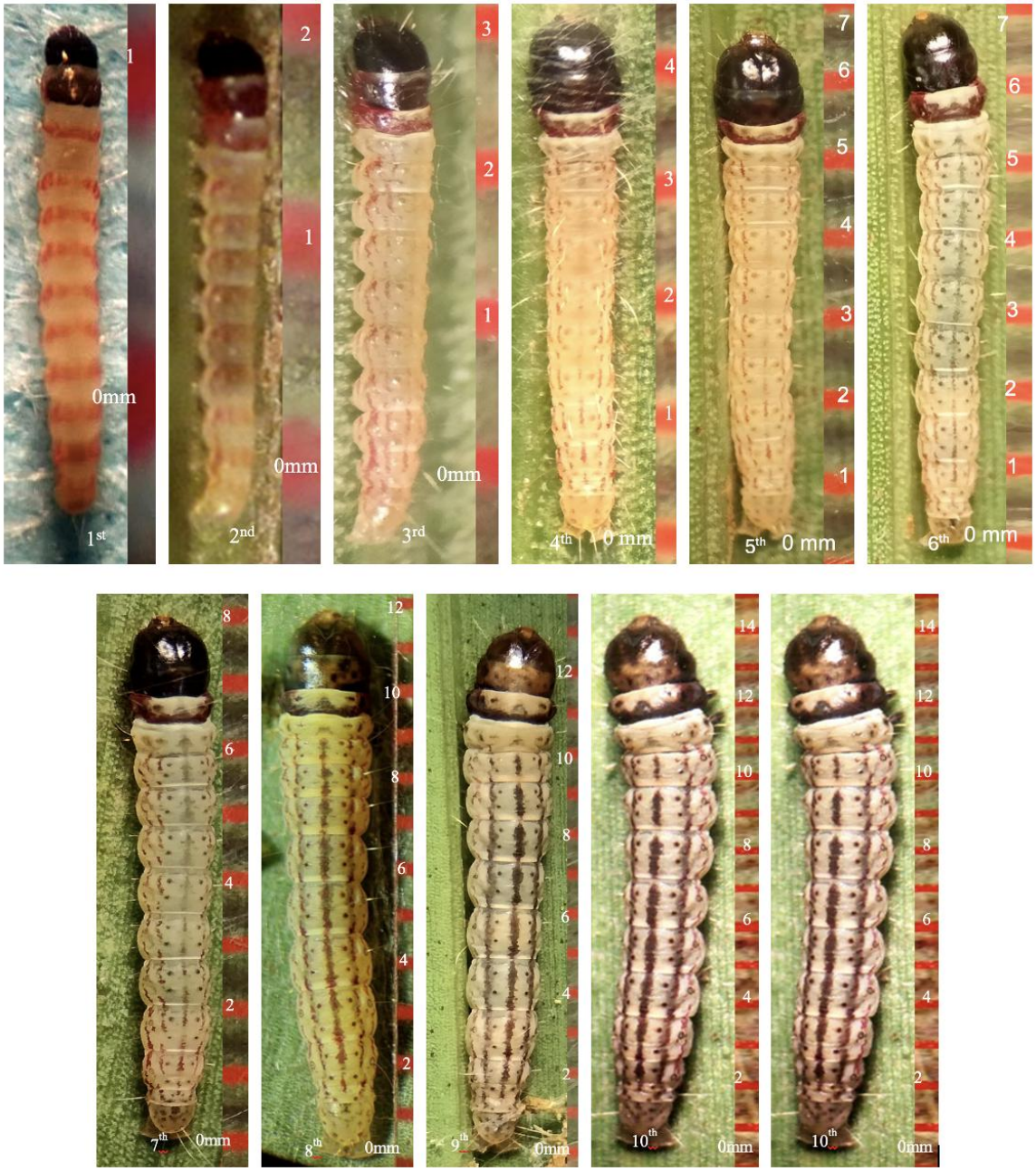

Figure 3. Morphology of *Opisina arenosella* larvae from 1 to 11 instar

Under the laboratory conditions, the color of the head capsules of *O. arenosella* larvae did not significantly change at different instars, being black or dark brown (Figure 4). The head capsules of *O. arenosella* larvae varied from 0.20±0.06 mm (width) and 0.20±0.06 mm (length) and reached a maximum size of 2.33±0.02 mm (width) and 2.40±0.01 mm (length) (Table 1, Figure 4). Along with the development of the larvae, the head capsule size of the larvae increased significantly steadily through the instars, except for those of the seventh to eighth instar larvae, which have remarkably clear differences. In a previous study, Mahadi and Ariffin (2018) reported that *O. arenosella* larvae were yellowish-green with black heads and distinct stripes clearly visible on their dorsal abdomen. At the latter stage of the development, the abdomens of the larvae turned light yellowish. In addition, the larvae had a length which increased from 1.5 to 14.4 mm, corresponding to a head width of 0.2 to 1.6 mm. Compared with Mahadi and Ariffin's (2018) publication, these results had numerous differences in the colors of *O. arenosella* larvae. In relation to these results, Cook and Saccheri (2013) pointed out that the color diversity of insects was related to their camouflage. In addition, temperature changes altered the color of larvae, as in the case of *Spodoptera exempta* (Lepidoptera: Noctuidae) (Aguilon et al. 2015).

