

# Morphology, biology, and damage behaviors of nutsedge borer (*Bactra venosana* Zeller) in Tra Vinh Province, Vietnam

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Manuscript received: 22 December 2021. Revision accepted: 19 January 2022.

**Abstract.** Nguyen HU, Phan QN, Dang HT, Son TTN, Nguyen HN, Nguyen THT. 2022. Morphology, biology, and damage behaviors of nutsedge borer (*Bactra venosana* Zeller) in Tra Vinh Province, Vietnam. *Biodiversitas* 23: 733-741. Nutsedge borer, *Bactra venosana* Zeller (Lepidoptera: Tortricidae), is a common pest of sedge in Tra Vinh Province. To control this species, its morphology, biology, and behaviors were investigated in Tra Vinh Province. The study was conducted with the aim of determining the information related to the *Bactra venosana*, as a basis for developing a strategy to manage this species by means of safety solutions. The study was carried out by surveying the behavior of nutsedge borer in sedge fields and determining their morphological and biological characteristics under laboratory conditions. The results showed that the eggs were  $1.06 \pm 0.17$  mm (in length),  $0.84 \pm 0.16$  (in width), oval, and white after turning pale yellow. Larvae progressed through 5 instars with body length and width of  $1.20 \pm 0.18$  mm to  $11.3 \pm 1.23$  mm and from  $0.25 \pm 0.05$  to  $1.05 \pm 0.25$  mm, respectively. Newly hatched larvae were dark yellow, and pupae were pale green. The male pupa was  $7.75 \pm 0.68$  mm long, while the female was  $8.46 \pm 0.93$  mm long, light green, and dark brown about to mature. The male adult of *B. venosana* was usually gray-brown and  $7.70 \pm 0.60$  mm in body length, whereas the female adult was silver-gray,  $8.41 \pm 0.86$  mm. The life cycle passed  $35.9 \pm 2.55$  days in laboratory conditions. Under field conditions, eggs were laid scattered on the surface of the host plants. Larvae bored inside the stems of sedge. Newly damaged sedge plants had no symptoms at first whose bodies got soft, turned from light green to pale yellow, and gradually wilted and died.

**Keywords:** *Cyperus*, Mekong Delta, sedge plant, Tortricidae

## INTRODUCTION

The sedge plant *Cyperus malaccensis* Lam. belongs to the genus *Cyperus*, family Cyperaceae with about 600 species distributed in many countries such as China, Indonesia, Japan, and Vietnam (Hibi et al. 2019). It is reported as a native plant at the mouth of the Min River, China (Wu et al. 2020), which additionally controls methane emissions associated with their production, oxidation, and conversion. (Tong et al. 2012). In northern Taiwan, sedges have been identified as an important component of mangroves (Hsieh et al. 2015). Several species in this family are used in folk medicine (Hibi et al. 2019). The studies stated that the extract of the *Cyperus rotundus* root had an analgesic effect on mice, and its rhizosphere contains bacteria with antimicrobial abilities (Ambarwati et al. 2019; Puspitasari et al. 2003). *Cyperus malaccensis*, which has become a vital fiber crop and grown in Japan and China, is the raw material for making mats (Shioya et al. 2019). The sedge plant fiber has already been used in some items such as ropes, furniture, paper and studied to make composite reinforcement for applications in engineering (Neuba et al. 2020). They can degrade or accumulate heavy metals, making them suitable for treating soils contaminated with some of these metals (Maleeya et al. 2015). Sun et al. (2019) pointed out that sedge accumulated much lead, copper, and zinc, to be used as

biological indicators in some cases. This plant was also considered to be used as a green and cost-effective microwave absorber (Liu et al. 2021). In Vietnam, besides dyke protection and saline soil improvement, sedge has high economic values for domestic demands as well as for export (Hung 2017). Products from sedge are very diverse such as making carpets, weaving mats, bags. The sedge is grown in four major regions: the North Coast, the North Central Coast, the South Central Coast and the Southern Coast, which the provinces of Thanh Hoa, Ninh Binh, Vinh Long, Long An, and Tra Vinh have large sedge areas and a great number of sedge mat craft villages (Hung 2017). Cang Long district is the main sedge growing area in Tra Vinh Province. Many cooperatives and villages producing sedge mats for export have been established here, helping many farmers increase their income by growing sedge and producing products from this plant (Ung 2017).

