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Biology and morphology of *Molippa cruenta* (Lepidoptera: Saturniidae) in the laboratory

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ABSTRACT. The goal of this contribution was to study the biology and morphology of all developmental stages of *Molippa cruenta* (Walker, 1855), a member of Hemileucinae (Saturniidae), endemic to southern Brazil and northeastern Argentina. Daily observations were carried out on individuals reared under controlled temperature (25 ± 1°C), relative humidity (70 ± 10%) and photophase (14 hours of light). The duration and viability of each developmental phase, as well as morphological and behavioral aspects of each stage, were evaluated. Under laboratory conditions, the life-cycle of *M. cruenta* was completed in 150 days, with mean periods for egg, larva, pupa and adult phases being 35, 71.95, 37.85, 19.17 and 4.81 days, respectively. The larval phase included six instars, with an average growth rate of 1.46. The study of the immature stages of *M. cruenta* revealed several morphological characteristics that are distinct from other species of the genus, highlighting the importance of these stages for taxonomic and phylogenetic studies.

KEY WORDS. Developmental biology; Hemileucinae; larvae; life-cycle.

Molippa Walker, 1885 includes 22 taxa restricted to the Neotropical region (Lemaire 2002). Published information on species of the genus is restricted to the adult stage, brief descriptions of the last larval instar of six species, and some biological aspects of *M. sabina* Walker, 1855 (Lemaire 2002, Specht *et al.* 2005, 2008) and *M. similina* Jones, 1907, cited by Bourquin (1949) as *M. sabina* (Specht *et al.* 2008).

Molippa cruenta (Walker, 1855), a synonym of *M. semirosea* (Weymer, 1907), is endemic to the southeast and south of Brazil and adjacent areas of northeastern Argentina. In Brazil, this species has been recorded from the states of Minas Gerais, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul; in Argentina, it is known from Missiones (Lemaire 2002). Published information on this species is limited to geographical records (Weymer 1907, Biezanko 1986, Corseuil *et al.* 2002, Nunes *et al.* 2003, Specht *et al.* 2005), descriptions of the adult and morphological aspects of the immature stages and host plants in captivity (Lemaire 2002, Specht *et al.* 2008). In this paper, we contribute to the understanding of the taxonomy of *M. cruenta* by providing information on the biology and detailed descriptions of the immature stages of this species.

MATERIAL AND METHODS

Initially, two egg masses (136 eggs total) were obtained from a female collected in Bento Gonçalves, Rio Grande do Sul, Brazil. Laboratory rearing was conducted in a controlled environment (temperature = 25 ± 1°C, relative humidity = 70 ± 10% and 14 hours of photophase). Measurements were made with digital calipers (precision = 0.01 mm), and a stereo microscope equipped with an ocular micrometer. The mass of the pupa was measured with a semi-analytical scale (precision = 0.01 g).

The two egg masses were separated into individual Petri dishes, where they remained until eclosion. Petri dishes were lined with filter paper moistened daily with distilled water. In addition to morphological descriptions, the height of each egg was also measured (from the micropilar area to the opposite pole, which is fixed to the substrate).

After hatching, the larvae from each Petri dish were transferred to netted insect cages (width = 50 cm, depth = 42 cm and height = 80 cm) where they were maintained up to the prepupal phase. Leaves from a variety of host plants in different families were offered to the larvae, but only mate tea (*Ilex paraguariensis* St. Hill. – Aquifoliaceae) and "Ipê-amarelo" (*Tabebuia pulcherrima* Sandwrht – Bignoniaceae) were accepted. Given the wide availability of *I. paraguariensis*, this plant was used throughout the larval stage. In order to avoid sagging of the leaves, the petioles were put in 50 ml vials containing distilled water. Shed head capsules were collected to measure the distance between the frontal setae (Podoler & Klein 1978). The instars were differentiated as in Parra & Haddad (1989), using the frequency distribution of the distance between the frontal setae, at intervals of 0.01 mm; in the end, a curve of distribu-

tion of frequencies was constructed, in order to ascertain the fit to the model proposed by Dyar (1890).

During the prepupal period, when the larvae stopped feeding and began looking for places to pupate, individuals were separated into 500 ml glass containers. Filter paper moistened with distilled water was used to line the bottom of the glasses, which were closed with voile. A small incision was made on the cocoon after its construction, in order to allow for determination of the exact time of pupation.

On the second day after pupation, when the cuticle had already hardened, the cocoons were opened and the pupae were removed for sexing, as in Butt & Cantu (1962). The mass, height and longest width between the pterotecae were recorded.

