



## Biology and description of immature stages of Gymnetis rufilateris (Illiger, 1800) (Coleoptera: Cetoniidae: Cetoniinae)

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Abstract: Larvae, pupae and adults of Gymnetis rufilateris (Illiger, 1800) (Coleoptera: Cetoniidae: Cetoniinae) were collected in the municipality of Rochedo, Mato Grosso do Sul State. Studies on biology and morphology were conducted at the Laboratory of Entomology of the Universidade Estadual de Mato Grosso do Sul in Aquidauana, Mato Grosso do Sul, Brazil. Females of couples formed in the laboratory oviposited and allowed the biology studies. The eggs lasted for 15.5 days. Larvae of the 1st, 2nd and 3rd instars lasted 13.5, 30.6 and 113.1 days, respectively. The pupal period lasted 12.8 days. The egg-to-adult period lasts 186.8 days. The larvae fed on decomposing feces of poultry. In the laboratory, adults fed on ripe banana (Musa sp.) (Musaceae). Descriptions of the 3rd larval instar and pupa of G. rufilateris are presented. A key to known larvae of Gymnetis is also included.

Keywords: Biology, behavior, Gymnetini, immature stages, morphology.

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Resumo: Larvas, pupas e adultos de Gymnetis rufilateris (Illiger, 1800) (Coleoptera: Cetoniidae: Cetoniinae) foram coletados em Rochedo, MS. Estudos sobre a biologia e morfologia foram conduzidos no Laboratório de Entomologia da Universidade Estadual de Mato Grosso do Sul em Aquidauana, MS. Fêmeas de casais formados em laboratório ovipositaram e foram iniciados os estudos de biologia. O período embrionário dura 15,5 dias. As larvas de primeiro, segundo e terceiro instares duram 13,5, 30,6 e 113,1 dias, respectivamente. O período pupal dura 12,8 dias. O período de ovo a adulto completa-se em 186,8 dias. As larvas se alimentam de fezes de aves em decomposição. Em laboratório, os adultos foram alimentados com pedaços de banana (*Musa sp.*) (Musaceae) madura. As descrições da larva de terceiro instar e pupa de G. rufilateris são apresentadas. Uma chave para larvas de Gymnetis conhecidas também está incluída.

Palavras-chave: Biologia, comportamento, Gymnetini, estágios imaturos, morfologia.

#### Introduction

For Gymnetis MacLeay, 1819 (Coleoptera: Cetoniidae: Cetoniinae), 26 species are known, occurring from the United States to Argentina (Morón & Arce 2002). The coloration of adults is variable, including intraspecific variation, as observed by Morón (1995), Antonie (2001), Solís (2004), Orozco & Pardo-Locarno (2004) and Di Iorio (2013). According to Micó et al. (2008), Morón (2010) and Cherman & Morón (2014), Cetoniidae gather enough characteristics to be considered as a family different from Scarabaeidae and Melolonthidae. The Gymnetis is comprised by rougly 30 species (Krikken 1984). For the genera of Gymnetini, Antoine (2001) proposed accurately the use of the old nomenclature and considered Paragymnetis Schürhoff, 1937 and Gymnetosoma Martínez, 1949 as Gymnetis MacLeay, 1819.

In Colombia, Orozco & Parco-Locarno (2004) reared Gymnetis holosericea (Voet, 1779) in laboratory and observed that the egg-toadult period is completed in seven months and adult insects showed mutilation behavior and for G. pantherina Blanchard, 1837, the larvae show cannibalism. In studies conducted in Colombia, Neita et al. (2006) collected adults of G. coturnix (Burmeister, 1842) with fruit traps or on flowers of Annona muricata L. (Annonaceae), and larvae are found in decaying logs of Brosimum utile (Kunth) Oken (Moraceae). Morón & Arce (2002) collected adults of G. hebraica difficilis Burmeister, 1842 and G. flavomarginata sallei Schaum, 1849 (= G. sallei) in rotten banana (Musa sp.) (Musaceae) in a forest in Mexico.