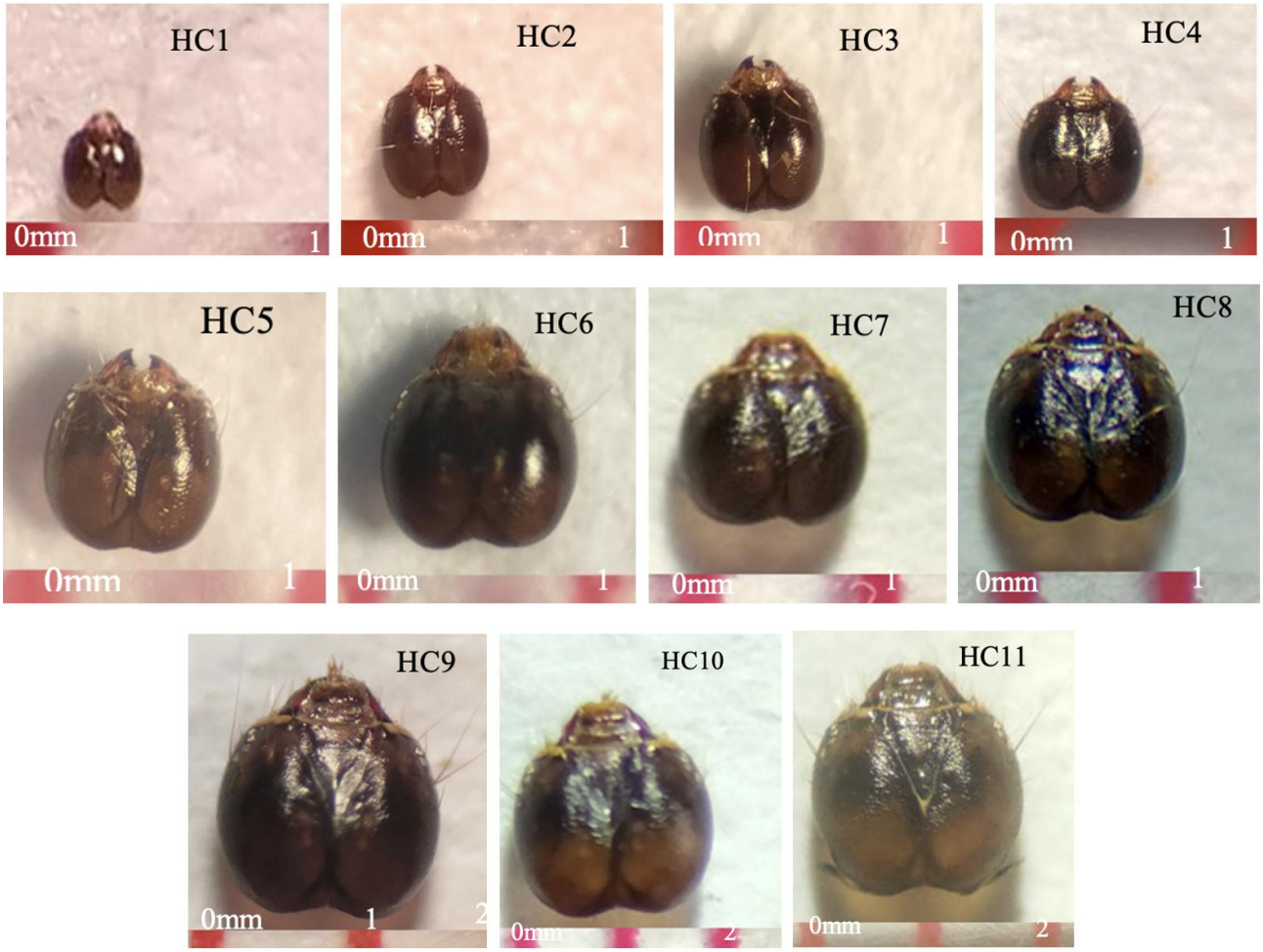

Figure 4. Head Capsules (HC) of *Opisina arenosella* larvae from the 1st-11th instar (mm)

Opisina arenosella pupae had an average size of 11.33±1.25 mm (length) and 2.63±0.38 mm (width) for females, while the results for males were 9.30±0.99 mm and 2.07±0.30 mm. Females were usually larger than males. The pupae were initially grayish-white (Figure 5.A), then changed to reddish-brown (Figure 5.B) and blackish-brown at the end of the pupal stage (Figures 5.D and 5.E).

The male pupa has an external genital opening which is a small slit, while the female's genital opening is longer and wider than the male's with small tubercles on either side (Figure 6).

Mahadi and Ariffin (2018) similarly described female pupaeas larger than male pupae with lengths of 10.06 mm and 8.23 mm, respectively, and differed in the position of the genital opening. In the study of *O. arenosella* pupae, besides the morphological characteristics consistent with previous publications, the results additionally determined the color changes of pupae during the development period and described the differences between male and female genital opening at this stage. Some research results have also shown that the external genital opening is one of the common features for sex differentiation of insects in the pupal stage of the Lepidoptera order, such as *Hyphantria cunea* (Lepidoptera: Erebidae) (Tuncer and Aker 2017), *Mythimna separata* (Lepidoptera: Noctuidae) (Lin et al. 2020), and *Bactra venosana* (Lepidoptera: Tortricidae) (Nguyen et al. 2022).

