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Bionomic Aspects of *Cerodirphia opis* (Schaus, 1892) (Lepidoptera: Saturniidae, Hemileucinae) and Identification of Its Natural Enemies †

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Abstract: The forestry sector occupies a prominent place among the economic segments in Brazil, with the cultivation of *Eucalyptus* sp. Among the numerous insects that can damage eucalyptus crops, the Saturniidae family has been noted as a secondary and main pest. The objective is to describe some bionomic aspects of *Cerodirphia opis* (Schaus, 1892) and to identify its natural enemies in the laboratory. Larvae were collected in stands of *Eucalyptus* sp. and raised in nylon mesh cages. Biological parameters were obtained under temperature conditions: 25 ± 1ºC, RH 80 ± 10% and 14-hour photophase, with daily observations. Only 11% of viability was observed in the larval stage. 32 males and 7 females were obtained, which represents a sex ratio of 0.18. The length of male pupae ranged from 20.57 mm to 26.46 mm, width ranged from 8.28 to 11.07 mm and weight from 0.31 to 1.930 g. The females were 10.42 to 12.85 mm, 8.28 to 11.07 mm and 1.91 to 1.93 g respectively. The parasitoid of larvae *Megaselia scalaris* (Loew) (Diptera: Phoridae) was found as a natural enemy, being the first recorded in Saturniidae.

Keywords: Biology; Eucalyptus defoliator and parasitoids

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1. Introduction

Originally from Australia, eucalyptus has become one of the most important resources of the forest economy in Brazil. The selection of species, the use of hybrids and genetic research put the country at the forefront in the implantation of clonal forest stands, implemented the most advanced cultivation and management technology, with expressive results in productivity and profitability [1]. In Brazil, according to Ibá [2] in 2016, 7.84 million ha of planted forests were reported for commercial purposes, which represents less than 1% of the national territory. Bahia is a reference in the Brazilian forest-based sector, with 612.2 thousand hectares planted with eucalyptus, which places it in 4th place in the national ranking. It is the state with the largest planted area, only behind Minas Gerais, São Paulo and Mato Grosso do Sul [3]. The genus Eucalyptus native to Australia (Myrtaceae) was independent in Brazil for the production of raw material for the forestry sector. It has about 600 species, many varieties and hybrids. Immature Lepidoptera of several eucalyptus defoliating species are responsible for great damage [4], and in case of successive advances, they can paralyze plant growth [5]. The Saturniidae family is among the main representatives of moths, dimensioning and variables. Saturniidae is one of the best studied and well-resolved Lepidoptera families from a taxonomic point of view, so it has been used for several studies, such as ecology, biodiversity and biogeography [6]. This family includes moths of medium to very large size, with a robust and densely hairy

body, with different wing patterns, varying in shapes, ornamentation and coloration [7]. In Brazil, this family is represented by five of the nine subfamilies: Arsenurinae, Ceratocampinae, Hemileucinae, Oxyteninae and Saturniinae [8]. Most species 9 have nocturnal and billiard habits, especially among the Saturniinae and Hemileucinae, they are diurnal [7]. Species belonging to the subfamily Hemileucinae have great economic importance due to the damage brought to agriculture and medical importance because they cause clinical complications, sometimes fatal, both in the larval stage and in the adult stage [7]. Currently, the genus Cerodirphia Michener, 1949, comprises about 30 species of medium-sized moths, distributed from western Mexico to Bolivia and southeastern Brazil [9]. A species *Cerodirphia opis* (Schaus, 1892) (Lepidoptera: Saturniidae; Hemeleucinae) has not yet been recorded in Bahia, nor as a defoliator of eucalyptus. The work aims to describe some bionomic aspects of the species *Cerodirphia opis* (Schaus, 1892) and identification of its parasitoids in laboratory.

2. Material and Methods

2.1. Insect Collection Area

The larvae of *Cerodirphia opis* (Lepidoptera, Saturniidae, Hemileucinae) were collected in different instars in the city of Esplanada - BA, located at 140 meters altitude, has the following geographic coordinates: Latitude: 11° 46' 51" South and Longitude: 37° 56' 50" West. According to Köppen's classification, the type the predominant climate in the area is Am, tropical rainy monsoon climate, with month less rainy with precipitation less than 60 mm and the coldest month with average temperature above 18 °C and, to a lesser extent, Aw', with hot climate with coldest month with an average temperature above 18 °C and driest month with precipitation less than 60 mm and rainy season with peaks in autumn. Due to the urticance of the larvae, they were collected from the branches of *Eucalyptus* sp. with the aid of pruning shears and metal tweezers in different plots, belonging to Bracell (Bracell Bahia Florestal Ltda), in the Maçaranduba Project in September of the year 2019 (FIGURE 1A, 1B). The larvae were placed in buckets plastics with a capacity of 100L, containing eucalyptus branches which were covered with anti-aphid screen for ventilation and conducted to the Laboratory of Forest Entomology at the Federal University of Recôncavo da Bahia (UFRB) at Cruz das Almas *campus* – BA about 200 km. For the collection and maintenance of the larvae in captivity, the methodology was followed used by LORINI [10], applying modifications when necessary.