Regarding the morphology of Gymnetis immatures, there are descriptions of the 3<sup>rd</sup> larval instar and pupa of G. flavomarginata sallei

Schaum, 1849 (Richter 1966, Morón & Arce 2002), 3<sup>rd</sup> larval instar of *G. hebraica difficilis* Burmeister, 1842 (Morón & Arce 2002), 3<sup>rd</sup> larval instar and pupa of *G. (Paragymnetis) chalcipes* (Gory & Percheron, 1833) (Morelli 2000) and 3<sup>rd</sup> larval instar and pupa of *G. holosericea* (Voet, 1779) and *G. pantherina* Blanchard, 1837 (Orozco & Parco-Locarno 2004).

In Brazil, works carried out on *Gymnetis* are restricted to studies on the occurrence and diversity of the species (Morón 2004, Ratcliffe 2004, Gonçalves & Louzada 2005, Rodrigues et al. 2013, Puker et al. 2014). In this work, we present for the first time information on the biology and descriptions of 3<sup>rd</sup> instar larva and pupa of *G. rufilateris* (Illiger, 1800).

#### **Material and Methods**

We collected adults, pupae and larvae of *G. rufilateris* between 22-25 December 2012 and 15-20 March 2013 at Bela Vista farm, in the municipality of Rochedo, Mato Grosso do Sul, in a poultry shed (4 x 5 m) in decomposing feces. The samples were sent for identification at the Laboratory of Entomology at the Universidade Estadual de Mato Grosso do Sul (UEMS) in Aquidauana.

We collected 104 larvae of *G. rufilateris* (four of  $1^{st}$  instar, 24 of  $2^{nd}$  instar and 76 of  $3^{rd}$  instar). In the laboratory, the larvae were kept in plastic containers of 500 mL with 2/3 of their volume completed with organic material collected at the site where the larvae were found.

We collected 47 pupae and seven adults from the in plastic containers that was closed with voile fabric. The couples were kept in plastic containers of 4,000 mL, which was covered with voile fabric to prevent them from escaping. Inside the container, we added approximately 2,000 mL of organic material collected from the poultry shed, and ripe banana to feed the adults.

In the containers with adults, we carried out daily observations of mating behavior. Each two days, we inspected and screened material for egg extraction. We collected 200 eggs, which were put in Petri dishes containing a layer of soil, and subsequently placed in a temperature-controlled chamber, which remained with photophase of 12 hours and temperature of  $26 \pm 2^{\circ}$  C. We measured egg width and length with a caliper and the weight on an analytical scale.

After hatching, the larvae were kept in 250 mL containers with organic material and feces of poultry. The larvae remained in the containers until emergence of adults. For larvae and pupae, we performed measurements with a caliper, obtaining information of body length, thorax width and cephalic capsule width. The weight was verified on a digital analytical scale. To monitor the development and duration of larval stage, 50 newly hatched larvae were kept in containers properly numbered 1-50 and we measured the width of the cephalic capsule every three days.

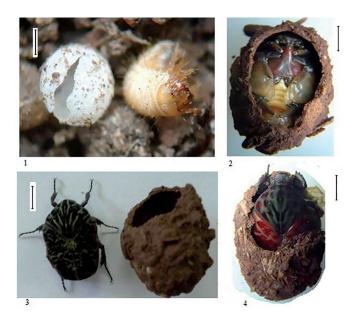
Some adults were mounted with an entomological pin and, through comparisons with insects preserved in the entomological pinned collection of the Universidade Estadual de Mato Grosso do Sul, we obtained their identification.

Ten laboratory-reared larvae were killed in boiling water and preserved in alcohol for description. Larvae and body parts were drawn on stereomicroscope coupled with a camera lucida. Mouthparts were mounted on slides in Hoyer liquid. Descriptions of the larvae were obtained from observations of characters, following the terminology used by Costa et al. (1988) and Morón & Arce (2002). We measured and preserved in alcohol 10 larvae and nine pupae laboratory-reared by Felix P. Garcia on April 24, 2013. The specimens are deposited at UEMS and Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZSP).

### **Results**

**Biology.** In the poultry shed, *G. rufilateris* was usually found up to 50 cm from the sides of the shed walls at 10-20 cm deep. The organic material where the insects developed was humid and easy to handle, unlike the organic material inside the shed, which was dry and compact. Several larvae, pupae and adults were found in this substrate, configuring as suitable food for larval development of *G. rufilateris*.

