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Biomorphometry of *Automeris liberia* Cramer (Lepidoptera: Saturniidae) in oil palm in the Amazon

Biomorfometria de *Automeris liberia* Cramer (Lepidoptera: Saturniidae) em palma de óleo, Amazônia Oriental

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Highlights

There is variation in the biological cycle between males and females.
There is marked sexual dimorphism in the species in the pupae and adult stages.
It is possible to breed this species on a natural diet under laboratory condition.

Abstract

Automeris liberia Cramer are found in South America, with documented sightings in Ecuador, Peru, some regions of Mexico and, more recently, the Brazilian Amazon. These moths cause damage to several plant species. We designed an experiment to study the biology and morphometry of *A. liberia* in the laboratory and provide information for the management of the pest in oil palm cultivation in the Brazilian Amazon. Caterpillars for rearing were collected from commercial areas of oil palm cultivation, where they cause defoliation of the plant. They were fed a natural diet of oil palm leaves of the *Tenera* variety and observed from second generation onwards. Sex, behavior, posture, mass, body length, wingspan, body diameter, sex ratio, average growth ratio, and antenna were quantified. The embryonic stage lasted 14 days, followed by seven larval instar stages over 36 days. The pupal stage lasted 21 days. Adults had a longevity of 4.5 and 6 days and a total biological cycle of 78.5 and 80 days, for males and females, respectively. The growth ratio was 1.49. The antennae showed sexual dimorphism, with bipectinate morphology in males and filiform in females. In the adult stage, morphological variables were evaluated (body length, thoracic diameter, wingspan, forewing length, forewing height, hindwing length, hindwing height, antenna length, number of antennomeres, and body mass), and they showed significant differences between males and females

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(Newman-Keuls test, P < 0.05).
Key words: *Elaeis guineenses* Jacq. Leaf stripper caterpillar. Biological cycle.

Resumo

A espécie *Automeris liberia* Cramer tem ocorrência na América do Sul, com registro no Equador, Peru, algumas regiões no México e, mais recentemente, na Amazônia brasileira, causando danos a várias espécies de plantas. Objetivou-se estudar pioneiramente a biologia e a morfometria de *A. liberia* em laboratório, visando subsidiar informações para o manejo da praga na cultura da palma de óleo, na Amazônia brasileira. A criação, foi oriunda de lagartas coletadas em áreas comerciais de palma de óleo, onde as quais estavam causando desfolhamentos. Foram alimentadas com dieta natural a base de folhas de palma de óleo da var. *Tenera.* As observações foram iniciadas a partir da segunda geração. Foram avaliados sexagem, comportamento, posturas, massa, comprimento, envergadura, diâmetro do corpo, razão sexual, razão da média de crescimento e antena. O estágio embrionário durou 14 dias e o larval apresentou sete instares em 36 dias. O estágio pupal foi de 21 dias. Os adultos apresentaram longevidade de 4,5 e 6 dias e ciclo biológico total de 78,5 e 80 dias, para machos e fêmeas, respectivamente. A razão de crescimento foi de 1,49. As antenas apresentaram dimorfismo sexual, sendo do macho tipo bipectinada e da fêmea filiforme. Na fase adulta foram avaliadas variáveis morfológicas (comprimento do corpo, diâmetro do tórax, envergadura, comprimento da asa anterior, altura da asa anterior, comprimento da asa posterior, altura da asa posterior, comprimento da antena, número de antenômeros e massa corpórea) entre machos e fêmeas, as quais apresentaram diferença estatística pelo teste Newman-Keuls (P < 0,05).
Palavras-chave: *Elaeis guineenses.* Lagarta desfolhadora. Ciclo biológico.

Introduction

Butterflies and moths are important species in tropical ecosystems as they interact with local vegetation and fauna (Basset et al., 2017; Goldstein, 2017; Johnson et al., 2017; Mitter, Davis, & Cummings, 2017). However, they can cause significant damage to host plants, since more than half of the lepidopterans of agronomic interest are polyphagous (Formentini, Gómez, Moraes, Barros, & Specht 2015).

The genus *Automeris* has 135 species (Specht, Formentini, & Corseuil, 2006). They are characterized by a circular black or red macula resembling an eye on the hindwing. One of these is the species *Automeris liberia* Cramer, also known as "target moth", belonging to the family Saturniidae (Hemileucinae). An important characteristic of this species is the erucism caused by its caterpillars' bristles leading to severe burns on human skin.

