

Biology-Zoology

Biological cycle of *Philaethria pygmalion* (Fruhstorfer, 1912) (Lepidoptera, Nymphalidae, Heliconiinae)

Ciclo biológico de *Philaethria pygmalion* (Fruhstorfer, 1912)
(Lepidoptera, Nymphalidae, Heliconiinae)

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ABSTRACT

Butterflies are important components of biodiversity, both in terms of the number of species and the functions they perform, in relationships that are almost always specific to the host plants. Rearing these organisms in butterfly houses, in addition to protecting the species, allows visitors to develop environmental awareness by getting to know them and learning more about their habitat, habits, importance, etc. *Philaethria pygmalion* (Fruhstorfer, 1912) is a species of butterfly distributed throughout Brazil, with an intense standard greenish-yellow color on the dorsal part of the wings, in contrast to the brown ventral side. Females lay their eggs on plants of the genus *Passiflora* L., where their caterpillars hatch, feed, and grow. The objective of this study was to monitor the larval stages and life cycle of *Philaethria pygmalion* fed on leaves of *Passiflora hexagonocarpa* Barb. Rodr. under laboratory conditions. For this purpose, a fertile female was collected using a puçá (insect net) in the urban area of the city of Santarém (PA), Brazil. After collection, the eggs were taken to the insectary for oviposition. The eggs were monitored, and the hatching rate was 64.2% (68 eggs). An embryonic period of 5.79 days (± 0.41) was recorded. The caterpillars went through five larval stages in a period of 19.01 days (± 1.17). The pupal period lasted 10.65 days (± 0.66). The survival rate of the adult phase was 45.6% (31 adults) and 54.4% (37 fixed), in addition to the immature stages being described and compared to other species of the genus. This study supports the rearing of this species in a butterfly house, making it an attraction for environmental education.

Keywords: Insects; Butterfly house; Amazon

RESUMO

Borboletas são importantes componentes da biodiversidade seja em número de espécies ou pela função que exercem, em relações quase sempre específicas em relação às plantas hospedeiras.

A criação desses organismos em borboletários além de proteger a espécie permite trabalhar no visitante uma sensibilização ambiental ao conhecê-las e saber um pouco mais sobre seu habitat, seus hábitos, sua importância etc. *Philaethria pygmalion* (Fruhstorfer, 1912) é uma espécie de borboleta com distribuição por todo o Brasil, com intensa coloração padrão verde-amarelado na parte dorsal das asas, em oposição ventral marrom. As fêmeas ovipositam em plantas do gênero *Passiflora* L., nas quais suas lagartas eclodem, se alimentam e crescem. Assim, o objetivo deste estudo foi acompanhar os estádios larvais e o ciclo de vida de *Philaethria pygmalion* alimentadas com folhas de *Passiflora hexagonocarpa* Barb. Rodr. em condições laboratoriais. Para isso, uma fêmea fértil foi coletada com puçá na área urbana da cidade de Santarém (PA), Brasil. Após a coleta foi levada ao insetário para a oviposição. Os ovos foram acompanhados e a taxa de eclosão foi de 64,2% (68 ovos). Foi registrado um período embrionário de 5,79 dias ($\pm 0,41$). As lagartas passaram por cinco estádios larvais num período de 19,01 dias ($\pm 1,17$). O período pupal foi de 10,65 dias ($\pm 0,66$). A taxa de sobrevivência da fase adulta foi de 45,6% (31 adultos) e 54,4% (37 fixados), além de os estágios imaturos serem descritos e comparados a outras espécies do gênero. Este estudo subsidia a criação desta espécie em borboletário, sendo um atrativo de educação ambiental.

Palavras-chave: Insetos; Borboletário; Amazônia

1 INTRODUCTION

Butterflies are known for their beauty. During their clumsy flights or while landing on flowers, they often reveal fascinating colors and patterns (Morrel, 1960; Peggie & Amir, 2006). More than just beautiful, these organisms are ecologically important as pollinators and are considered bioindicators of habitat changes and environmental impacts, typically caused by the loss of host plants or rising temperatures (Dessuy & Morais, 2007; Norradihah et al., 2018). Therefore, butterflies can also serve as umbrella species for conservation purposes (New, 1997).