After emergence, moths of the same age were separated into 10 couples and maintained inside cylindrical PVC cages (height and diameter = 20 cm), covered with a plastic film basally and white voile on the upper portion. The cages were lined with filter paper, moistened daily with distilled water, and provided with a stem of the host plant for oviposition. Longevity, fecundity and wingspan were noted.

Morphometric data and mass were analyzed after calculating the respective means and standard-errors. The t-test with a confidence level of 95% was used for comparisons between sexes, pupae and adults. The sex ratio was calculated as the number of females/number of females + number of males.

RESULTS

Egg (Figs 1 and 2) sub-oval, highest along the longitudinal axis (between the micropilar area and the opposite pole, which is fixed to the substrate); egg flattened laterally, slightly concave on one longitudinal plane; coloration pearl-white immediately after oviposition, micropilar area turning black with time, chorion becoming transparent, allowing visualization of the developing embryo. The distance between the micropile and the opposite pole averaged 1.203 ± 0.035 mm (n = 5). The embryonary period (Tab. I) corresponded to 23.381% of the total development and the viability of the egg masses obtained from the female collected in the field was 98.529%.

Six instars were present (Tab. II) with an average growth rate of 1.459. Larval cephalic capsule (Fig. 3) black, integument and scoli light yellow upon eclosion, turning greenish within a few hours and remaining greenish up to the end of the larval stage.

Table II. Mean and standard deviation of the distance between the frontal setae of the cephalic capsule of *M. cruenta*, by instar. Larvae were fed mate tea, and maintained under 25 ± 1°C temperature; 70 ± 10% relative humidity and 14 hours of photophase.

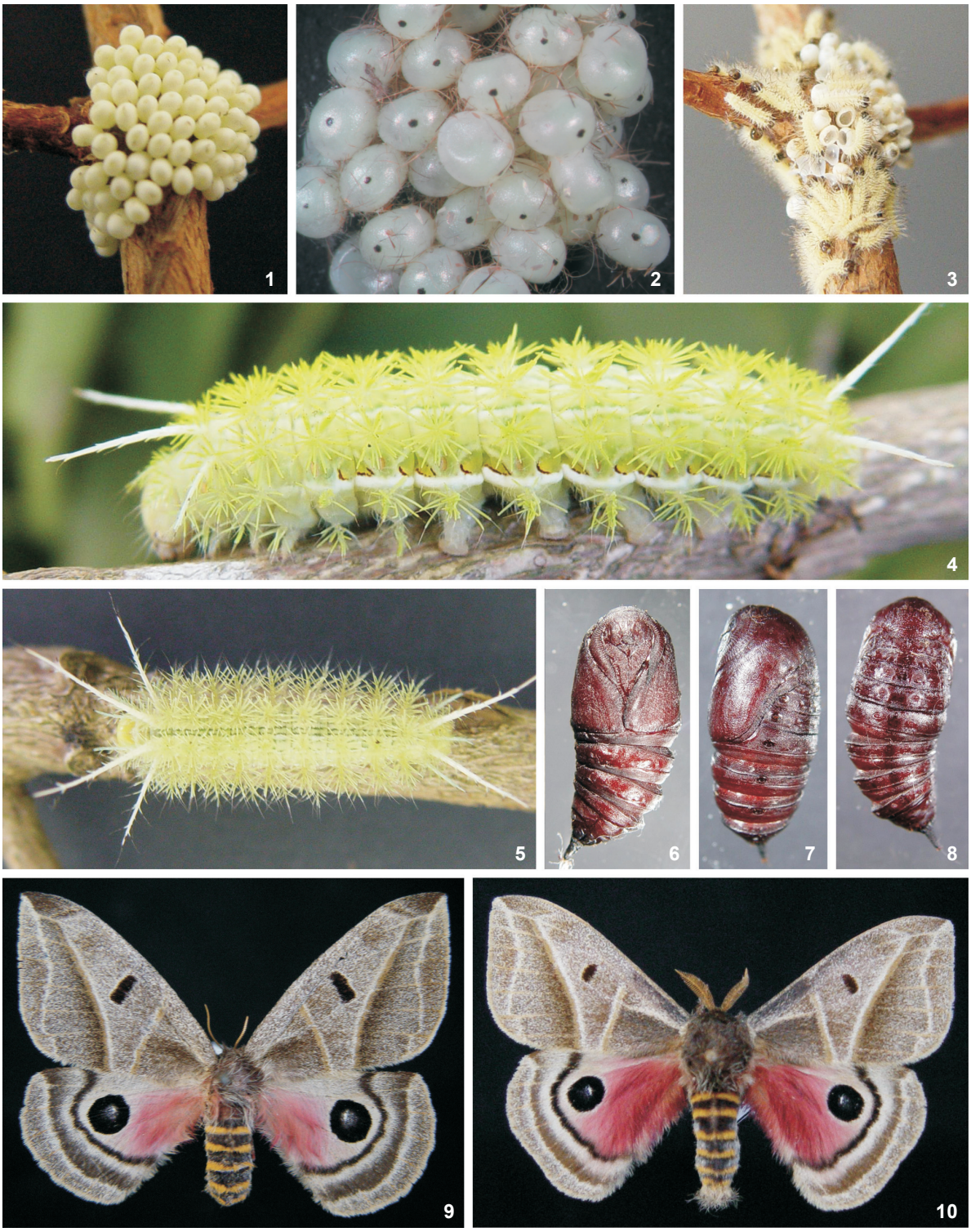
Instar	Distance between frontal setae	Growth ratio
I	0.120 ± 0.021	–
II	0.176 ± 0.032	1.463
III	0.258 ± 0.061	1.470
IV	0.378 ± 0.118	1.465
V	0.550 ± 0.178	1.455
VI	0.795 ± 0.239	1.445