The species is found in Ecuador, Peru, and some regions of Mexico and Trinidad and Tobago (Cock, 2005). Damage caused by *Automeris* spp. has been observed in banana, avocado, cotton, coffee, eucalyptus, mango, and citrus in south, southeast, and northeast Brazil. In the northeast it is found in the states of Alagoas, Ceará, and Maranhão (Zanúncio, Fagundes, Araújo, & Evaristo, 1992). Santos, Casagrande and Mielke (2015) described the genus in the municipalities of Vossoroca and Tijucas do Sul, in the state of Paraná. Silva et al. (2019) reported the presence of *A. liberia* in Eastern Amazonia, Pará state,

where it attacked oil palm (*Elaeis guineensis* Jacq) plants, the most important source of vegetable oil for human consumption (Wahid, Abdullah & Henson, 2004).

Few studies have been conducted on the biological cycle and hosts of *Automeris* spp. Some of the prominent ones are by Furtado and Lemaire (1999); Cock (2005); Specht et al. (2006); Specht, Formentini and Corseuil (2007); Pérez, Domínguez, Fleischer, García and Torres (2017), and Baz, González, Camargo, Sánchez and Bobadilla (2018). Therefore, the objective of this experiment was to study the biology and morphometry of *A. liberia* in the laboratory, on a natural diet of oil palm leaves, to provide important biological information for pest management in oil palm cultivation in the Brazilian Amazon.

Materials and Methods

Collection

A. liberia caterpillars were collected from natural populations in the municipalities of Tailândia, Moju, Acará, and Tomé-Açú, in the northeastern region of Pará (2° 31'33.9 "S 48° 45'49.1" W), Eastern Amazonia, Brazil.

Laboratory rearing

A. liberia caterpillars were reared in the Laboratory of Applied Entomology (LEA), Amazon Rural Federal University (1° 45"59.82 'S 48 ° 43" 77.16' W).They were placed individually in translucent 500 mL polypropylene plastic pots, kept at 26 ± 1°C, covered with voile fabric and attached by plastic alloy. The diet, with daily changes, was composed of oil palm leaves of the *Tenera* variety.

For greater durability, the leaves were stored at 20°C. For feeding, the base of the leaflets were moistened with a cotton swab dipped in distilled water to maintain turgidity.

The morphometric analysis of the pest was performed along with the description of its biological cycle in the laboratory from the second generation. These analyses were conducted in two consecutive generations, starting with the pupal stage, when it was possible to have equal time.

In the adult phase, body length (BL), thoracic diameter (TØ), wingspan (W), forewing length (FL), forewing height (FH), hindwing length (HL), hindwing height (HH), antenna length (AL), type and number of antennomeres (NA), body mass, behavior, and longevity (days) were evaluated.

In the embryonic phase (egg), the eggs were collected from the adult housing cages; they had dimensions of 2.15 x 1.85 x 3.80 m. The eggs were placed in 150 x 20 mm petri dishes that were lined with a filter paper. These were moistened daily and kept in a climate-controlled chamber (26 ± 1°C), with 70% humidity and 12-hour photophase. Length, lower and upper width, mass, coloration, and number of eggs per spawn were determined.

The different larval stages were identified from the time of hatching and morphological and biometric characteristics such as coloration, erucism, length, width, mass, and the time spent in each instar were quantified.

The cephalic capsules of the larvae were measured using an Olympus stereomicroscopic magnifying glass with an attached camera and Toupview software (x86). Biometric analysis was performed according to the protocol described by Parra

and Haddad (1989) and growth was based on the linear model of Dyar (1890).

In the pre-pupal period, when the caterpillars stopped feeding and searched for a place to form the cocoon, the leaves were removed, and tufts of cotton moistened with distilled water were placed inside the container to maintain the necessary humidity. In the pupal stage, mass, thoracic diameter, body length, and sex (Butt & Cantu, 1962) were evaluated. The sex ratio (SR) was calculated according to equation 1 below (Posso, Cardona, Valor, & Moreles, 1989):

$$SR = \frac{\text{number of females}}{(\text{number of males} + \text{number of females})}$$

In the adult phase, the moths were placed in two categories of cages, which differed mainly in size, depending on the purpose. The first cage was larger, measuring 2.15 x 1.85 x 3.80 m, and its sides and ceiling were covered with voile fabric. Three 3-month-old oil palm seedlings were placed inside these cages so that the adults could find a suitable environment for reproduction and oviposition.