Adults

The average size of *O. arenosella* adults was 10.7±1.47 mm (body length), 6.82±0.65 mm (antennae length), and 24.3±2.31 mm (wingspan) for females. The results for males were 8.62±0.72 mm (body length), 6.13±0.42 mm (antennae length) and 19.32±2.12 mm (wingspan). Most of the females were larger than the males (Table 2).

Table 2. The size (mean±SD) of black-headed caterpillar adults was reared in the laboratory of Tra Vinh University, Vietnam, in 2023 (T: 28-31°C, RH: 60-75%)

Development stage	Samples	Average sizes (mm)		
		Body length	Wingspan	Antennae length
Female adult	50	10.7±1.47	24.3±2.31	6.82±0.65
Male adult	50	8.62±0.72	19.32±2.12	6.13±0.42

Figure 5. A. Morphology *Opisina arenosella* pupae when newly pupated, B. 2-4 days, C. 5-6 days, D and E. Elose to adult emergence

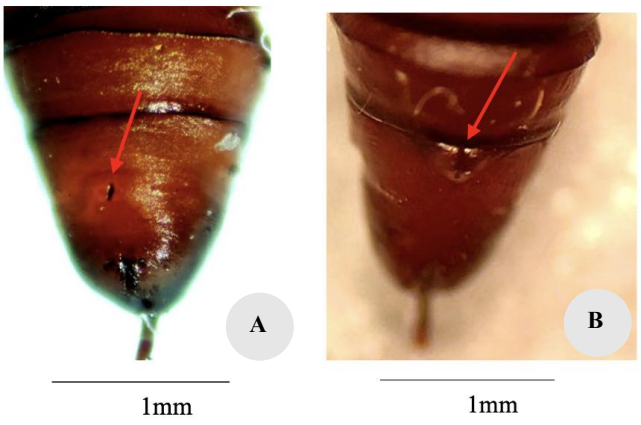

Figure 6. Genital opening of *Opisina arenosella* pupa. A. Male, B. Female

Male adults had light gray and shiny wings (Figure 7.A) while adult female wings were darker grey (Figure 7.C). This is the characteristic that distinguishes between male and female adults. In addition, the difference between male and female adults was clearly shown in the last abdominal segment. In male adults, the terminal abdominal segment was covered with long, gray-white hair (Figure 7.B) while in females, the terminal abdominal segment was covered with short and flat hair (Figure 7.D). The male's aedeagus was yellowish-brown and approximately 1.00 mm long (Figures 7.E and 7.F)