Tortricidae is one of the large families of the order Lepidoptera and is mostly pests (Jerome et al. 2012; Özdem 2020). *Bactra* Stephens, 1834 (Olethreutinae: Bactrini) is a common genus of the family Tortricidae (Lepidoptera) and has been recognized as a major pest on the family Cyperaceae (Vargas and Vargas-Ortiz 2019). The nutsedge borer, *Bactra venosana* (Zeller, 1847), is common in many parts of the world, such as Africa, Europe, and Asia (Sohn et al. 2015). They have been suggested as one of the agents to control nutsedge in some

countries (Das et al. 2015). *B. venosana* is one of many species of the family Tortricidae that have been mentioned in Iran (Alipanah 2015) and recorded in Korea, Fiji Island (Razowski 2016; Clayton 2017). In Vietnam, *B. venosana* is also one of the important pests of sedge in Tra Vinh Province and some other places such as Ninh Binh, Thanh Hoa province, etc. Damage symptoms are yellowing and dying sedges (Figen et al. 2012) with possible damage up to 85% (Hung 2017). Our previous studies in Tra Vinh Province have recorded that the density of the nutsedge borer was 52.1 individuals/m<sup>2</sup> (Ung et al. 2017). The damages have shown that *B. venosana* possibly causes significant effects on sedge productivity and quality. At present, studies on this pest in Trà Vinh Province have not been made available. Therefore, the morphology, biology and behaviors of *B. venosana* were studied to serve as a database for the management of this pest.

## MATERIALS AND METHODS

### Study areas

Field studies were investigated in Cang Long district, which has the largest sedge areas in Tra Vinh Province, Vietnam (Figure 1). The morphology, biology and damage behaviors were carried out in the laboratory of Tra Vinh University. The duration of these studies was from September 2019 to August 2020.

### Sources of insects

Sources of insects for studying were obtained in Tra Vinh Province of Vietnam. The sedges which were found to contain nutsedge borer were collected from the fields and reared in the laboratory until maturity for morphological and biological research.

### Study on the morphology and biology of *B. venosana*

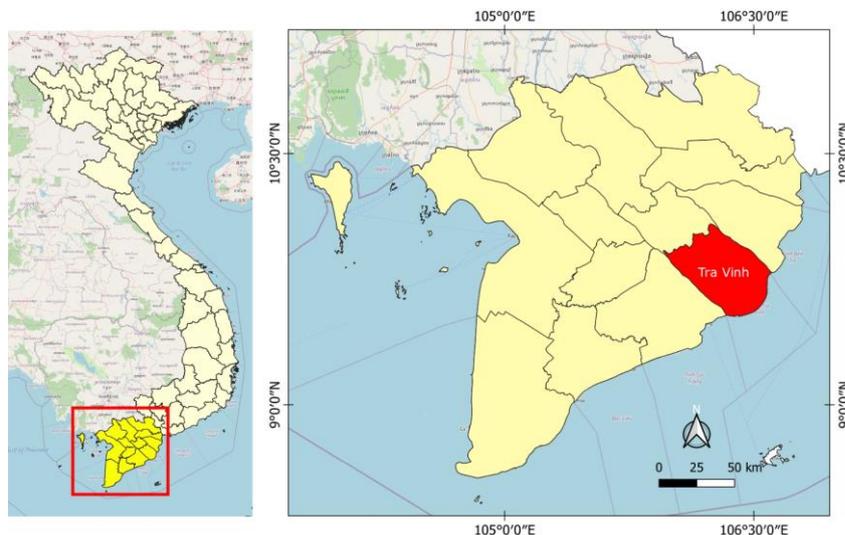
*Bactra venosana* individuals were initially collected from infected sedges in the fields in Tra Vinh Province and were maintained in the laboratory ( $T = 30 \pm 1^\circ\text{C}$ ,  $\text{RH} = 74$

$\pm 4\%$ ). After emerging, the adults were put and paired in transparent plastic boxes with sizes of 12.5 x 16.5 x 6.0 cm (in length, width, and height) covered by white chiffon fabric, perforated caps. These adults were reared on sedge stem pieces for mating and oviposition substrate with cotton soaked in a 10% honey solution. Studies were started on newly laid eggs within 24 hours and sedge stem pieces as the food was renewed daily to determine the exact time when new eggs were laid. A total of 100 100 individuals were examined and measured under a stereomicroscope. Accordingly, their morphological and biological characteristics were carried out with a total of 100 eggs, 100 larvae, 59 female pupae, 41 male pupae, 59 females and 41 adult males in total. The life cycle was surveyed on 59 adult pairs in the laboratory.