Smaller cages of 40 x 40 x 40 cm size were used to observe the behavior of adult pairs. The number of eggs per laying, mating behavior, and longevity of the adults were determined.

The data was statistically analyzed using the Neuwman-Keuls test ($p< 0.05$) to evaluate possible morphometric differences between males and females.

Results and Discussion

The biological cycle of *A. liberia* in the laboratory spanned over 78.5 and 80 days for males and females, respectively (Table 1). This cycle was shorter than that observed for *A. granulosa* (Furtado & Lemaire, 1999) and *A. illustris* (Specht et al., 2006).

Table 1
Duration (days) of the biological cycle of *Automeris liberia* (Lepidoptera: Saturniidae), fed with natural diet of oil palm leaves, Tenera variety, in the laboratory. Eastern Amazonia, Pará, Brazil

Stages	Time (days)
Embryonic (n=100)	14 ± 0,02
Larval (n=80)	36 ± 0,23
Pre-pupa (n=40)	3 ± 0,10
Male pupa (n=20)	21 ± 0,2 A
Female pupa (n=25)	21 ± 0,24 A
Male longevity (n=18)	4,5 ± 0,19 b
Female longevity (n=25)	6 ± 0,21 a
Male cycle	78,5
Female cycle	80

Upper case letters compare means of male and female pupae, lower case letters longevity. Different letters indicate statistical differences by Newman & Keuls test, p<0.05.

Embryonic period

The eggs were found adhered to substrates, such as leaves and the fabric (voile) that covered the cage. Gregarious egg laying was observed, with an average of 24 ± 0.02 eggs per cluster; they were united by adherence to the substrate and to the sides of adjacent eggs (Figure 1A). The spawning was similar to that observed in other species of the genus (Lemaire, 2002), especially *A. naranja* (Specht et al., 2007) and A. illustris (Specht et al., 2006). Egg attachment was similar to that of *A. naranja* eggs but differed from that of *A. illustris* eggs, which are only attached to the substrate.

A. Liberia eggs are trapezoidal in shape, slightly flattened laterally, and they resemble a heart at the end of the micropyle. They are white in color with a light green micropyle, which becomes blackish in the center with a green halo around it when the larva is close to hatching (Figure 1B). The central, upper, and lower diameters and height were measured; they were 1.68 ± 0.02; 1.35 ± 0.02; 1.13 ± 0.02 and 1.89 ± 0.02 mm, respectively. The embryonic period until hatching was 14 days (Table 1); this was similar to that of A. granulosa, which had 13 days of incubation (Furtado & Lemaire, 1999), but it was different from those of *Lonomia obliqua* (Lorine, 1999) and *A. illustris* with 17 and 10.6 days, respectively (Specht et al, 2006).

The egg viability was 64 ± 0.62%. No difference in viability was found between the eggs from females placed in the smaller cages containing individual pairs and those from females in the large cage with a greater number of subjects.

Caterpillars

In the first 48 hours after hatching, the caterpillars did not eat the leaves offered to them. This was probably due to the fact that they fed on the remaining chorion of the eggs (Lemaire, 2002), which is characteristic of individuals of the subfamily Hemileucinae and other lepidopterans. Leaf consumption always starts at the edges and preferentially at the abaxial part. This is followed by the first ecdysis, which characterizes the end of the first instar (Figure 1D).

In the first instar the larvae were very small and measured about 4.72 ± 0.38 mm, while in the second stage, the larvae grew by 1 mm, measuring 5.64 ± 0.45 mm. The caterpillars in both stages were greenish yellow and had long bristles with dark edges. They showed gregarious behavior, similar to *A. granulosa* of the same age (Furtado & Lemaire, 1999). Their bristles did not show urticating action when in contact with the skin, all the way until the fifth instar. Their legs had black bands covered by white bristles.

The third and fourth instars did not show marked morphological changes compared to the second. However, the caterpillar presented intense green coloration, which remained until the pre-pupal stage.