The larval stage represented 48.1% of the total development, with survival above 90%. The slightest movements or changes in luminosity caused the larvae to stop feeding and remain motionless for about half an hour. The larvae were extremely gregarious during the first three instars, feeding together on the upper surface of the leaves, and moving about in a straight line. During the fourth and fifth instars, a few larvae remained together, but all of them fed individually during the final instar, preferentially at night. First instar (Fig. 3) with bifurcated dorsal and subdorsal scoli on the three thoracic segments, and the bifurcated dorsal scoli on abdominal segments 8 and 9, each branch of such bifurcated scoli bearing a pronounced apical seta; remaining scoli simple, with a short apical seta.

The most pronounced morphological modifications of the scoli shields occurred during the second instar, with the development of several spines, giving the larvae their typical spiny appearance; during this instar, further development of the dorsal and subdorsal scoli of the mesothorax and the uromere 9 became apparent. These scoli became longer, more robust, bearing less developed spines and ending apically in

Table I. Mean duration, standard error and percent survival of *M. cruenta*. Larvae were fed mate tea, and maintained under 25 ± 1°C temperature; 70 ± 10% relative humidity and 14 hours of photophase.

Developmental stage	N	Duration (days)	N	Survival (%)
Egg	136	35.000 ± 0	136 (134)	98.529
Larva	74	71.947 ± 7.892	74 (68)	91.892
Pupa	56	37.854 ± 1.598	56 (56)	100
Adult – female	14	5.128 ± 0.976	–	–
Adult – male	16	4.531 ± 0.911	–	–
Total	–	149.692	–	90.540



Figures 1-10. *Molippa cruenta*: (1) egg mass; (2) eggs in different positions; (3) larvae immediately after eclosion; (4) last larval instar, lateral aspect; (5) last larval instar, dorsal aspect; (6) pupa, ventral aspect; (7) pupa, lateral aspect; (8) pupa, dorsal aspect; (9) adult female; (10) adult male.

filaments, contrasting with the rigid setae present at the apices of the remaining scoli. The cephalic capsule became light green and remained so until the end of the larval phase.

The third instar was characterized by further elongation of the subdorsal and dorsal scoli of the mesothorax and uromere 9, already twice as long as the remaining body scoli in the second instar.

The main modifications that took place during the remaining instars included a further contrast between the dorsal and subdorsal scoli of the mesothorax and uromere 9, and intensification of the coloration.

Last instar (Figs 4 and 5), as described by Lemaire (2002) based on a photograph, with integument and larval cephalic capsule light green. Dorsal line (Fig. 5) darker than integument, extended from the mesothorax to the uromere 8, interrupted by the median dorsal scolus. Subdorsal line extended from the mesothorax to the uromere 8, whitish with apple-green margins that are more pronounced dorsally. Sub-spiracular line white, more pronounced from the metathorax to the uromere 8; each yellowish spiracle can be seen inside a V-shaped figure formed by folds of the integument. Predominantly semi-circular, brownish marks present on each side, between the sub-spiracular line and the V-shaped figure. Dorsal and subdorsal prothoracic scoli relatively narrow and slightly longer than the remaining scoli on the body, with spines bearing rigid apical setae. Dorsal and subdorsal mesothoracic scoli four and five times longer than remaining scoli on the body, respectively, bearing rigid setae that inoculate venom only at their base, at the same level as the remaining, shorter scoli. Spines on the median and distal regions of the long scoli ending as fragile filaments. Dorsal scoli of the uromere 8 approximately four times as long as remaining scoli; subdorsal scoli of the uromere 8 and mediodorsal scoli of the uromere 9 approximately twice as long as remaining scoli; as described for the mesothorax, all these with spines bearing hard setae basally and very thin and flexible setae elsewhere. Remaining scoli on the metathorax and abdomen short, rosette-shaped with spines bearing hard apical setae and long, filiform basal setae.