Figure 1. This is a figure. Schemes follow the same formatting.

2.2. Creation in Laboratory

In the Entomology Laboratory (FL04), located in the Forest Engineering Complex (CEF) of the Federal University of Recôncavo da Bahia (UFRB), the breeding was kept in an air-conditioned room at a temperature of 25 ± 2 °C, relative humidity of 70 ± 10% and 12 h photophase. Biological studies were started with 350 larvae. At the time of collection (25/IV/2019), the larvae were in second, third, fourth, fifth instars, due to the gregarious habit they were placed in cages with nylon mesh on the sides for ventilation with a

wooden bottom (100, 0 cm high × 50.0 cm wide × 50.0 cm long) being separated into groups of approximately 50 individuals according to their size. The larvae were fed ad libitum daily with new eucalyptus branches collected at the UFRB campus. To maintain the turgidity of the leaves, the ends of the branches were introduced in 100mL Erlenmeier with distilled water. At the end of the last instar, to facilitate puffing, a 40 cm-diameter basin with autoclaved sand and dry leaves was placed in each cage to reproduce what they found in the natural environment. In order to obtain the natural enemies, the last instar specimens that showed lethargy, turgidity, secretions and differentiated color were selected, signs that indicate a pathological state in insects [11,12]. Prepupae with little movement and malformed pupae were also separated.

3. Results and Discussion

3.1. Larvae Life Cycle

It should be noted here that there are few records on biology for the species including work provided by Lemaire [13], Lampe [14] and Meister [15]. Only one piece of information was found in the form of a poster presented at the Congress. Brazilian of Zoology occurred in Londrina - PR Specht et al [16]. Among the 350 larvae selected for breeding, 39 reached the prepupae stage, obtaining a viability of only 11%. Four pupae died (apparent dehydration, intact but withered structure; there were no parasitoids). Specht et al., [16] studying the biology of *Automeris illustris* (Walker, 1855), a polyphytophagous hemileucine found a viability of 98.09%, ie eight times greater than that found in this work. The larvae of *C. opis* started the pupation on 08/X/2019, therefore 23 days after field collection. Pupation usually occurs within a cocoon of silk stuck to dry leaves found in the substrate in the work by Pereira, et al. [17] who performed the biology of a Saturnidae, the duration (days) and larval viability (%) *of D. moderata* were 53.00 ± 0.09 and 80.00 ± 0.99 and for larvae of this species reared with *E. cloeziana* with six instars. The fully sclerotized cuticle does not expand, therefore, the growth of hard parts only occurs when there is a "mute", when a new one cuticle is produced and expanded. As a result of this, the growth of these sclerotized parts happens in a series of steps. Based on this fact, Dyar defined a rule, which predicts the degree of growth of insects. is the rule by Dyar who says that "the cephalic capsule of larvae grows in geometric progression increasing in width with each ecdysis, at a constant ratio on average, 1.4". The determination of the number of instars, based on this rule, has been widely used. Despite this, it has received a lot of criticism, as it does not apply to certain groups of insects. Among the rules that predict how much insect growth, there is Pyzibram's rule that the weight is doubled during instar, and every ecdisis all dimensions are increased by the ratio of 1.26. Specht et al. [16] found eight instars for the species *Cerodirphia opis* larval when fed on white cinnamon leaves (Nectandra lanceolata Ness et Mart ex Ness-Lauracea). The larval stage lasted 66.83±5.72 days, of which 5.71±1.12 23 corresponded to the prepupae period and width of the cephalic capsule ranged from 1.16 to 4.09 mm. The average growth rate was 1.22 at each instar. In one of the few citations on Cerodirphia biology, Zanuncio et al., [18] determined an average duration of 87.4 days, the instar number was eight and the prepupae lasted for 14.9 ± 0.18 days, pupae lasted for 3 months in condition of laboratory. Featuring two annual generations. The duration of the pupal period was according to the authors long when compared to other species of larvae eucalyptus defoliators.