In the fifth instar, the caterpillars can cause erucism. This makes them dangerous to manage in the laboratory as well as in the field as the caterpillars are found on the adaxial part of the oil palm leaves and can adhere to the clothing of the people in plantations. This increases the chances of caterpillars falling to the ground, which can cause accidents. This characteristic intensifies with time until the pupal stage. At the same time, a longitudinal white band appears on the sides of the larva (Figure 1E).

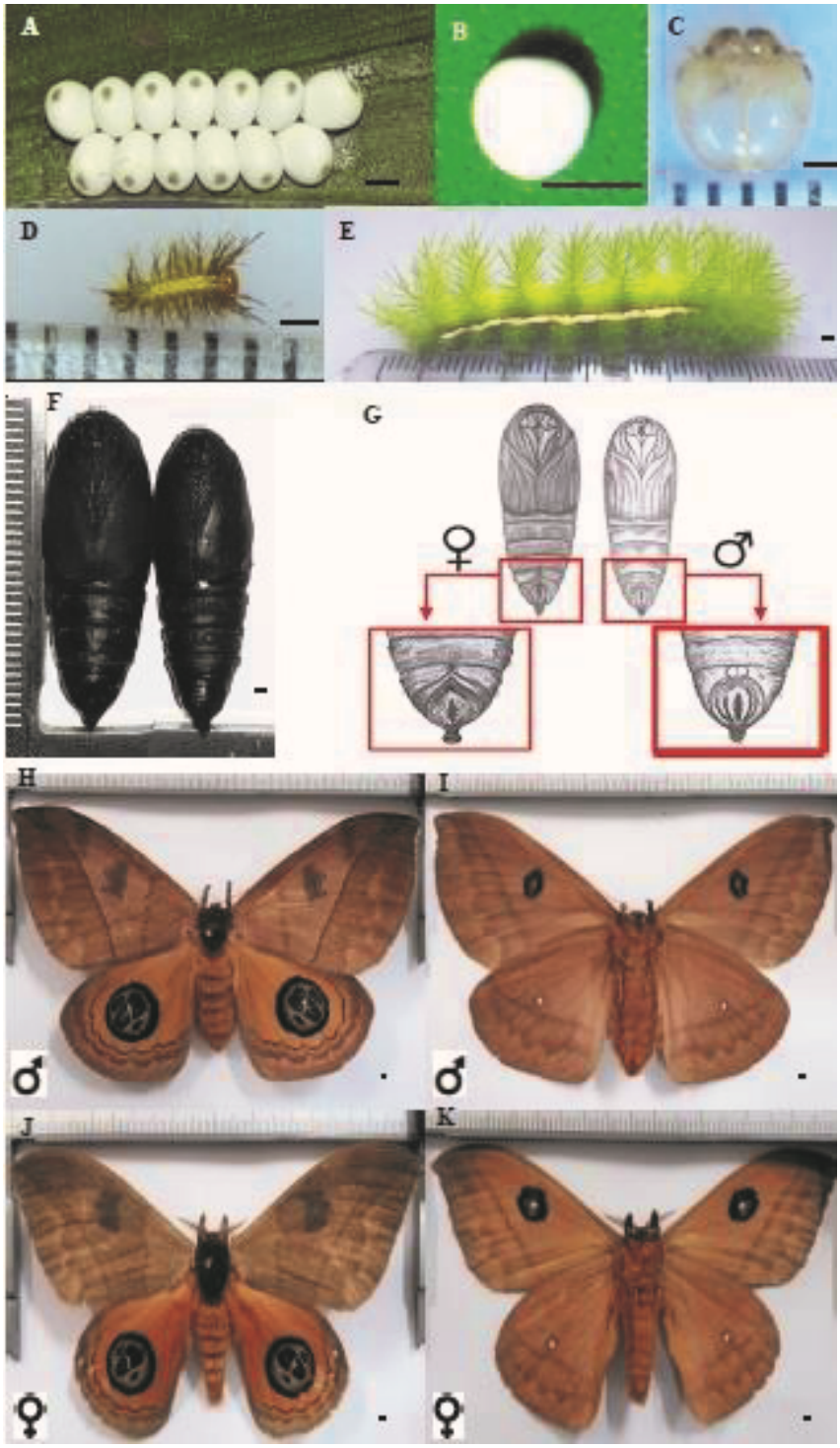

Figure 1. Life cycle of *Automeris liberia.*
Gregarious spawning (A); Individual egg (B); Cephalic capsule (C); First-stage caterpillar (D); Seventh-stage caterpillar (E); Pupae, with female on the left and male on the right (F); Schematic drawing showing sexual dimorphism among pupae (G); adult individuals. Male, dorsal view (H); male, ventral view (I); female, dorsal view (J); female, ventral view (K). Scale bar = 1 mm.

