Hindawi Publishing Corporation
Psyche
Volume 2014, Article ID 328030, 6 pages
http://dx.doi.org/10.1155/2014/328030

Research Article

Immature Stages and Life Cycle of the Wasp Moth, *Cosmosoma auge* (Lepidoptera: Erebidae: Arctiinae) under Laboratory Conditions

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Received 24 February 2014; Revised 20 May 2014; Accepted 29 May 2014; Published 9 July 2014

Academic Editor: Kent S. Shelby

Cosmosoma auge (Linnaeus 1767) (Lepidoptera: Erebidae) is a Neotropical arctiid moth common in Cuban mountainous areas; however, its life cycle remains unknown. In this work, *C. auge* life cycle is described for the first time; also, immature stages are described using a Cuban population. Larvae were obtained from gravid wild females caught in Viñales National Park and were fed with fresh leaves of its host plant, the climbing hempweed *Mikania micrantha* Kunth (Asterales: Asteraceae), which is a new host plant record. Eggs are hemispherical and hatching occurred five days after laying. Larval period had six instars and lasted between 20 and 22 days. First and last larval stages are easily distinguishable from others. First stage has body covered by chalazae and last stage has body covered by verrucae as other stages but has a tuft on each side of A1 and A7. Eggs and larvae features agree with Arctiinae pattern. Pupal stage lasted eight days, and, in general, females emerge before males as a result of pupal stage duration differences between sexes.

1. Introduction

Cosmosoma is a large Neotropical moth genus with approximately 155 species [1], and some of them are broadly distributed. Wasp moth *Cosmosoma auge* L. (Lepidoptera: Erebidae) occurs in Central America, South America, and the Caribbean islands. In Cuba, it occurs throughout the main island with big populations in the main mountainous areas. As in other moth taxa, knowledge about immature stages of this genus is lacking and there are no descriptions of their life cycle. Dyar [2] described *C. myrodora* immature stages from a Florida population and Castillo [3] described *C. myrodora* larval and pupal stages duration which were fed with three *Mikania* species, but no instar duration or larval morphology were taken into account. Wild larval ecological traits are also unknown for *Cosmosoma* species. In this paper we describe life cycle and larval stages for *Cosmosoma auge* from a Cuban population. We also report a new host plant for this species and some immature ecological traits. Other host plants reported for *C. auge* in HOSTS databases are *Cecropia peltata* (Cecropiaceae), *Ipomoea* sp. (Convolvulaceae), *Lagenaria siceraria* (Cucurbitaceae), *Mikania pachyphylla*, *M. parviflora,* and *M. scandens* (Asterales: Asteraceae) [4].

2. Materials and Methods

2.1. Collecting and Rearing. Larvae were obtained from seven wild *Cosmosoma auge* females collected at Viñales National Park in September 2011. As Peterson [5] suggested, females were kept confined together in a plastic jar until they laid eggs. Newly hatched larvae were placed in plastic Petri dishes (100 × 10 mm) and they were provided with fresh host plant leaves daily. Larvae that hatched the same day were placed together in groups of 30 individuals. When growth desynchronization occurred, larvae were sorted in other Petri dishes in order to keep the same age groups to detect individual variations in stages length. Larvae were reared in captivity at Cojimar, Eastern Havana, under natural photoperiod, humidity, and temperature between September 29th and October 21st 2011.

Daily temperature fluctuated between 24 and 28°C with average of 26°C, and the relative humidity varied from 54 to 93% with average of 79%. Both abiotic variables were provided by Casablanca Meteorological Station, Havana.

Host plant identification and ecological traits descriptions were made with botanical samples and animals from Sierra del Rosario Biosphere Reserve. Some individuals ($n = 3$) were fed with *Cecropia peltata* to prove its suitability as host plant.

2.2. Life Cycle and Description of Immature Stages. Egg laying patterns were described from wild clutches found on the host plant leaves. Duration of the egg stage was measured and proportion of infertile eggs was also recorded. Egg shape and dimension were described using eggs obtained from wild females caught in Sierra del Rosario Biosphere Reserve. Two base diameters and height were measured using an ocular micrometrer (0.05 mm precision) attached to a stereomicroscope Olympus. Egg volume ($N = 36$) was calculated following García-Barros [6]. Surface relief and micropilar details were observed with a SEM (250x magnification).