Among the strategies for species conservation are butterfly aviaries (Hughes & Bennett, 1991; Morton, 1991; Pearce-Kelly et al., 1998). These facilities allow for the recreation of habitats suitable for butterflies and their caterpillars, enabling visitors to engage directly with the insects while learning about their importance, life cycle, and the resources essential for their survival (Silva et al., 2013). Such experiences can raise public awareness and promote environmental education. However, to ensure the effectiveness of butterfly aviaries, it is essential to have a comprehensive understanding of the biological and ecological aspects of the species involved.

The genus *Philaethria* Bilberg, 1820 (Lepidoptera: Nymphalidae: Heliconiinae) currently comprises ten species (Constantino & Salazar, 2010). These species are recognized by their intense yellowish-green coloration on the upper surface of the wings, with forewings that are more elongated and hindwings that resemble the typical shape found in Heliconiinae. Oviposition and caterpillars feeding occur on host plants of the genus *Passiflora* L. (Benson et al., 1975).

The species *P. pygmalion* (Fruhstorfer, 1912) was originally described as *Metamandana dido pygmalion*, based on specimens from Óbidos (Pará), in northern Brazil. However, this species is now known to have a wide geographic distribution across Brazil (Barão et al., 2014). Adults are typically observed flying in open areas and do not frequent wet environments (Constantino & Salazar, 2010).

Philaethria pygmalion oviposits *Passiflora coccinea* Aubl., *P. feroana* Arms, *P. hexagonocarpa* Barb. Rodr., *P. mansoi* (Mart.) (Passifloraceae) in Brazil (Benson et al., 1975). Eggs are laid singly on the abaxial and adaxial surfaces of mature leaves, as well as on dry or green tendrils (Dell'Erba et al., 2005). The caterpillars are solitary and adopt a characteristic hook-shaped posture while feeding or at rest (Constantino & Salazar, 2010). In contrast to the spiny caterpillars, the pupae lack spines and resemble bird droppings (DeVries, 1987).

With a view toward the potential inclusion of this species in a butterfly house within an environmental education school, the objective of this study was to monitor the larval stages and life cycle of *Philaethria pygmalion* fed with *Passiflora hexagonocarpa* leaves under laboratory conditions.

2 MATERIAL AND METHODS

2.1 Collection of the Female Specimen

A single fertile female of *P. pygmalion* was collected using an entomological net, in the community of Cucurunã (02°28'03" S, 54°46'38" W), located in the city of

Santarém, Pará, Brazil. After collection, the butterfly was placed in a plastic container and subsequently released into an insectary measuring 1.80 m in width and 1.80 m in height (Figure 1A). A second generation of this butterfly was also monitored within the butterfly house. Upon completion of the life cycle, the butterflies were released into their natural habitat.

Figure 1A – D. Creation of *Philaethria pygmalion*. A– Insectary measuring 1.80 m in width and 1.80 m in height, where the fertile female was placed for oviposition on *Passiflora hexagonocarpa*. B– Packaging of eggs in Petri dishes for caterpillar hatching under laboratory conditions. C– Fungal proliferation on eggs under laboratory conditions, at 1.6× magnification. D– Newly hatched caterpillar feeding on the eggshell on the screen, at 4.0× magnification. Scale bar = 4 mm



Source: Authors' private collection (October 2023)

2.2 Host plant

Branches of *P. hexagonocarpa* (Figure 2) were collected from the same location as the butterfly to serve as host plants for oviposition and caterpillar feeding. The turgidity of the leaves was maintained by placing the branches in plastic containers with water, partially submerging the stems.

Figure 2 – Branch with fruit of *Passiflora hexagonocarpa*



Source: Authors' private collection (October 2023)

2.3 Cycle in the laboratory

After oviposition, the host plant branches were transported to the laboratory, where each was trimmed to isolate the section containing the egg. These sections were then placed in Petri dishes lined with moistened paper towels (Figure 1B). Each dish was properly labeled with an identification number corresponding to its origin, facilitating the tracking of each collected egg. Once the caterpillars hatched, the Petri dishes were observed daily for food replacement (*P. hexagonocarpa* leaves), cleaning (removal of frass and food residues), and monitoring of larval growth and ecdysis.

From the third instar onwards, due to the increase in body size, the caterpillars were transferred to 500 mL jars with perforated lids to allow air circulation. During the creation process, the laboratory temperature was maintained at 25°C. At each instar change, the cephalic capsules were collected, measured, and stored in 2.0 mL microtubes containing Dietrich's solution for 48 hours. After this period, the capsules were preserved in 70% ethanol. The material examined is stored in LETIA, UFOPA (Laboratório de Ecologia e Taxonomia de Invertebrados Aquáticos, da Universidade Federal do Oeste do Pará, Campus de Santarém).