The sixth instar was distinguished by the appearance of a red longitudinal band at the upper edge of the already present white band. Finally, in the seventh instar, another red band appeared on the lower edge of the lateral white bands (Figure 1E).

At the end of the larval stage, cessation of feeding was observed, which is characteristic of the pre-pupal stage. At this stage, the individual begins to weave the cocoon with amber colored silk threads and joins the leaves for protection from natural enemies and to provide shelter.

The cephalic capsules were light green throughout the larval cycle. They had bristles with urticating characteristics, branching at the tips (Table 2). They were collected to confirm the exchange of ecdysis and growth of the larvae (Figure 2C). The mean growth ratio was 1.49 times at each instar. This was corroborated by Dyar (1890) and was similar to those found by Gonçalves, Barbosa and Palucha (2020) for *Dirphia moderata,* a lepidopteran of the same family, and for *A. illustris* (Specht et al, 2006).

Table 2
Body length (mm), thoracic diameter (mm), mass (g) cephalic capsule width and growth ratio of larval stages of *Automeris liberia* (Lepidoptera: Saturniidae), from rearing on a natural diet of *Tenera* variety oil palm leaves, in the laboratory. Eastern Amazonia, Pará, Brazil

	Caterpillar			**Cephalic** capsule	
	Body length (mm)	Thoracic Diameter (mm)	Mass (g)	Width (mm)	Growth ratio
1st instar	4,72 ± 0,38 A	0,71 ± 0,02 A	-	0,63 ±0.05 A	-
2nd instar	5,64 ± 0,45 A	1,36 ± 0,04 AB	0,01 ± 0,00	0.92 ± 0.08 AB	1,46
3rd instar	7,06 ± 0,52 A	2,02 ± 0,05 B	0,02 ± 0,00	1,66 ± 0.01 B	1,80
4th instar	13,61 ± 1,41 B	2,83 ± 0,20 C	0,05 ± 0,00	2,35 ± 0.01 C	1,41
5th instar	21,56 ±1,69 C	5,32 ±0,26 D	0,20 ±0,03 A	3,44 ± 0.01 D	1,46
6th instar	32,36 ±4,0 D	7,18 ±0,24 E	0,55 ±0,06 B	4,97 ± 0.08 E	1,44
7th instar	50,21 ± 1,36 E	9,05 ±0,59 F	1,97 ±0,16 C	6,91 ± 0,02 F	1,39
Mean	19,30	4,06	0,46	2,98	1,49

Different letters in the columns indicate statistical differences between means (Newman-Keuls test, p<0.05).

No cannibalism was observed when several caterpillars were placed in the same container. This behavior is corroborated by the observations of Lemaire (2002).

No significant differences were found in body length for the first three larval instars, although there was growth above 1 mm in each instar. However, there was an increase in length of almost 300% from the fourth to the seventh instar. The diameter of the thorax, mass, and length of the cephalic capsule, in the same period, showed differences (Table 2); they increased at each change of ecdysis, confirming the growth of the insect. Specht et al. (2007) reported similar results for *A.*

naranja, with differences observed in the last four instars.

The larval phase lasted 36 days. This is important information for pest management in oil palm culture in the Amazon because the at the larval phase, the pest is the most active. It indicates the duration of the defoliation period that can occur in severe attacks in the Brazilian Eastern Amazon.