3.2. Pupae

The pre-emptying and the bloating took place in the soil (sand) between the dry leaves (Figure 2a,b). The average pre-employment period observed was 2.9 days, maximum of 4 and minimum of 2, with viability in this period of 70%. The period observed pupal was an average of 23 days. Garcia [19] working with *Cerodirphia rosacordis* (cited as *Dirphia rosacordis*) in pequi found pupal viability of 69.2% and the sex ratio of 0.48. According to Zanuncio et al. [20] *C. rosacardis* (cited as *Dirphia rosacordis*) presented a period of 74 days for the larval stage, below 87.5 days, created in eucalyptus leaves, according to

these authors the cycle mean total egg to adult for *C. rosacordis* was 129 days (4 months). Too it was observed that in the first four instars there was a mortality of 51.2% of the larvae, while the observed larval viability was 46.3%. In this work the mortality in the total larval stage was 80%.

Figure 2. Behavior of *Cerodirphia opis* (Schaus) with 24 hours (**A**) and with 48 hours (**B**).

Figure 3. Newly formed *Cerodirphia opis* (Schaus) pupae (**A**) with detail of the color range from light brown to black (**B**).

The pupa is obtect, bare and dark brown; it is sclerotinized, matte, and with a darker cephalic and pterotheca region. It has a cremaster with several simple hooks (Figure 3A). Typical general appearance of Saturniidae; cylindrical that tends to the ellipsoid at the ends; from the abdominal segment A7 there is a clear taper, rounded A10 at the end; no ornamentation whatsoever; color varies in prepupae stage from yellow then to light brown going to dark brown almost black with the intersegmental abdominal regions in the lighter joint regions; matte appearance; between the appendices (wings, antennae, jaws and legs) the color may be reddish (Figure 3b). The length of male pupae ranged from 20.57 mm to 26.46 mm; the width ranged from 8.28 to 11.07 mm and the weight from 0.31 to 1.930 g. For females, these measurements were 10.42 to 12.85 mm, 8.28 to 11.07 mm and 1.91 to 1.93 g, respectively. In pupae morphometry, both for body length and width, the pupae females were, on average, statistically higher than males (Table 1).

Usually, weight gain is faster during the first instars and, ultimately, it is at least doubled every instar [21], a fact verified in the results shown in Table 1. The pupae of males and females can be separated by shape, size and, mainly, by the characteristics of the abdominal segments VIII and IX. In males (Figure 4b), the genital orifice has the shape of an ellipsoid slit and is contained in the abdominal segment IX, therefore, it does not

extend into the VIII. In females (Figure 4a), the genital segment is contained between the abdominal segments VIII-IX, giving the impression of two ellipsoid slits, in VIII and IX respectively. As for the anal orifice, this corresponds to a slit totally contained in the segment X of pupae of both sexes. Female pupae were significantly larger than male pupae. The insect's weight gain is an important parameter for assessing rebirth [21], since it is a factor that is directly correlated with the quantity and quality of food eaten. However, it is not a good parameter for determining the number of instars [22]. The moths emerged between 02/XI/ 2019 to 18/XI/2019. Of the 39 pupae so far, only seven moths (six males and one female) have emerged. The emergence of imagos was recorded between 6:00 pm and 7:00 am, therefore, between dusk and dawn. Lorini [23] found that most adults of *L. obliqua* also emerged during scotophase. After leaving the cocoon, the moths, with their wings still short, held onto the cocoon itself or on the wall of the container where the pupae were placed, then landed on the nylon screen and remained there. with the wings suspended for about 1:30 h until complete extension of the wings and stiffening (Figure 4). Eventually they flew after a few times or remained motionless over the cocoon or screen until nightfall the next day. These behaviors were also observed by Neves [24]. Upon emergence, adults of *C. opis* have folded wings and are small in size, taking approximately 60 min to fully extend them and reach normal wingspan (Figures 4 A,B.)

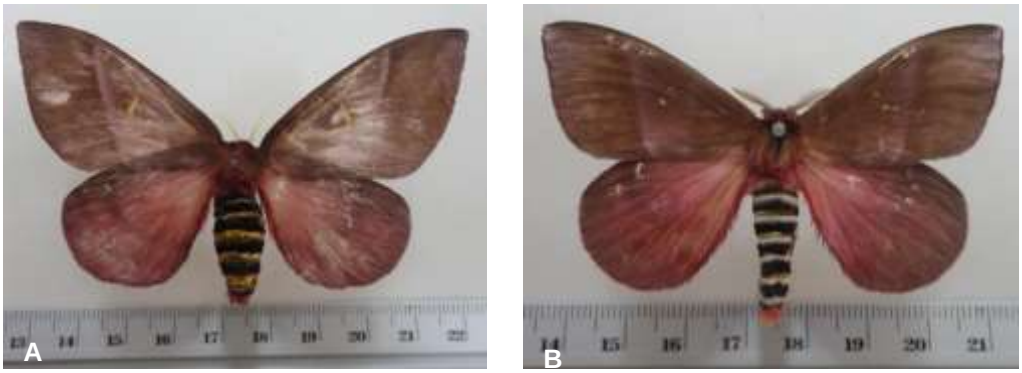

Figure 4. Adults of *Cerodhirphia opis* (Schaus): female (**a**); male (**b**) obtained in the laboratory.