2.4 Study of life cycle phases and photographic documentation

Morphological observations of live caterpillars (e.g., color pattern and length) were conducted under a stereomicroscope (LEICA S8 APO). Photographs were taken using a LEICA CM120 HD camera attached to the stereomicroscope and a Nikon Coolpix L820 camera.

2.5 Identification and terminology

The identification of the adult *Philaethria pygmalion* was based on external morphology, following the description and key of Constantino & Salazar (2010). For the terminology of the morphology of immature stages, Barão & Moreira (2010) were used. And, the identification of the host plant (*Passiflora hexagonocarpa*) was based on the morphology of the leaves, flowers, and fruits, following the key of Escobar (1994).

3 RESULTS

3.1 Oviposition

A total of 106 eggs were obtained from the fertile female, with oviposition occurring on sunny days between 7 a.m. and 2 p.m. The eggs were laid individually

on various parts of the *P. hexagonocarpa* plant, including dry and green tendrils, both adaxial and abaxial leaf surfaces. In some cases, multiple eggs were observed on the same leaf or tendril. Additional eggs were also deposited on the top and sides of the insectary screen.

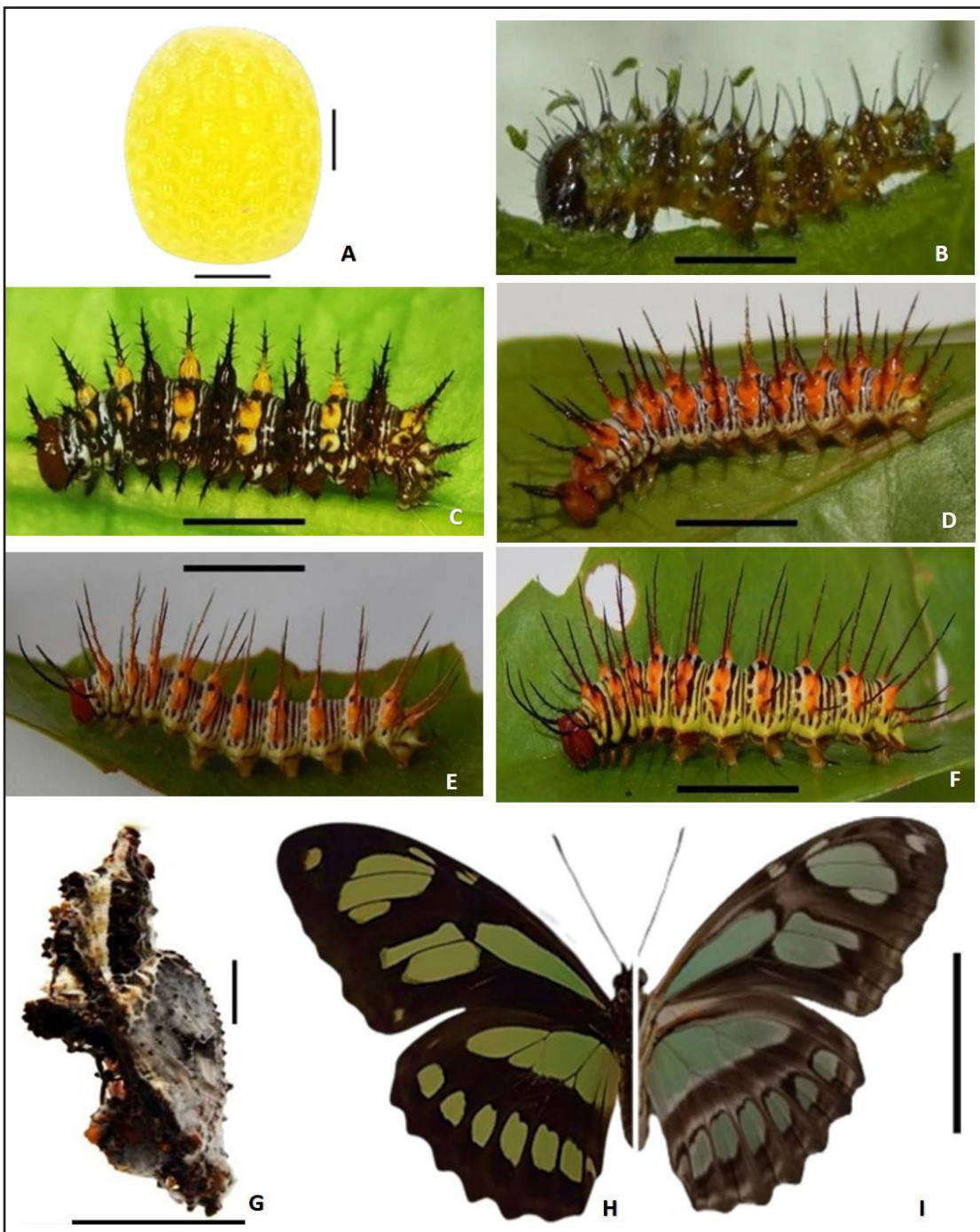
The hatching success rate was 64.2% (n = 68). The failure to hatch (n = 28) was likely due to fungal proliferation (Figure 1C), although ten of these eggs were preserved for documentation. Among the hatched eggs, the survival rate to the adult stage was 45.6% (31 adults), 17 females and 14 males, and 54.4% (37 attached caterpillars).