Larvae individuals were reared in small round plastic transparent boxes of 7.5 x 7.5 x 5.0 cm with a fresh sedge stem piece to study each instar's number and duration (Figure 2). The number of larval instars was recorded after each molt based on the finding of head capsules (Grunert et al. 2015). This method was also reported in the other studies, according to which the head capsule was also an instar indicator of *Monochamus alternatus* (Coleoptera) (Go et al. 2019) and *Dendrolimus pini* L. (Lepidoptera: Lasiocampidae) (Sukovata 2019), etc. Nutsedge borer *B. venosana* was identified according to the method of Youdeowei (1977), Walker and Crosby (1988) and Upton and Mantle (2010).

### Investigation on the damage symptoms and behaviors of *B. venosana* in the sedge fields

The symptoms and behaviors of *B. venosana* on sedge were carried out on 100 sedge fields with an area of over 2,000 m<sup>2</sup>. The study was assessed by randomly surveying five different locations in the sedge fields (four sites in the peripheral and the other one in the central areas), with each location of one square meter. Symptoms of this pest on sedges were determined by field observations and aided by a microscope. Behaviors of this species were also routinely investigated in the sedge fields for additional results.



**Figure 1.** Map of Vietnam, Mekong Delta, and study area: Tra Vinh Province



**Figure 2.** Experimental arrangement for surveying the development of eggs, larvae and pupae of *B. venosana* in the laboratory

When the last larval instars were prepared to transform to the pupal stage, they were kept inside the sedge stem pieces and put in plastic boxes until they emerged into adults. After their emerged from pupae, adults were reared in boxes like the original adults and checked daily to monitor the time of laying and the number of eggs. Morphological and biological characteristics were implemented by surveying and following from 100 new eggs to the consummation of the stages.

**Data analysis**

The results of the investigation on morphology, biology, and damage behaviors of *B. venosana* were entered and calculated with mean ± SE using Microsoft Office Excel.

**RESULTS AND DISCUSSION**

**Morphological characteristics of *B. venosana* reared under laboratory conditions of Tra Vinh University, Vietnam.**

*Eggs*

The *B. venosana* eggs were average 1.06 ± 0.17 mm (length) and 0.84 ± 0.16 mm (width), oval, shiny, semi-

transparent, then changed to yellowish color. The black larva's head can be found clearly underneath the eggshell before hatching (Figure 3).

In previous research in other provinces, Hung (2017) also indicated that eggs were oval, about 1.2 x 0.8 mm in size, white changed into yellow color after 1-2 days and a big black dot when preparing to hatch.

*Larvae*

The larvae of *B. venosana* had a blackhead, body length from 1.20 ± 0.18 mm to 11.3 ± 1.23 mm, and body width from 0.25 ± 0.05 to 1.05 ± 0.25 mm. After hatching, they were dark yellow, then changed to yellow-brown, and in the last instar, the larvae's body turned into the light green before pupation. The average size of the larval head capsule increased from 0.26 to 0.81 mm (length) and from 0.25 to 0.64 mm (width) (Table 1, Figure 4). Compared with Hung's research (2017), there was a difference in the color of larvae. Accordingly, the body length *B. venosana* larvae increased gradually from 2.25 ± 0.15 mm to 16.7 ± 0.06 mm while the width was from 0.19 ± 0.27 mm 2.03 ± 0.01 mm. Newly hatched eggs were yellow, then turned to milky white, while the last larvae instars were shiny yellow. The color diversity of insects was related to many factors like camouflage (Cook and Saccheri 2013) and climate change (Bastide et al. 2014). As for the color change of larvae, Aguilon et al. (2015) observed differences in the coloration of larvae of *Spodoptera exempta* (Lepidoptera: Noctuidae) at different temperatures, which has been noted as the variety of insects adaptation to climate change.

In addition, under a stereo microscope, the larval testes could be seen at the 3rd instar, milky white then turned into reddish-brown with the larvae developing (Figure 5). This is also one of the common features of larvae of the Tortricidae family. Based on this characteristic, the sex of many species was easily determined from the larval stage. Specifically, a study on the morphology of *Grapholita molesta* (Lepidoptera, Tortricidae) also recorded that larvae testes were found to be kidney-shaped on the dorsum of the third instar (Zhang et al. 2017). In the research results on *Cydia pomonella* (Lepidoptera, Tortricidae), Zada et al. (2019) published purple-red testes of male larvae instar, which could be seen through the skin of the abdominal segment.