During the prepupal phase, characterized by interruption in feeding activities and the search for a place to build a cocoon, the larvae become darker and smaller in size. They spent approximately half of this phase producing rough, amber-colored silk threads to join leaves and other plant parts together in order to build a partially resistant, impermeable, one-walled cocoon.

Pupae (Figs 6-8) soft and reddish immediately after pupation, becoming darker, shiny-brown and hardened after a few hours. Round, slightly flattened anteroventrally; the visible portions of the uromeres have external projections that correspond to the locations of the larval scoli and pseudopodia. Apex of the pterotecae 0.45 times the total length of the pupa, reaching the posterior end of the third spiracular projection. Spiracles of the seventh uromere black, located on a projection of the tegument. Cremaster projecting from the dorsal portion of the uromere 11, with proximal (basal) portion round and typically thin, 0.782 ± 0.032 mm (n = 12) in diameter. Cremaster 2.159 ± 0.314 mm long (n = 12), wrinkled at base and becoming smooth toward the apex; apex of cremaster straight, bearing irregular hooks.

A total of 29 female and 27 male pupae were obtained, corresponding to a sex ratio of 0.518. During this phase, the females were significantly larger (p> 0.05) and heavier than the males (Tab. III). The duration of the pupal period (Tab. I) corresponded to 25.288% of the total development, with a 100% survival rate.

Table III. Mean and standard deviation of the length, in mm, and the mass, in g, of the pupae of *M. cruenta*, grouped by gender. Larvae were fed mate tea, and maintained under 25 ± 1°C temperature; 70 ± 10% relative humidity and 14 hours of photophase.

Measurements	Females (n = 12)	Males (n = 12)
Length *	23.272 ± 0.423	20.658 ± 0.737
Width *	9.258 ± 0.182	8.341 ± 0.532
Mass *	0.920 ± 0.077	0.716 ± 0.033

* Indicates significant differences according to the t-test with a confidence level of 95%.

There were no significant discrepancies in adult longevity, which corresponded to 3.213% of the total development. Fecundity was 153.565 ± 35.673 (n = 10) eggs per female. Females turned out significantly larger than the males, with wingspan of 66.804 ± 1.345 and 51.982 ± 0.925 mm, respectively.

DISCUSSION

The flattened, tablet-shaped, pale yellow eggs with white vertex of *M. cruenta* are similar to those of *Lonomia obliqua* Walker, 1855 (Lorini 1999, 2008, Lorini & Corseuil 2001), *Periga circumstans* Walker, 1855 (Specht *et al.* 2008) and *M. similina* (Bourquin 1949). The embryonic period (Tab. I) of *M. cruenta* was found to be twice as long as recorded for several Hemileucinae reared under the same temperature regimen (25°C). Incubation generally lasts about two weeks in Hemileucinae, corresponding to approximately 12% of the total development (e.g. Lorini 1999, 2008, Specht *et al.* 2006b, 2007b). Longer incubation periods, however, have been recorded for species of *Hylesia* Hübner, [1820] (e.g. Santos *et al.* 1988, 1996, Specht *et al.* 2006a, 2007a). Eggs of some species in this genus experience diapause and, unlike the eggs of *M. cruenta*, are normally covered with urticant setae from the female abdomen, which confer some protection against predators and parasitoids (e.g. Diaz 2005). The high viability of the eggs obtained from the female collected in the field was consistent with literature data (e.g. Lemaire 2002, Specht *et al.* 2006b).

The presence of six larval instars (Tab. II) with mean growth rate of 1.459 suggests mean growth rates that are close to each other, around 1.4 for each instar in the Hemileucinae. This is consistent with predictions based on the rule of Dyar (1890), and similar to results obtained with representatives of *Automeris* Hübner, [1819] (Specht *et al*. 2006b, 2007b) and *Hylesia* (Specht *et al*. 2006a, 2007a).