Pupas

The pre-pupal period was 3 ± 0.10 days long and the pupae formed cocoons for protection, as described by Lemaire (2002) for genus *Automeris.* The pupae were obtect or banded with small amber bristles all over the body. They showed sexual dimorphism, with large females, measuring 2.75 ± 0.04 cm in length, 1.07 ± 0.05 cm in thoracic diameter, and 2.21 ± 0.12 g in mass and small males, measuring 2.51 ± 0.03 cm in length, 1.02 ± 0.01 cm in thoracic diameter, and mass of 1.51 ± 0.06 g. The pupal stage duration of *A. liberia* showed no difference between the sexes, with 21 ± 0.2 and 21 ± 0.24 days ($p<0.05$) for males and females, respectively. However, it was lower than the duration observed for A. granulosa (Furtado & Lemaire, 1999), which was 26 days, and higher than that reported by Specht et al. (2006) for *A. illustris,* which was 19 days.

The pupa presented simple cremaster without any modification and terminal bristle tuft; a characteristic also found in *A. granulosa* (Furtado & Lemaire, 1999).

The occurrence of diapause was observed in some subjects, as they took longer to emerge, approximately 20 days after the others. This delay may be attributed to a temperature imbalance (17 ºC) that occurred in the laboratory. This situation is common in other species of the genus (Lemaire, 2002) and is also the most common way for these lepidopterans to spend the winter, especially in the northern hemisphere. This observation is important because it shows that the temperature in the field may affect the number of generations per year. Temperature is inversely proportional to the presence of adults, i.e., in geographical areas where low temperatures occur, the insects may go into pupal diapause and reappear as soon as the temperature rises.

Sexing in pupae showed sexual differences in the last abdominal segments. The eighth segment of the female pupa is divided by the ninth segment, causing the copulatory pouch to open in this segment. In the male, the eighth segment has no division, but the ninth segment has two round pads on each side of the ventral line (Figure 1G).

Adults

In this phase, *A. liberia* showed marked sexual dimorphism in size, morphometry, and antenna types. Females were larger in size, which usually occurs in lepidopterans (Albertoni, Mielke, & Duarte, 2018), while males were smaller and more intensely colored.

The antennae were differentiated, being filiform in females and bipectinate in males. Longevity did not differ between males and females, with 4.5 and 6 days, respectively. However, it was lower than that observed by Specht et al. (2006) for *A. illustris,* which was 7.83 days. These differences are attributed to climatic, geographic, genetic, and dietary variations of the populations studied.

The present study found that the occurrence of emergence was preferentially in the period between 16 and 22 hours. D'Almeida (1944) observed similar results in *A. aurantica,* indicating that it is a characteristic of the genus. It was not possible to identify the mating period among the individuals, due to the agitation of the couples, but copulation probably occurred in the twilight period when their movements were more intense.

The body of adults showed many bristles, with the dorsal thorax dorsally having dark bristles and the rest of the body having beige colored bristles.

The wings were dark beige, with a transverse band on the forewing from the anal margin to the apex and a zigzag band near the frenulum. Both females and males had an irregular dark macula between the two bands (Figures 1H, J). The hindwings were orange in color, differentiated by the brown undulating stripes on their edges, separated from the beige coloration on the anal edge of the wing. The hindwings had an ocelli imitating a large dark eye on each wing, with lighter shades of color in the center.

In both males and females, the ventral side of the wings was also beige. In the center of the anterior wing, there was a dark circular macula in males and one with irregular edges in females, whose center had a white dot and two transverse bands, one zigzag and the other straight, in the anal direction to the apex of the wing (Figures 1I, K). These characteristics are corroborated by Cock's (2005) description of the species.

On the ventral hindwing, there were two stripes with the same characteristics as the forewings, however it presented only a white point in the center of the wing.

The males' abdomen had an intense orange coloration on the back and belly. It had a smaller width than the females' abdomen and the last segment was truncated. The head and thorax were dark brown on the dorsal side, while the ventral part followed the same coloration as the abdomen.

The females had a larger abdomen and a less intense orange dorsal coloration, while their ventral side was beige. Their thorax and head were light brown on the dorsal side. The last segment of the abdomen had a pointed end due to the presence of the aculeus for oviposition.

Morphometric parameters and mass differed between the sexes, with females having the largest measurements compared to males (Figure 2). Despite the difference in antenna types between males and females, the number of antennomeres did not differ, and they had 30 articles. The sex ratio was 0.55, similar to that of *A. ilustris* (Specht et al. 2006). In biology studies, this information is important because it defines the proportion of males in a population and is considered an evolutionarily stable strategy (Born & Lima, 2005).