**Table 1.** Average sizes (mean ± SD) of *Bactra venosana* larvae

Development stage	Samples	Average sizes (mm)			
		Head capsule length	Head capsule width	Body length	Body width
1st Instar	100	0.26 ± 0.05	0.25 ± 0.06	1.20 ± 0.18	0.25 ± 0.05
2nd Instar	100	0.27 ± 0.05	0.26 ± 0.06	4.39 ± 0.77	0.34 ± 0.07
3rd Instar	100	0.42 ± 0.09	0.39 ± 0.07	6.15 ± 0.61	0.54 ± 0.16
4th Instar	100	0.60 ± 0.08	0.47 ± 0.09	7.89 ± 0.64	0.71 ± 0.16
5th Instar	100	0.81 ± 0.09	0.64 ± 0.08	11.3 ± 1.23	1.05 ± 0.25

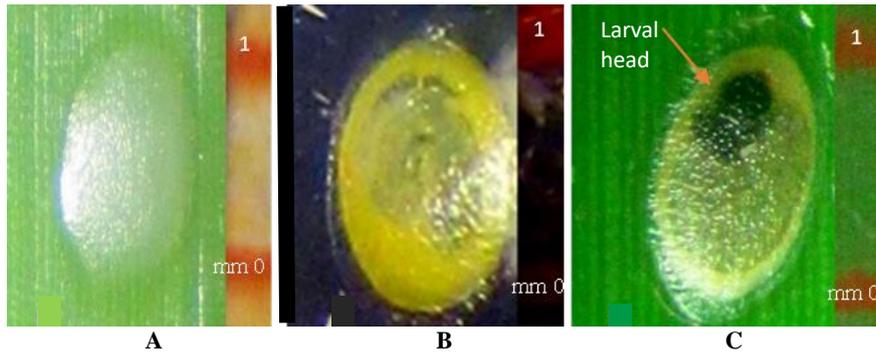


Figure 3. Morphology of *Bactra venosana* eggs (mm): newly laid egg (A), mature egg (B) and pre-hatched egg (C)

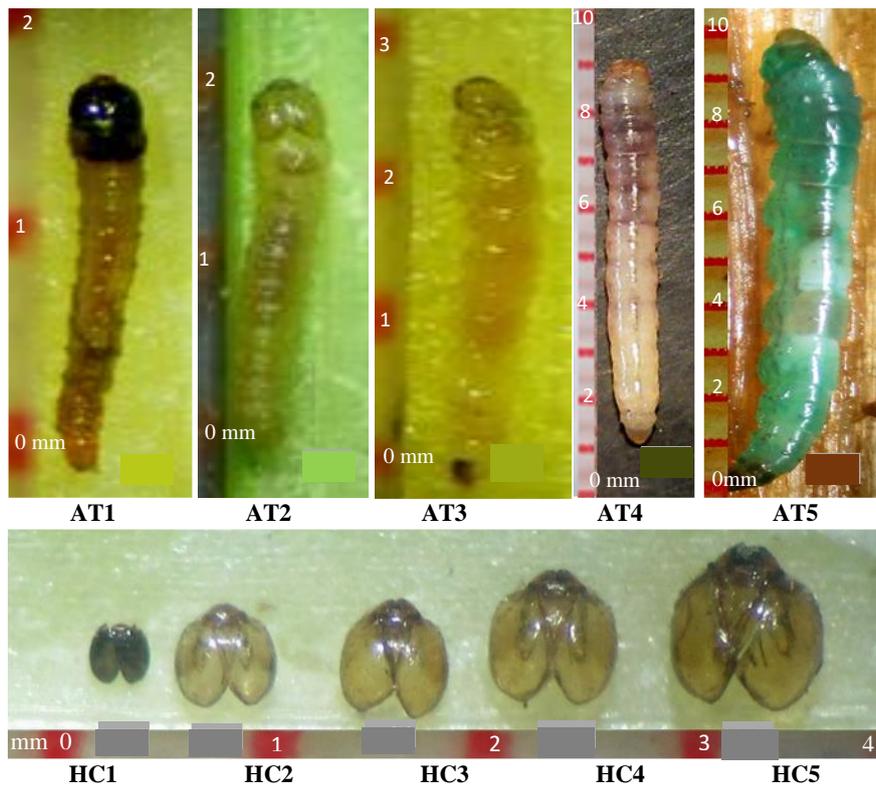


Figure 4. Morphological characteristics of *Bactra venosana* larvae and larval head capsules (HC) from the 1<sup>st</sup> to 5<sup>th</sup> instar (mm)