Newly-laid eggs had average sizes of  $1.0 \times 0.8 \text{ mm}$  and near hatching time, average sizes reached  $2.5 \times 3.0 \text{ mm}$  (n = 50). The embryonic period lasted on average 15.5 days and  $1^{st}$  instar larvae, remained in that stadium for 13.5 days, with cephalic capsule width of 1.35 mm (Table 1 and Figure 1). The larvae had 6.3 mm of body length, 1.94 mm thorax width and 13.34 mg weight (Table 2). Larvae in the  $2^{nd}$  instar remained in this stadium for 30.6 days (Table 1), with 2.06 mm cephalic capsule width. Body length was 16.6 mm, thorax width 4.48 mm and weight 257.05 mg (Table 2). Larvae in the  $3^{rd}$  instar remained in that stadium for 113.1 days (Table 1), with 3.6 mm of cephalic capsule width. They were 29.7 mm long, 6.7 mm wide and weighed 1,352.34 mg (Table 2).



**Figures 1-4.** *Gymnetis rufilateris* (Illiger, 1800). 1) Chorion and first instar larva. 2) Pupa inside pupal chamber, 3) Adult and pupal chamber, 4) Newly emerged adult inside pupal chamber. Scale bars: Fig. 1, 1 mm; 2-4, 5 mm.

At the end of the third instar, the larvae acquire a whitish color and construct a pupal chamber, where they remained. The pupal chamber was constructed with organic material, soil, own excrement and probably saliva. The chambers had an average size of 23.84 mm long and 17.48 mm wide (Figures 2-4).

<b>Table 1.</b> Duration (mean $\pm$ SE	E) of developmental stages of	Gymnetis rufilateris (laborato	ory conditions, $26 \pm 1^{\circ}$ C, 12-h photoperiod).

Stage	<b>Duration (days)</b>	Interval of variation (days)	N	Viability (%)
Egg	$15.5 \pm 0.04$	15 - 16	200	100
1 <sup>st</sup> instar	$13.5 \pm 0.16$	12 - 17	200	100
2 <sup>nd</sup> instar	$30.6\pm0.20$	30 - 35	180	90
3 <sup>rd</sup> instar	$113.1 \pm 3.91$	102 - 138	142	77.8
Pupae	$12.8 \pm 0.55$	10 - 16	138	97.2
Adult	$38.5 \pm 0.25$	32 - 45	138	100
Egg-to-adult	$186.8 \pm 1.25$	102 - 214	138	69

**Table 2.** Mean value ( $\pm$  SE) for length, width, and weight of developmental stages of *Gymnetis rufilateris* (in laboratory,  $26 \pm 1^{\circ}$ C, 12-h photoperiod).

Stage	Length (mm)		Width (mm)		Weight (mg)	
	$Mean \pm SE$	Interval	$Mean \pm SE$	Interval	$Mean \pm SE$	Interval
Egg	$1.0 \pm 0.02$	0.9 - 1.1	$0.8 \pm 0.02$	0.7 - 1.0	-	-
1st instar	$6.3 \pm 0.24$	4.5 - 12.0	$1.9 \pm 0.01$	1.4 - 3.8	$13.34 \pm 0.01$	6.1 - 80.0
2 <sup>nd</sup> instar	$16.6 \pm 0.85$	9.9 - 22.4	$4.5 \pm 0.21$	2.6 - 6.0	$257.06 \pm 32.83$	60 - 544
3 <sup>rd</sup> instar	$29.7 \pm 0.58$	19.3 - 34.3	$6.7 \pm 0.15$	5.3 - 8.3	$1,352.34 \pm 71.11$	380 - 2,153
Pupa	$23.8 \pm 0.26$	18.5 - 27.7	$17.5 \pm 0.25$	12.8 - 20.6	$697.55 \pm 77.99$	436 - 1,211

The pupae remained in this stage for 12.8 days on average with 23.8 mm long and 17.5 mm wide (Table 1 and Figure 2). The egg-to-adult period of *G. rufilateris* was completed in 186.8 (102 - 214) days on average.

After emergence adults have predominantly red color (Figure 4). After sclerotized, adults head were predominantly slightly green with shades of black. On the pronotum and elytra, dark-green color predominated with black streaks and red color predominated in spots on the edges (Figure 3). Adults of *G. rufilateris* showed average longevity of 38.5 days (n = 50).