The results of the survey on the morphology of the *O. arenosella* adult with gray-white forewings, the female being larger and the antennae longer than the male, were also described by the publication of Mahadi and Ariffin (2018). In this study results, the color difference as well as the wingspan length of male and female adults were added. In addition, the ability to easily distinguish between males and females through external morphology is also one of the important characteristics for conducting further studies, such as sterile insect techniques (Bourtzis and Vreysen 2021), application of sex pheromones (Bhanu et al. 2018; Muniyappa et al. 2018) to control this species damage, etc.

Biological characteristics of *Opisina arenosella*

Number of eggs laid by each female and hatching rate

Each *O. arenosella* female adult lays an average of 173.9±37.2 eggs with the hatching rate equivalent to 88.5±7.0 eggs during its entire development period. Le et al. (2023) similarly pointed out that *O. arenosella* females start laying eggs roughly around 1.2 days after emergence with an average of 278.8 eggs during their entire development period. Previously, Kumara et al. (2015) showed this insect could lay 273.63 eggs/ female.

Opisina arenosella*'s development time*

The results in Table 3 showed that under the laboratory conditions (T: 28-31°C, 68-75%), the average development time of eggs was 5.62±1.70 days, larvae 38.7±1.89 days. The *O. arenosella* larvae varied from 5 to 11 instars, of which, the proportion of larvae pupating at instar 8 accounted for the highest proportion (35%). Simultaneously, the development time of pupae was 8.02±1.18 days (male pupae) and 8.05 ±0.85 days (female pupae), adults 8.49±2.89 days (male adults) and 9.35±3.36 days (female adults). The results summarized in Table 3 emphasized/ demonstrated that the development time of *O.*

arenosella eggs was 5.62±1.70 days while that of the larvae was 38.7±1.89 days, (with 5 to 11 instars). Variation in insect instar has also been reported in previous studies. For example, larvae of *Diocalandra frumenti* (Coleoptera: Curculionidae) ranged from 8 to 19 instars (Nguyen et al. 2020), *Tenebrio molitor* (Coleoptera: Tenebrionide) larvae had 14 to 18 instars (Morales-Ramos et al. 2015), larvae of *Helicoverpa armigera* and *Helicoverpa zea* ranged from 3 to 7 instars and 5 to 7 instars, respectively (Barbosa et al. 2016).

Adult females started laying eggs at 2.71±0.57 days after emergence and lasted about 3.05±0.40 days. Thus, the black-headed caterpillar had an average life cycle of 67.3±7.30 days under the laboratory conditions (T: 28-31°C, RH: 60-75%) (Table 3, Figure 8). There have been previous publications on the biological characteristics of *O. arenosella* larvae. Specifically, research results by Muralimohan et al. (2013) also showed that at 26°C, the total development time of *O. arenosella* from egg to female adult was 58.32 days, longer than that of male (54.20 days). In addition, Le et al. (2023) emphasized that the total development time of *O. arenosella* from egg to adult was 57.7 days (female) and 52.6 days (male) (at 28°C). Meanwhile, the average development times of egg, larva, and pupa were 6.1 days, 41.2 days and 3.6 days, respectively. Regarding the larval age of this species, Le et al. (2023) pointed out the lifespan of adult females was 4.9 days, while males had a lifespan of 10.7 days, longer than that of females.