The percentage of adult emergence was 82.03 ± 0.06 %, lower than that observed by Specht et al. (2007) for *A. naranja.*

The inactive period of *A. liberia* (egg, pre-pupa, and pupa) lasted 38 days, and the active period (larva and adult) constituted more than 50% of the biological cycle, with 40.5 and 42 days, for males and females, respectively. It is noteworthy that the active period, larval phase (45% of the cycle), is a long period, when the insect can cause greater damage to the oil palm crop.

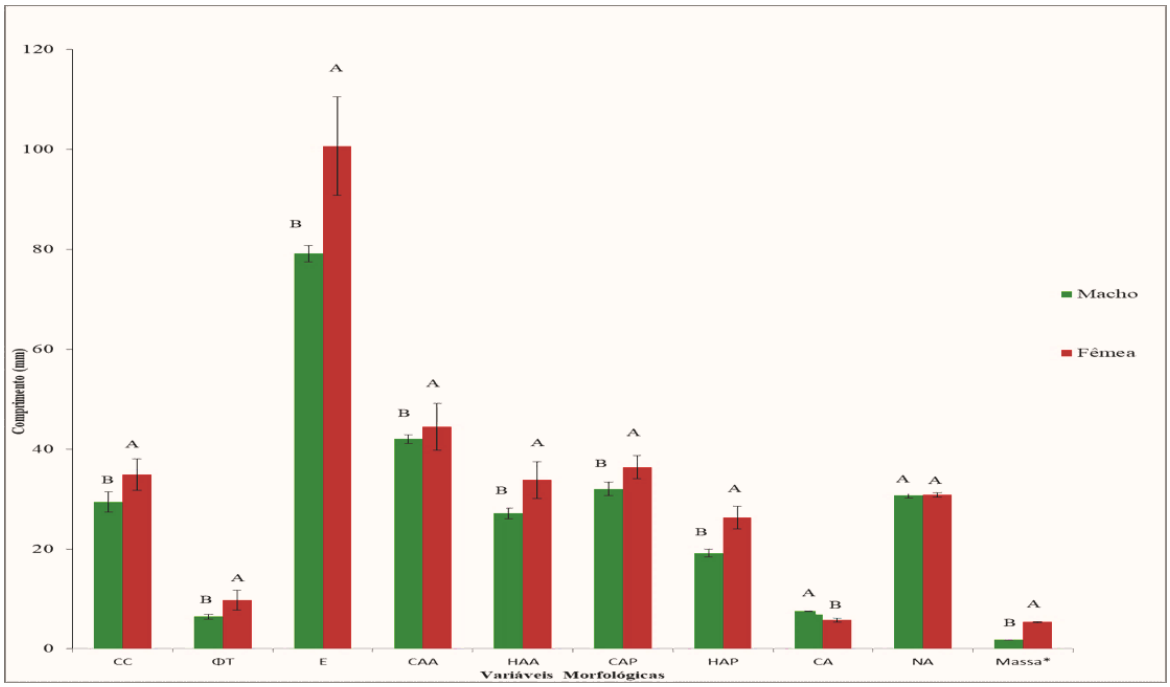

Figure 2. Morphometric parameters of adults of *Automeris liberia.* Body length (BL), thoracic diameter (TØ), wingspan (W), forewing length (FL), forewing height (FH), hindwing length (HL), hindwing height (HH), antenna length (AL), number of antennomeres (NA) and body mass (g). Different letters indicate statistical difference between means (Newman-Keuls test, $p<0.05$), bars represent the standard deviation between means, n=10. Real mass values were multiplied by 5 to facilitate visualization on the graphic scale.
Embedded text:
Comprimento (mm) – Length (mm)
Variáveis Morfológicas - Morphological Variables
CC – BL / ØT – TØ / E – W / CAA – FL / HAA – FH / CAP – HL / HAP – HH / CA – AL / NA – NA Massa – Mass
Macho – Male / Fêmea – Female.

Conclusion

The biological cycle of *Automeris liberia* Cramer is 78.5 and 80 days for males and females. Sexual dimorphism occurs in the pupal and adult stages. The species showed greatest activity during the seven larval instar stages (45% of the total cycle), lasting for 36 days, which is enough time to cause great damage to oil palm cultivation if the attack is severe. It is possible to sexually distinguish males and females in the pupal stage and to raise them in the laboratory on a natural diet.

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