The long duration of the larval phase (Tab. I) is in agreement with reports on most Hemileucinae, which display diverse anti-predator strategies, including mimetic coloration, cryptic habits, gregarious behavior, and structures that produce and inoculate urticant substances (Bernays & Janzen 1988). The elevated survival rate of this phase can be attributed to its characteristic tameness, which also greatly facilitated specimen maintenance in the laboratory. The fact that larvae remain motionless and interrupt their feeding in the presence of any movement or alteration in light intensity indicates that they are extremely sensitive to external stimuli, especially visual (variations in luminosity), auditory and tactile stimuli. This feature, previously described for other Hemileucinae (Hodge 1972, Specht *et al*. 2006a,b) was most often observed during maintenance activities like changing the food. The non-aggressive behavior of *M. cruenta* differs from what has been described for larvae of *Automeris* (Specht *et al*. 2006b) and *Hylesia* (Specht *et al*. 2006a), who react to certain stimuli by detaching themselves from the host plant and contorting their bodies aggressively.

The remarkably gregarious behavior of *M. cruenta* during the first three instars is common among other representatives of the Hemileucinae. Most members of the subfamily feed at night and use their mimetic appearance to remain unnoticed during the day (Lemaire 2002).

The setal shield of the first larval instar (Fig. 3) of *M. cruenta* agrees with the pattern described for the subfamily. The major modifications occur in the second instar, as reported for all Hemileucinae, and include the development of ramifications of the scoli, giving the larvae a typical spiny appearance (Lemaire 2002). The pronounced development of the dorsal and subdorsal scoli of the mesothorax and uromere 9, starting in the third instar, makes the larva of *M. cruenta* distinct from all remaining Hemileucinae, including other species of *Molippa* (e.g. Bourquin 1949, Lemaire 2002, Specht *et al*. 2008). The only known larva with a potentially similar appearance is that of *Molippa latemedia* (Druce, 1890), as suggested by Lemaire (2002).

The remaining short, rosette-shaped thoracic and abdominal scoli with spines bearing hard and urticant apical setae, and fewer spines with long and filiform basal setae are similar to those described for *Hyperchiria incisa* Walker, 1855 (Barth 1954) and *L. obliqua* (Veiga *et al*. 2001).

The one-walled, resistant and impermeable cocoon, made by joining leaves and other plant parts with rough silk threads are similar to those described for *M. similina* (Bourquin 1949) and various other Hemileucinae (Lemaire 2002).

The shiny appearance of the entire pupal integument of *M. cruenta* contrasts with that of *M. similina* (Bourquin 1949). The latter has the thorax and dorsum corrugated and rough, similar in appearance to *Automeris illustris* (Walker, 1855) (Specht *et al*. 2006b) and *A. naranja* Schaus, 1898 (Specht *et al*. 2007b). It is important to note that the presence of a long cremaster in *M. cruenta* contrasts with the short cremaster of *M. similina* and other Hemileucinae (Lemaire 2002). Only species that pupate on the ground, such as *L. obliqua* (Lorini & Corseuil 2001) and *P. circumstans* (Specht *et al*. 2008), are known to have a long cremaster. In contrast with *M. cruenta*, however, the cremaster of these species do not have hooks, since they do not build a cocoon.

The significantly larger size of the female (Tab. III) agrees with the significant sexual dimorphism already described for the adults (Lemaire 2002). The larval survival rate of 100% can be attributed to adequate diet and hygiene, coupled with the fact that the larvae are very ease to rear. The duration of the larval phase, corresponding to ca. 1/4 of the total development time has also been recorded for several Hemileucinae that do not display pupal diapause (e.g. Specht *et al*. 2006a, 2008).

The adult phase, corresponding to the sexual maturity and reproduction, is very short. Members of the Hemileucinae do not feed as adults, and the main activities in this phase are related to reproduction, such as location and attraction of the opposite sex, copulation, location of host plant and oviposition (Lemaire 2002).

Fecundity was relatively low in this species when compared to some species of *Hylesia* (Santos *et al*. 1988, 1996, Specht *et al*. 2006a) and *Automeris* (Specht *et al*. 2006b), but similar to fecundity reports for *L. obliqua* (Lorini *et al*. 2004).

Similarly to what has been shown for other groups of Lepidoptera (Freitas & Brown Jr 2005), the study of the immature stages of *M. cruenta* has revealed biological and morphological features that can be used in the generic placement and specific characterization of this species. Immatures of *M. cruenta* display several characteristics that are distinct from those found in other species of the genus. Based on our morphological study, a reassessment of the generic placement of this species, once more comparative information is available, will be forthcoming.

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