Soon after hatching, the caterpillars consumed their eggshells before beginning to feed on the leaf tissue (Figure 1D).

3.2 Embryonic stage

The egg is initially yellow in color and gradually becomes white and transparent during embryonic development, just before hatching. It has a flat base and an oval shape, which is slightly flattened at the tip (Figure 3A). The average egg height was 1.233 mm (± 0.091 mm), with an average diameter of 1.008 mm (± 0.006 mm), with 20 vertical and 8 horizontal ridges. The average duration of the embryonic period was 5.79 days (n = 68); the standard deviation was ± 0.41 ; the number of days (minimum – maximum) ranged from 5 to 6 days. The eggs were deposited individually on tendrils, stipules, old leaves, and on the insectarium screen.

Figure 3A – I. Life cycle of *Philaethria pygmalion*. A – Egg, at 4.0× magnification. B – First instar caterpillar, side view, at 1.60× magnification. C – Second instar caterpillar, side view, at 1.25× magnification. D – Third instar caterpillar, side view. E – Fourth instar caterpillar, side view. F – Fifth instar, side view. G – Pupa, side view. H, I. – Adult. (H) Dorsal view. (I) Ventral view. Scale bar = A – 1.233 mm × 1.008 mm; B – 5 mm; C – 8 mm; D – 14 mm; E – 14 mm; F – 32 mm; G – 30 mm. H, I. – 52 mm



Source: Authors' private collection (October 2023)

3.3 The larval stage

The larval stage undergoes significant morphological changes between instars. The larval period lasted an average of 19.01 days, with five molts ($n = 37$); standard deviation was ± 1.17 ; number of days minimum – maximum was 17 – 21 days. The total duration of the pre-imaginal cycle was, on average, 35.45 days ($n = 31$); standard deviation was ± 1.18 ; number of days minimum – maximum was 34 – 37 days.

First Instar. The first instar caterpillar has a dark brown head capsule, yellow tegument with caramel-colored transverse stripes, and white spots on the thorax and abdominal segments upon hatching. The average head capsule width was 0.702 mm ($n = 10$); standard deviation was $\pm 0,024$. After feeding, greenish intestinal content becomes evident along the body, and black bristles with dilated apices in the shape of transparent drops appear. The thoracic legs are black (Figure 3B). The average body width was 5.09 mm ($n = 10$); standard deviation was ± 0.52 . The average duration of this instar was 3.64 days ($n = 56$); standard deviation was ± 0.80 ; number of days minimum – maximum was 3 – 5 days.

Second instar. The second instar caterpillar has a brown head capsule, with an average width of 1,108 mm ($n = 10$), standard deviation of ± 0.010 ; a pair of short scoli, brown at the base and black in the remainder, with the reduced apex recurved backward in lateral view and diminished recurved inward in frontal view (this characteristic is maintained until the fifth instar), with an average length of 1,828 mm ($n = 10$), standard deviation was ± 0.212 . The thoracic and abdominal segments are brown, and the rings are white and black. The scoli emerging from the orange base are yellow with black apices, while the scoli from the brown base are black. At the orange base, the dorsal and suprspiracular scoli are yellow with black apices, while the scoli at the brown base are black. The spiracles are translucent black (Figure 3C). The average body length was 7.76 mm ($n = 10$); the standard deviation was ± 0.69 . The average duration of the

second instar was 3.58 days (n = 52); the standard deviation was ± 0.70 ; the number of days (minimum – maximum) was 3 to 5 days.