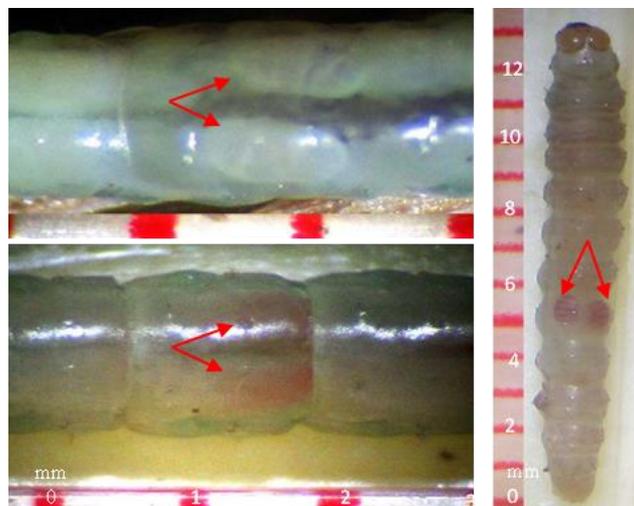


Figure 5. Pairs of male larval testes at the dorsal side were observed under the stereo microscope

**Pupae**

The pupae of the nutsedge borer had a light green color similar to that of the last larval instar and gradually turned to yellowish-brown. Before emerging, they were dark brown. The average male and female pupae lengths were  $7.75 \pm 0.68$  mm and  $8.46 \pm 0.93$  mm, respectively (Table 2, Figures 6A, B, C). In addition, male and female pupae were distinguished by the structure of the genital opening. While the male genital opening was like a short slit between two lumps, a longitudinal notch could be found obviously on the ventral surface of the female pupa next to the genital pore (Figures 6D, E).

The color of the *B. venosana* pupae in this study was different from the research results of Hung (2017). At the new pupation stage, their body was milky white, then changed to yellowish when preparing to emerge. This could be due to being reared in different conditions. Related to this information, some previous results have been mentioned. For example, in the study on *Papilio polytes* (Lepidoptera, Papilionidae), Yoda et al. (2020) presented the color polymorphism of *Papilio polytes* pupae related to habitat factors and Yamanaka (2012) indicated that the color of *Polygonia c-aureum* pupae (Lepidoptera: Nymphalidae) was controlled by environmental factors such as temperature and photoperiod.

In another study, Tuncer and Aker (2017) indicated that male and female pupae of *H. cunea* (Lepidoptera: Erebidae) had distinct posterior abdominal differences; and genital slits in females were twice as long as in males. Lin et al. (2020) studied *Mythimna separata* (Lepidoptera: Noctuidae) and described that the abdominal segments contained the sex organs, which were the morphological features distinguishing females from males, especially the genital openings.

In this case, it was shaped like a longitudinal suture on the female pupae's abdominal segments and as a short slit covered by two semicircular lumps on the male pupae. Nowadays, sterile insect techniques have become common in pest management programs by mating disruption (Marec 2019; Bourtzis and Vreysen 2021), in which early sex determination of insects is very important. Accordingly, distinguishing male and female pupae through morphological characteristics will contribute to the easier application of this technique.