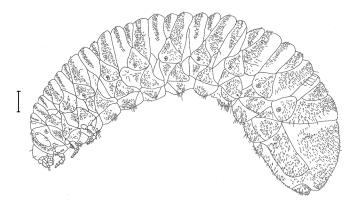
Copulation in the laboratory was observed with a duration 150 seconds (n = 1). Four other copulation attempts were observed in the laboratory. Copulation and copulation attempts occurred between 07:30 and 09:00 a.m. Copulation showed the following behavior: the male meets the female and touches this with antennae and tarsi of the first pair of legs. Then the male climbs onto the female, positions the final portion of the abdomen lined with female pygidium, exposes the aedeagus and inserts it into the female genital chamber. After copulation, the male climbs off the female and both separate.

In copulation attempts, the male climbs onto the female, exposes the aedeagus and initiates rapid movement with the abdomen, where the exposed aedeagus touches quickly on the female pygidium, in an attempt to initiate copulation. This behavior lasts 4 minutes on average and, immediately ceases. Next, the male climbs off the female, retracts the aedeagus, walks near the female, climbs onto the female again and repeats the behavior for 4 minute again (n = 4). If the female does not accept the male for copulation, the male climbs off the female, retracts the aedeagus and both separate. During the 4 minute attempts of copulation, the male show intense rhythmic activity back and forth with the aedeagus exposed.

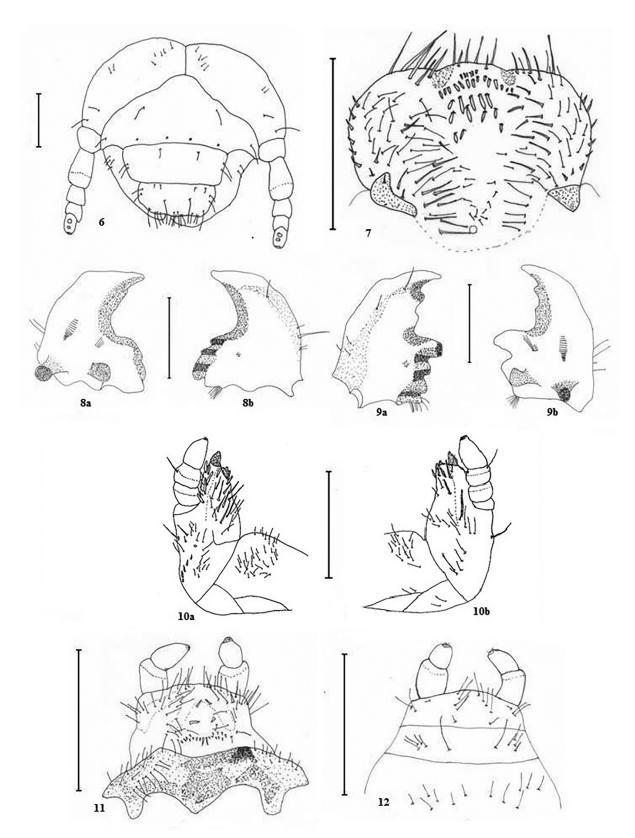
Ripe bananas were offered to adults, which remained feeding for 2-3 hours. According to information from Mr. Nilson Falco, owner of Bela Vista Farm, the 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae of *G. rufilateris* are used as excellent baits for deep-sea fishing of piraputanga (*Brycon microlepis* Perugia, 1897) (Characidae).