Third instar. The caterpillar in the third instar has a red cephalic capsule, with an average width of 1,317 mm (n = 10), with a standard deviation of ± 0.005 ; a pair of long scoli with a red base and the rest black (this characteristic is maintained until the fifth instar), with an average length of 6,469 mm (n = 10), the standard deviation was ± 0.666 . The thoracic and abdominal segments have irregular white and black rings, with yellowish-green sides. Orange-red scoli with black apices emerge from the base. The spiracles are black with a raised edge (Figure 3D). The average body length was 14.3 mm (n = 10); the standard deviation was ± 0.67 . The average duration of the third instar was 2.87 days (n = 46). The reference standard was a standard deviation of ± 0.86 ; the number of days (minimum and maximum) was 2 to 5 days.

Fourth instar. The fourth instar caterpillar has a bright red head capsule, with an average width of 2.135 mm (n = 10), with a standard deviation of ± 0.013 ; average length of 13.396 mm (n = 10), standard deviation was ± 0.991 . The thoracic and abdominal segments have irregular white and black rings, while the lateral segments are yellowish-green. The base of each scoli is orange, and the dorsal scoli are large, red, with black apices (Figure 3E). The average body length was 24.3 mm (n = 10); the standard deviation was ± 0.95 . The average duration of the fourth instar was 4.41 days (n = 41); the standard deviation was ± 1.14 ; the minimum and maximum number of days was 2 to 6 days.

Fifth instar. The fifth instar caterpillar has a bright red head capsule, with an average width of 4.215 mm (n = 10), with a standard deviation of ± 0.069 ; a pair of long scoli with an average length of 21.912 mm (n = 10), the standard deviation was ± 0.899 . The thoracic and abdominal segments have irregular white and black rings, while the dorsal and lateral segments are yellowish-green. The anal plate is yellowish. The dorsal, supralateral, and anal scoli are dark red with black apices, and the subspiracular scoli are yellow with black apices (Figure 3F). The average body width was 32.1 mm (n = 10);

the standard deviation was ± 1.60 . The average duration of the fifth instar was 4.51 days ($n = 37$); The standard deviation was ± 0.90 ; the minimum and maximum number of days ranged from 3 to 7 days.

3.4 The pupal stage

Pupal Stage. The pupa presents a mixture of grayish, brown, beige, reddish-orange, white, black, and light yellow coloration. : It is covered in warts and, on the prothorax and metathorax, has three pairs of silvery keels, resembling bird droppings (Figure 3G). The average pupal length was 29.8 mm ($n = 10$) and the average duration of the pupal period was 10.65 days ($n = 31$); the standard deviation was ± 0.66 ; the number of days ranged from 10 to 12.

3.5 Adult

Adult (Figure 3H,I). The sex ratio was 17 females to 14 males, or 0.82:1.

4 DISCUSSION

The development of the biological cycle of *P. pygmalion* in laboratory conditions proved to be viable. Of the 106 eggs laid by a single female, 64.2% ($n = 68$) successfully hatched, indicating high offspring viability. The survival rate to the adult stage was 45.6% ($n = 31$), while 54.4% ($n = 37$, eggs and larvae) were preserved. A second rearing was carried out exclusively to preserve the stages that had not yet completed $n = 10$.

These results suggest that maintaining a *P. pygmalion* in an insectarium is feasible. In this study, multiple eggs were deposited on a single leaf (both abaxial and adaxial surfaces), as well as on tendrils and the insectary screen. However, the species typically oviposits in isolation on different parts of the host plant (Dell'Erba et al., 2005; Constantino & Salazar, 2010). This behavior may be attributed to the limited availability of host plants or to the effects of a confined environment, potentially leading to

competition among caterpillars for food resources. Therefore, regular monitoring and removal of eggs and caterpillars (placing them individually in Petri dishes) is essential to ensure their survival and reduce intra-species competition. The egg's characteristics, such as color, size, and number of ridges, are similar to the results of Brown (1981) and Constantino & Salazar (2010); both studies included a description of the egg.

Of the 106 eggs, 28 did not hatch, likely due to fungal proliferation, a phenomenon also reported by Harrison et al. (2016) for *Lycaeides melissa samuelis* Nabokov, 1943 (Lycaenidae) collected in the wild. These authors observed a higher diversity of fungi in the eggs compared to other stages of the life cycle.