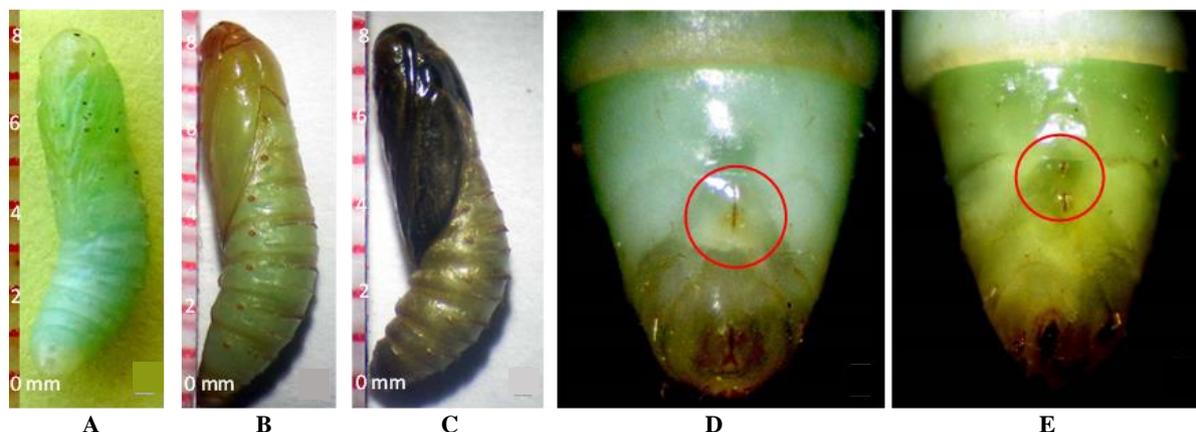
**Adults**

The adult nutsedge borer had threadlike and segmented antennae longer in females than males. The forewings were elongated, while the hindwings were oval and lighter in color and had long gray-yellow feathers in the outer margin area. Male adults were gray-brown with dark brown streaks spreading down the length of the wings, about  $7.70 \pm 0.60$  mm on average in body length and  $14.5 \pm 1.69$  mm in wingspan (Figures 7A, B). The adult female was silver-gray, averaging  $8.41 \pm 0.86$  mm in body length, with a  $16.4 \pm 1.48$  mm wingspan and darker yellow-gray markings on the wing margins (Figures 7D, E). This is similar to the results published by Hung (2017), in which the average body lengths of female and male adults were  $10.7 \pm 0.15$  mm and  $8.91 \pm 0.22$  mm, respectively. Previously, Sohn (2015) presented information on wings coloration when studying Olethreutinae species in Korea but with shorter wingspans (6.1-7.0 mm). These dimensions were also comparable to those pointed out by Figen et al. (2012) on *B. venosana* that the adult length and wingspan were 5-6 mm and 13-14 mm while those of the female were 6.0-7.0 mm and 14-16 mm. Wing size has been recorded as a size indicator of many species in the order Lepidoptera (Erlacher and Erlacher 2016; Gentile et al. 2021).

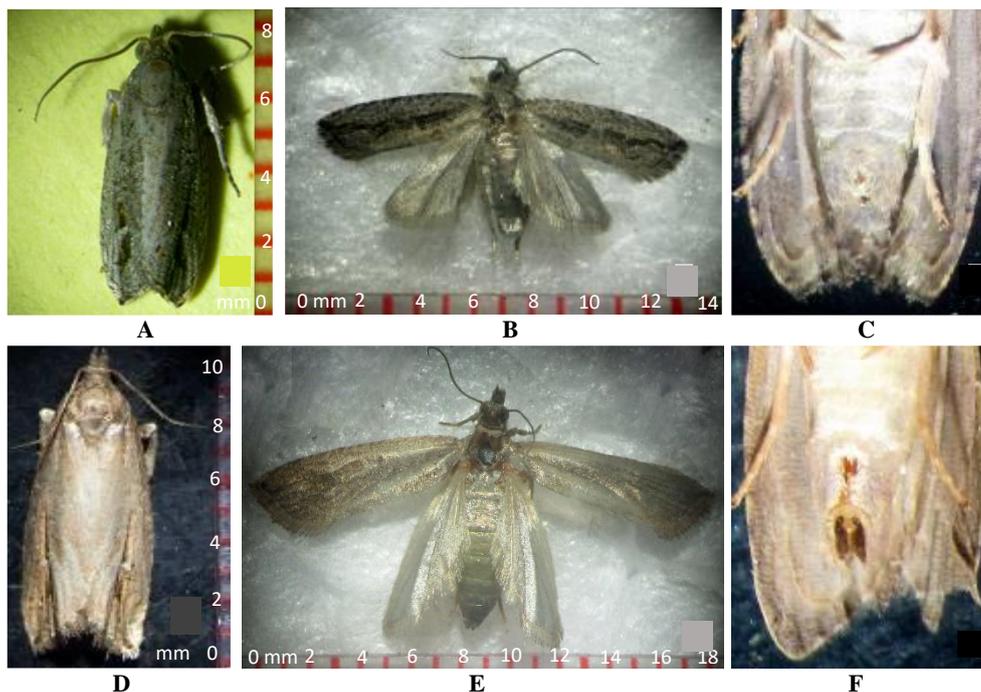
*Bactra venosana* male and female adults were easily distinguished by the color of the wings and the last abdominal segment. The female adults, whose external genitalia (Figure 7F) can clearly be seen, had a larger rounded abdomen than that of male adults with absent genitalia (Figure 7C).

**Table 2.** Body lengths (mean  $\pm$  SD) of *Bactra venosana*: pupae, adults, and wingspans of adults

Development stage	Samples	Average sizes (mm)		
		Body length	Wingspan	Antennae length
Male pupae	41	$7.75 \pm 0.68$	-	-
Female pupae	59	$8.46 \pm 0.93$	-	-
Male adult	41	$7.70 \pm 0.60$	$14.5 \pm 1.69$	$3.62 \pm 0.56$
Female adult	59	$8.41 \pm 0.86$	$16.4 \pm 1.48$	$3.75 \pm 0.58$



**Figure 6.** The color of *Bactra venosana* pupae (A, B, C) and the genital pore of male pupa (D), female pupa (E)



**Figure 7.** Morphological characteristics of *Bactra venosana* adults: male (A, B, C) and female (D, E, F)

#### Biological characteristics of *B. venosana* reared under laboratory conditions of Tra Vinh University, Vietnam.

The results of the study on 100 individuals showed that the average developmental periods of the immature stages of *B. venosana* were:  $5.32 \pm 0.69$  days (eggs),  $21.7 \pm 1.87$  days (larvae passing five instars),  $7.13 \pm 0.95$  days (female pupae), and  $6.76 \pm 1.36$  (male pupae). The average lifespans of female (59 individuals) and male (41 individuals) adults were  $6.46 \pm 1.82$  days and  $6.20 \pm 1.89$  days. In the laboratory, the time taken for the *B. venosana* life cycle to complete was calculated at  $35.9 \pm 2.55$  days (Table 3).