## *Gymnetis rufilateris* (Illiger, 1800) 3<sup>rd</sup> instar larva

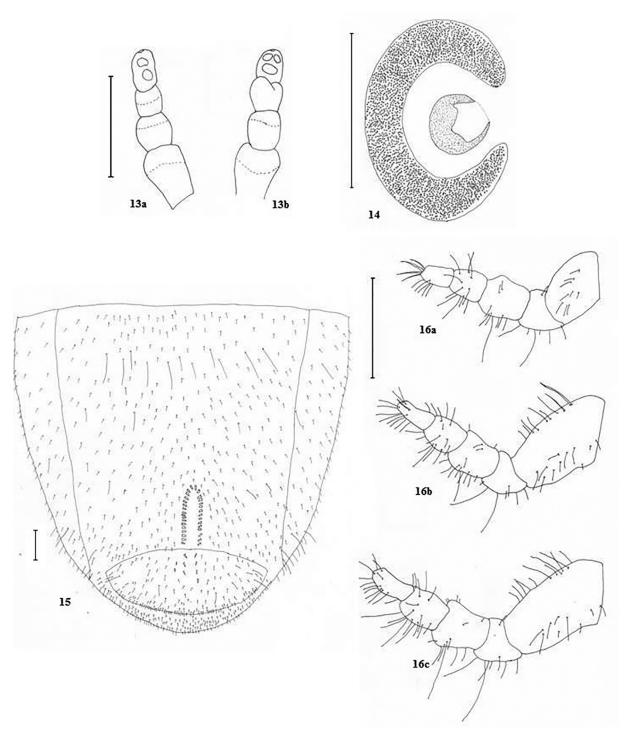
**Description.** Dorsal length 29.7 (20.3 - 35.4) mm on average, prothoracic width 6.7 mm, head capsule width 3.6 mm (Figure 5). **Head** (Figure 6): Hypognathous, strongly sclerotized, light brown. The epicranial arms is slightly sinuous in its initial third. Setae with the following distribution: five dorsoepicranial pairs adjacent to coronal suture, two pairs near the antennifer, one pair in the postero-frontal region, and one pair in the anterior frontal angle. Stemmata absent. Well-sclerotized antennifer about half of length of pedicel. Antennae (Figures 5, 6, 13) long, 4 articles, well-sclerotized, articles I-III enlarging to apex, short sensorial cone at apex on article III, article IV (Figure 13) with two dorsal and three ventral sensorial spots. Clypeus (Figure 6) trapezoidal, with one posterior clypeal setae and two exterior clypeal setae. Labrum (Figure 6) anterior border trilobate, 5-6 anterior marginal setae on each side, posterior-lateral margin with three setae per side, basal region with two groups of four short setae.



**Figure 5.** *Gymnetis rufilateris* (Illiger, 1800), third instar (lateral view). Scale bar: 2 mm.



Figures 6-12. Gymnetis rufilateris (Illiger, 1800), third instar larva. 6) Head, frontal view; 7) Epipharynx; 8) Right mandible, a) ventral view, b) dorsal view; 9) Left mandible, a) dorsal view, b) ventral view; 10) Left maxilla, a) dorsal view, b) ventral view; 11) Hypopharynx; 12) Labium. Scale bars: 1 mm.



**Figures 13-16.** *Gymnetis rufilateris* (Illiger, 1800), third instar larva. **13)** Antennal apex, a) dorsal view, b) ventral view; **14)** Thoracic spiracle; **15)** Urosternite X; **16)** Left legs, a) prothoracic, b) mesothoracic, c) metathoracic. Scale bars: Fig. 14, 0.5 mm; Figs. 13, 15-16, 1 mm.

Epipharynx (Figure 7): Clithra present. Corypha with 6 setae. Haptomeral process present, with distal projection and 14 heli. Above heli 6 small setae. Behind the heli, 12 long setae irregularly distributed. Acroparia with 6 long setae on left side, 7 on right side. Acanthoparia with 6 setae on left and on right. Chaetoparia with short spiniform setae, 51 setae on left side, 43 on right side. Dexiotorma transverse, short sclerotized plate. Laeotorma present. Sensorial cone present.

Right mandible (Figure 8ab) with scissorial teeth S1 and S2 fused, separated from S3 by a furrow. Dorsal molar area with four setae. Dorsal surface with a large setae below base of S3 scissorial. Scrobe with four setae. Brustia with nine setae. Ventral region with well-marked stridulatory area consisting of 17 subparallel ridges. Well-developed ventral process. Four setae in molar region, with two basolateral setae.

Left mandible (Figure 9ab) with tooth S1 separated from S2 scissorial tooth by incised furrow. Base of scissorial teeth S2 with a long setae. Scrobe with three setae. Dorsomolar region with about five setae. Brustia with 10 setae. Ventral surface with stridulatory area with 17 well-defined ridges. Ventral process present. Well-developed molar lobe. Molar region with 6-7 setae.

Maxillae (Figure 10ab) symmetrical. Cardo long, with a few setae and stipes with various setae. Galea and lacinia fused, forming mala. Mala with three unci, one apical and two pre-apical. Palpifer slightly shorter than palpomere I. Palp 3-segmented, segment II with a setae. Maxilla with rows containing five acute denticles and an intricate distal process.

Hypopharynx (Figure 11). Sclerome strongly sclerotized, asymmetric, with right side more prominent. Left lobe with 18 setae, right lobe with 10 short setae. Posterior left margin with 18 long, slender setae and posterior right margin with 17 long, slender setae. Glossa with a transverse row of small and short setae.