Table 3. Development time (mean±SD) of *Opisina arenosella* at laboratory conditions (T: 28-31°C, RH: 60-75%)

Development stage	Samples	Average duration (days)	Pupation rate (%)
Eggs to 1st instar	100	5.62±1.70	-
Larvae	-	38.7±1.89	-
1st to 2nd instar	100	5.22±2.10	-
2nd to 3rd instar	100	6.07±2.76	-
3rd to 4th instar	100	4.51±1.53	-
4th to 5th instar	100	4.38±1.60	5
5th to 6th instar	100	4.24±1.81	9
6th to 7th instar	73	4.48±1.91	20
7th to 8th instar	64	4.65±1.76	35
8st to 9th instar	51	5.29±2.40	22
9th to 10th instar	27	5.25±1.94	7
10st to 11th instar	7	5.63±1.85	2
11st to pupa	2	6.00±1.41	-
Male pupae	45	8.02±1.18	-
Female pupae	55	8.05±0.85	-
Male adult longevity	45	8.49±2.89	-
Female adult longevity	55	9.35±3.36	-
Pre-oviposition	55	2.71±0.57	-
Oviposition time	55	3.05±0.40	-
Life cycle	55	75.6±26.9	-

Figure 7. Morphology of *Opisina arenosella* adults: male (A. Dorsal view, B. Ventral view) and female (C. Dorsal view, D. Ventral view) and E, F. Male's aedeagus

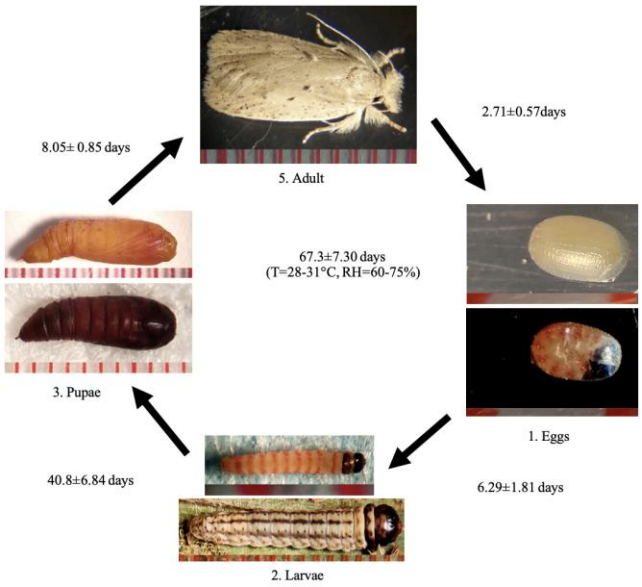

Figure 8. Life cycle of *Opisina arenosella* was monitored under laboratory conditions (T: 28-31°C, RH: 60-75%)

Muralimohan et al. (2013) reported that under 30ºC, the lifespans of *O. arenosella* adult males and females were similar (5.38 and 4.76 days, respectively). Some research results have formerly shown that the larvae of this species had 5 instars (Perera et al. 1988) and 8 instars (Mahadi and Ariffin 2018; Le et al. 2023). In this study, when the larvae were monitored under the laboratory conditions, they underwent 5-11 instars. *Opisina arenosella*'s biological characteristics had more significant differences than previous studies, especially the instar diversity and development time at the larval stage. This shows the strong adaptability of this species to different living conditions, which is possibly one of the risk factors for the spread of the pest. Some studies on factors affecting insect development have also shown that insect lifespan is related to the temperature of the living environment (Muralimohan et al. 2013) or both temperature, relative humidity, and host plants (Lu et al. 2023). Consequently, the development time and instar of black-headed caterpillar larvae are remarkably diverse. This change can be influenced by external factors such as temperature, humidity, nutrition, etc. The fluctuation shows the adaptability of *O. arenosella* in different living conditions. It is one of the important characteristics that allows the species to adapt and spread and cause damage to host plants.

It has been concluded that *O. arenosella* has a wide range of morphological and biological characteristics, especially in the larval stage, which may help them adapt well to different living conditions. The small size of eggs and early larvae may be an obstacle to identify pests in the garden. Aspects of sterile insect techniques and the application of natural enemies to larvae under field conditions in different regions need further study for effective management of this insect pest. In addition, the small size of *O. arenosella* eggs and larvae may initially be an obstacle to detection in the orchards. Therefore, the application of Geographic Information Systems (GIS) should be considered as a solution to promptly determine the pest status for effective management.

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