**Table 3.** Average developmental duration (mean  $\pm$  SD) of *Bactra venosana* stages

Development stages	Samples	Average duration (days)
Eggs to 1 <sup>st</sup> instar	100	$5.32 \pm 0.69$
Larvae	100	$21.7 \pm 1.87$
1 <sup>st</sup> to 2 <sup>nd</sup> instar	100	$3.07 \pm 0.59$
2 <sup>nd</sup> to 3 <sup>rd</sup> instar	100	$3.10 \pm 0.98$
3 <sup>rd</sup> to 4 <sup>th</sup> instar	100	$3.74 \pm 1.67$
4 <sup>th</sup> to 5 <sup>th</sup> instar	100	$5.03 \pm 1.77$
5 <sup>th</sup> to pupa	100	$6.76 \pm 2.20$
Female pupae	59	$7.13 \pm 0.95$
Male pupae	41	$6.76 \pm 1.36$
Pre-oviposition	59	$1.59 \pm 0.50$
Oviposition	59	$4.83 \pm 1.87$
Female adult longevity	59	$6.46 \pm 1.82$
Male adult longevity	41	$6.20 \pm 1.89$
Life cycle	59	$35.9 \pm 2.55$

#### Number of eggs per female and hatching rate

The laboratory survey results showed that the time taken for the *B. venosana* life cycle to finish was calculated at  $35.9 \pm 2.55$  days (Table 3). Besides, the average number of eggs laid by each female adult of *B. venosana* was  $102 \pm 27.0$ , the hatching rate was  $80.6 \pm 9.62\%$ . Previously, Ganga and Jayanth (1995) indicated that in the temperature range from  $22.0^{\circ}\text{C}$  to  $26.0^{\circ}\text{C}$ , each female adult could lay 230 eggs in their lifespan. In a recent study by Hung (2017) in Thanh Hoa province, Vietnam, this result corresponded from approximately 47.9 to 69.13 eggs in different temperatures and humidity. This implies the number of eggs laid has a relatively large fluctuation in different rearing conditions.

Research results of Hung (2017) on biological characteristics of this species carried out in semi-natural conditions in Thanh Hoa province at different months of the year showed that the life cycles of this insect lasted  $33.1 \pm 0.82$  days,  $44.6 \pm 0.87$  days and  $55.8 \pm 0.90$  days corresponding to the temperature levels of  $29.8 \pm 0.7^{\circ}\text{C}$ ,  $25.9 \pm 0.89^{\circ}\text{C}$  and  $21.8 \pm 0.7^{\circ}\text{C}$ , respectively. On average, each female can lay at most 69.1 eggs in its lifespan at  $25.4^{\circ}\text{C}$ ,  $91.9\% \pm 0.8$  humidity and the hatching rate was quite high from 84.1 to 90.1%. This showed that the number of eggs of *B. venosana* was highly dependent on temperature conditions. Indeed, in a previous study, Hung (2017) reported that the lifespan of male adults ranged from 7.62 to 10.3 days and male adults were from 2.43 to 6.31 days depending on temperature and humidity conditions. Thus, with its relatively short life cycle, high egg/female adults ratio, and high hatching rate, *B. venosana* can become a serious pest in farmers' sedge fields.

Adult longevity and spawning times are important for identifying and predicting the likelihood of a pest outbreak. The number of eggs per female and hatching rate is an important factor in assessing the species' damage level in the next generation. Therefore, this species can completely be the main pest in sedge fields.

**Behaviors of *B. venosana***

In the field conditions in Tra Vinh Province, *B. venosana* eggs are laid scattered in many places on the sedge stems, but mainly below 10.0 cm above the ground (Figure 8A). Habib (1976) mentioned that this species laid eggs upper the surface of the *Cyperus rotundus*. This is similar to the study of Hung (2017) in which *B. venosana* eggs are often laid scattered around the sedge stems at 5.0-10.0 cm from the ground. Therefore, by observing in the fields, farmers can detect the early infection caused by the pest from the egg stage for timely prevention and treatment to reduce its damage (Table 4).