Upon hatching, the caterpillar initially feeds on the eggshell, which is rich in protein and can represent up to 50% of the caterpillar's body weight at this developmental stage (Barros-Bellanda & Zucoloto, 2001). Subsequently, it begins feeding on leaves, with a preference for young leaves during the initial feeding period (Rodrigues & Moreira, 1999).

The larval development time of *P. pygmalion* is similar to that reported for *P. dido* (Linnaeus, 1763) and *P. wernickei* (Röber, 1906), as observed by Young (1974) and Barão & Moreira (2010).

Species of the genus *Philaethria* are quite similar in coloration and wing pattern, as well as in the morphology of their immature stages (Constantino & Salazar, 2010). The morphology of the caterpillars, particularly in the first and second instars, such as coloration, thorax and abdomen structure, spiracles, and the arrangement of bristles and scoli, resembles that described by Barão & Moreira (2010) for *P. wernickei*. However, in *P. pygmalion*, the thoracic legs are black, differing from the brown legs observed in *P. wernickei* (Barão & Moreira, 2010). The egg of *P. pygmalion* also differs from that of *P. dido*; according to Beebe et al. (1960), the latter presents brown spots at the end of embryonic development.

The morphology of the third, fourth, and fifth instars of *P. pygmalion* exhibits coloration patterns similar to those described by Barão & Moreira (2010) for *P.*

wernickei. However, in *P. pygmalion*, each scoli has an orange base and large dorsal scoli with a red shaft and black apex, a combination also observed in *P. dido* by Beebe et al. (1960). The head capsule is bright red and the anal plate is yellowish, differing from *P. wernickei*, which has a yellowish-brown head capsule and grayish anal plate (Barão & Moreira, 2010). Brown (1981) noted that the fifth instar caterpillar had red scoli and a red base, but in the present study, individuals showed an orange base with red scoli and black apices, matching observations for *P. dido* (Beebe et al., 1960). The subspiracular scoli in *P. pygmalion* are yellow with black apices, consistent with patterns reported for both *P. dido* and *P. wernickei* (Beebe et al., 1960; Barão & Moreira, 2010). Brown (1981) and Constantino & Salazar (2010) describe the fifth instar larva of *P. pygmalion pygmalion*; however, in the first study, this larva is illustrated in black and white, while in the second, the larva of *P. pygmalion metaensis* Constantino & Salazar (2010) is illustrated in color for the first time. The larvae illustrated and described in these two studies are similar to our results.

Therefore, if the field collection is for obtaining the stages, it is recommended to collect caterpillars in the fourth and, especially, the fifth larval instar for rearing purposes, as they are more robust, adapt more easily to new environments, and are closer to pupation. However, for rearing, the most important thing is that the insectarium has at least the dimensions reported in this study.

The pupa has a grayish coloration composed of various colors, and bears three pairs of keels with silvery spots on the prothorax and metathorax. Similar structures were recently described by Barão & Moreira (2010) for the pupa of *P. wernickei*, although in that species the keels present golden rather than silvery spots. Our results are similar to the descriptions by Brown (1981) and Constantino & Salazar (2010).

We also observed that *Philaethria pygmalion* presents a contrast between the conspicuous larvae and the cryptic pupa; while the larvae are quite colorful, the pupa resembles bird droppings. Thus, the larvae, with their bright and visible color patterns that do not go unnoticed by potential predators, signal that they are acquired, harmful,

or dangerous to them (Endler, 1991). Conversely, the pupa, by having the appearance of bird droppings, employs a camouflage strategy, avoiding predation, as they are mistaken for inedible objects by their predators (Skelhorn et al., 2010; Lindstedt et al., 2019).

The sex ratio observed for *P. pygmalion* was 17 females to 14 males, or approximately 0.82:1. In most panmictic populations, crosses tend to be egalitarian between both sexes, with a sex ratio approaching 1:1 (Fisher, 1930).

The total pre-imaginal development time from egg to adult for *P. pygmalion* ranged from 34 to 37 days, which is similar to the 37 to 39 days observed for *P. dido* in Costa Rica (Young, 1974).

5 CONCLUSION

With the oviposition of the female kept in captivity, it was demonstrated that the rearing of *P. pygmalion* is viable in the laboratory. This work will subsidize the future butterfly house that will be implemented in Santarém, thus it will be possible to maintain the butterfly *P. pygmalion* as one of the species to be reared and exhibited, as well as contribute to environmental education, integrating the teaching, research, and extension tripod.

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