3.3. Sex Reason

32 males and 7 females were obtained, which represents a sex ratio of 0.18 as there was a greater number of males. Zanuncio et al., [18] analyzing the sex ratio of *D. moderata* shows that it was similar, with 0.48 and 0.46 when using exotic and native hosts *E. cloeziana* and *P. guajava*, respectively. The sex ratio of this insect was also 0.48 in E. urophylla, which demonstrates that eucalyptus species do not change this biological aspect. Pereira et al. [17] found a higher sex ratio of 0.48 and 0.46 for adults obtained with larvae fed by *E. cloeziana* and *P. guajava*. Pereira et al. [25] who obtained a sex ratio of 0.59 for *Hylesia paulex* and Specht et al. [16], observed a sex ratio of 0.505 for *A. illustris,* that is, about twice the number of females obtained in the present work.

3.4. Identification of Natural Enemies

Few studies on the biology and morphology of the species have not yet clarified the duration of the phases of its life cycle, as well as the characteristics of the differentials that identify the larva, especially in forest areas. In recent decades, the advance of deforestation, for the installation of extensive reforestation areas, probably changed the natural habitat of the larva, reducing thus the number of its natural enemies and favoring its closeness with the cultivated species. Therefore, it is necessary to study the natural biological control agents of *C. opis*, its dispersion and abundance, enabling actions of control of this species to prevent damage caused by the defoliation it is capable of causing. Through the

applied methodology and in accordance with the main objective proposed, it was possible to recognize the following natural enemies:

Parasitoids obtained from larvae from Esplanada-BA:

1- Larval parasitoids:

- *Megaselia scalaris* (Loew) (Diptera: Phoridae) – First record in Saturniidae. (Figure 5) The specimen was identified by Dr. Danilo Ament (USP)

- *Lespesia* sp. (Diptera: Tachinidae).

The dipteran of the family Phoridae *Megaselia scalaris* (Loew) was recorded parasitizing larvae at different instars. This fly is cosmopolitan and synanthropic, eclectic in its eating habits and acts as a detritivore, parasite, parasite facultative and parasitoid. The most important vector of Chagas disease in areas has already been reported parasitizing colonies of Triatoma brasiliensis in the laboratory. semi-arid regions of Brazil [26]. *M. scalaris* larvae were found if feeding on *Boophilus microplus* [27].

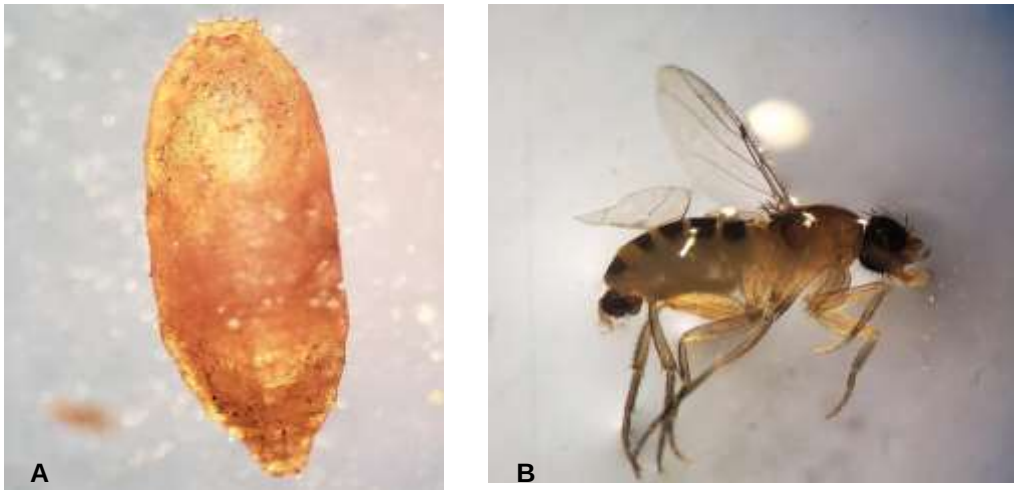

Figure 5. Exemplary of *Megaselia scalaris (*Loew). Pupa (**A**) and adult (**B**).

4 conclusions

The species *Cerodirphia opis* presented in the laboratory a high mortality in the phase larval, suggesting that insects are quite sensitive to packaging, in laboratory, and what changes in the methodology should be made in the next works.

- *Megaselia scalaris* and *Lespesia* sp. (Diptera, Tachinidae) as natural enemies of *Cerodirphia opis.*

- Biological studies of eucalyptus defoliators need more attention from researchers to assist in the Integrated Management of these species.

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