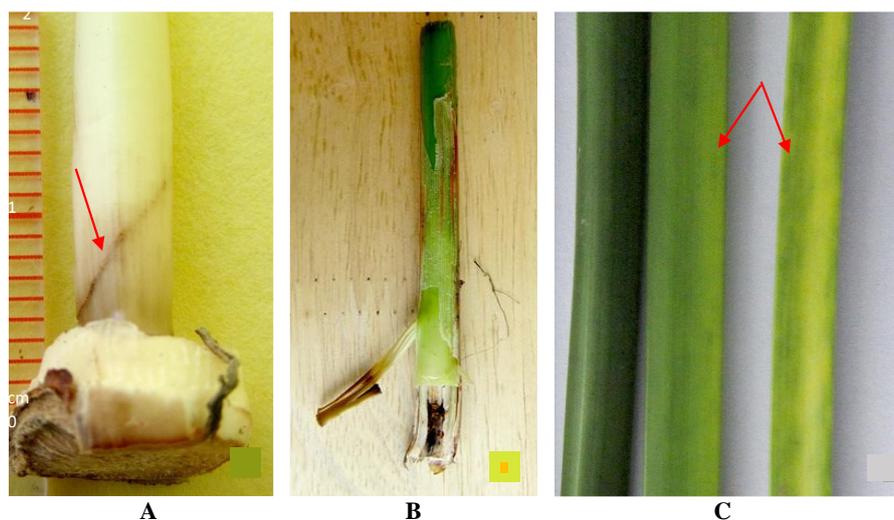
Before getting close to pupation, larvae created a round hole on the stem, which was the place where the adult exited after emergence (Figures 8B, C). Figen et al. (2012) determined that *B. venosana* made a hole near the root neck of the sedge plants before pupating. Hung (2017) also reported on this feature of *B. venosana* larvae in previous research.

Field survey results showed that each sedge plant usually contained only one *B. venosana* larva. This was also found in the publication of Figen et al. (2012) in an earlier study of *B.venosana* in Turkey. Larvae lived and damaged inside sedge stems, bored into a path around the base of the sedge stems and caused the growth point to become necrotic and turn black-brown (Figure 9A). This was noted in Hung's study (2017) on the damaging

behaviors of *B. venosna* larvae in Ninh Binh and Thanh Hoa provinces. In most of the surveyed cases in sedge fields in Tra Vinh Province, at the beginning of the damage, although there was damage inside the tissues, the sedge plants still showed no symptoms when being observed from the outside (Figures 9B). This has made it difficult to detect and prevent this harmful insect on time. Damage symptoms only showed up when the larvae invaded plants for a while. They included plants turning yellow, soft, gradually wilting, and dying (Figure 9C). In a previous study, it has been reported that the damage caused by larvae was that sedge plants could completely dry out (Figen et al. 2012). This is also consistent with pieces of information by Krishnamoorthy (2012) and Sohn et al. (2015) in that *B. venosana* larvae feed into the nutgrass stems. *B. venosana* larvae have been recorded to highly effectively control *C. rotundus*, a species of the genus *Cyperus*, in Australia (Peerzada 2017). Vargas and Vargas-Ortiz (2019) mentioned that larvae of the genus *Bactra* feed in the sedge stem. In India, the larvae have been found to bore into the stem of *Cyperus rotundus* and made their plants dwarf (Singh et al. 2020). These results emphasize the demand to observe and detect this pest at its infancy in sedge fields for high effectiveness control.

**Table 4.** *Bactra venosana* development stages at above-ground locations in farmers' sedge fields.

Development stages	Sample size	Locations above the ground (%)		
		0.0-5.0 cm	≥ 5.0-10.0 cm	≥ 10.0 cm
Eggs	100	47.7 ± 12.7	40.7 ± 11.2	11.7 ± 10.3
Larvae	100	72.3 ± 13.5	21.7 ± 11.4	6.10 ± 5.70
Pupae	100	44.2 ± 11.7	38.4 ± 9.00	17.4 ± 8.80



**Figure 9.** Damage behaviors of *Bactra venosana* larvae inside sedge (A, B) and symptoms observed from outside (C)



**Figure 8.** *Bactra venosana* development stages on sedge stem: eggs (A), larva (B), pupa (C) in sedge stems

In conclusion, the nutsedge borer *B. venosa* has relatively diverse morphological and biological characteristics in terms of color and development time. Therefore, its invasion and damage on sedges are often difficult to detect at an early stage. Although eggs are laid on the surface, they spend most of their life inside the sedge stems. Control by farming methods and biological agents such as parasitic wasps needs further research to manage this pest effectively and safely.

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