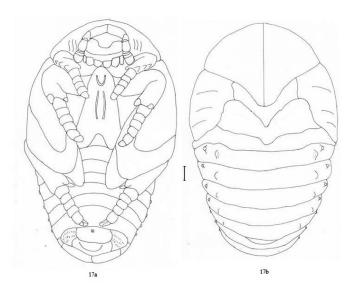
Labium (Fig. 12). Submentum with various setae. Mentum with 5 pairs of setae. Prementum with 5 pairs of setae on median region and 3 pairs of seta on lateral region. Labial palpi 2-segmented, small sensory hairs on apex.

Thorax: Thoracic segments with several dorsal and lateral setae disposed in rows. Pronotum with two lobes and a brown sclerotized plate on each side with three setae (Figure 5). Thoracic spiracle 0.54 mm long, 0.47 mm wide (Figure 14). Respiratory plate with about 19 holes across the middle section. Respiratory orifice with irregular shaped. Meso and metanotum each with three lobes, respectively. Legs. Coxa, trochanter, femur, tibia and tarsungulus of all legs with numerous, long, stout setae. Anterior and middle legs shorter than hind legs (Figure 16abc). Tarsungulus cylindrical rounded apically with 9-13 setae.

**Abdomen**: Ten-segmented. I-VII each with 3 dorsal lobes. Segments IX and X united. Spiracles I, II and IV 0.55 mm long, 0.41 mm wide; III and V 0.51 mm long, 0.42 mm wide; VI 0.60 mm long, 0.50 mm wide; VII 0.58 mm long, 0.43 mm wide; and VIII 0.57 long, 0.40 mm wide. Raster with one pair of palidia and with 15-17 setae in each pali. Palidia opened posteriorly and closed anteriorly. Lower anal lip with about 168 small, thick setae (Figure 15).

Pupa (Figure 17ab)

Length: 23.8 (18.5 - 27.7) mm, maximal width: 11 mm. Body elongated, exarate and light yellow. Completely glabrous. Antennae and mouthparts clearly separated. Ocular canthus, antennae, and compound well-differentiated eyes. Clypeus concavo. Pronotum trapezoidal.



**Figure 17.** *Gymnetis rufilateris* (Illiger, 1800), pupa, a) ventral view, b) dorsal view. Scale bar: 1 mm.

Meso- and metasternum with wide process with apex emerging between pro- and mesocoxa. Pterothecae free, compressed around the body and extended posteriorly to fifth abdominal segment. Pterothecae I superposed to pterothecae II. Prior margin of pterothecae I subposed by medial leg. Pterothecae II covering proximal half of posterior leg. Spurs of meso and metatibiae visible. All tarsomeres little defined on apex. Tergites convex. Seven spiracles visible dorsally. Elongated spiracle I, not prominent, partially protected. Spiracles II - IV tuberculiform, sclerotized peritreme. Spiracles V-VII closed, tuberculiform, prominent. Urogomphi absent. Female ventrite VIII with medial genital pore, male ventrite VIII with genital ampulla.

**Material Examined.** BRAZIL. Mato Grosso do Sul: Rochedo (Bela Vista Farm), on poultry feces, 22.XII.2012 - 20.III.2013, F. P. Garcia, 10 larvae, 9 pupae (measured) (UEMS), 3 larvae (3<sup>rd</sup> instar), 1 pupa, 2 adults (MZSP).

# Key to the Known Third Larval Instar of *Gymnetis* MacLeay, 1819 (Adapted from Orozco & Pardo-Locarno 2004)

### **Discussion**

Gymnetis rufilateris completed the cycle of 186.8 days (about 6 months), and showed no cannibalism behavior in the laboratory. Its life cycle is seven months shorter than that of *G. holosericae*, which also presented cannibalism behavior according Orozco & Locarno Parco (2004). Immature of *G. rufilateris* develop in decaying poultry droppings. These observations agree with Arce & Morón (1999), who reported that *Gymnetis* larvae develop in organic material.

The third instar larvae of *G. rufilateris* differ from those of other species of *Gymnetis* by having five dorsoepicranial pairs setae adjacent to coronal suture, antennomere IV with 2 dorsal and three ventral sensorial spots, spiracles of abdominal segments similar in size and palidium with a row of 15-17 pali opened posteriorly.

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