Petri dishes were checked daily for head capsule exuviae to establish larval instar duration. The head capsule width was measured (using the same ocular micrometer) directly on the larvae while resting and was defined as the distance between outermost ocelli. Two body lengths per stage were measured. One length was taken after molt (minimum length) and another in the premolt phase (maximum length), when larvae have emptied their gut and became light yellow. First and second instar larvae length were measured using the ocular micrometer mentioned above, and third to sixth instar larvae length were measured using a Vernier caliper with 0.05 mm precision.

Larvae that pupated the same day were placed together to measure the duration of the pupal stage. Pupae maximal length and width were measured using the same Vernier caliper. At emergence, adults' sex was recorded in order to detect differences in pupal stage duration among sex.

Stehr [7] and Scoble [8] were followed for morphological terminology. Photographs of larvae, pupae, and adults were taken using a macro mode of a 10.1 megapixels digital camera. Eggs, at least three individuals of each larval stage, two pupae, and cocoons were preserved in 75% alcohol and housed in Felipe Poey Natural History Museum, University of Havana. Fourth to sixth larval instars were killed in very hot water to prevent gut content decomposition.

2.3. Statistical Analysis. Mean and standard deviation of larval stages duration were calculated. Stage duration and pupal period length between sexes were compared using a randomization test from Monte Carlo algorithm, using the PopTools complement of Microsoft Excel; 10000 iterations were made.

3. Results

3.1. Host Plant and Ecological Considerations. Wild caterpillars were found feeding on climbing hempweed *Mikania micrantha* Kunth (Asterales: Asteraceae) and laboratory cohorts were successfully raised with it. Larvae fed with *Cecropia peltata* spent most of the time walking all over the Petri dish; they did not eat the leaves and died of starvation.

Table 1: Larval stages duration of *Cosmosoma auge* raised at Cojímar, Havana, under laboratory conditions (September-October 2011).

Instar	Duration (days)		Mean ± SD
1st ($N = 40$)	3 ($N = 15$)	4 ($N = 25$)	3.6 ± 0.49
2nd ($N = 40$)	2 ($N = 21$)	3 ($N = 19$)	2.5 ± 0.50
3rd ($N = 40$)	2 ($N = 13$)	3 ($N = 27$)	2.7 ± 0.47
4th ($N = 39$)	2 ($N = 8$)	3 ($N = 31$)	2.8 ± 0.44
5th ($N = 35$)	3 ($N = 22$)	4 ($N = 13$)	3.4 ± 0.59
6th ($N = 24$)	6 ($N = 7$)	7 ($N = 17$)	6.7 ± 0.46

Cosmosoma auge is a multivoltine species with multiple generations along the year; adults were caught almost every month. In the wild, eggs were laid singly ($n = 9$), in pairs ($n = 5$), and in trios ($n = 3$) on the underside of mature leaves of its host plant. Only two clusters with five eggs and two clusters with eight and ten eggs, respectively, were found. Eggs were always found in the basal portion of host plant leaves, near to veins.

First and second instar larvae fed on the leaf epidermis while other instar larvae fed on all the laminae but not on the 1st and 2nd order veins. Second instar larvae fed on shed skin as all the other larval instars did. In the wild, 4th and 6th instar larvae were found feeding on leaves underside at night. Always one larva per leaf was found. In the wild, larvae were apparently hidden during the day but in laboratory conditions larvae of all instar fed at day also. When larvae were disturbed they fell down holding themselves with a silk thread. In the wild, we found empty cocoons on the upperface and the underface of host plant leaves.

3.2. Life Cycle. A total number of 134 eggs were obtained and 47 of these (35%) did not hatch; but they were not infertile because in all cases a well-formed embryo could be seen through eggshell. Eggs ($n = 87$) hatched days after oviposition at any time of day five. Larval stage lasted 20 to 22 (21.5 ± 0.66) days and pupae ended their development 8 to 10 (8.6 ± 0.56) days later. Instar's duration was between two and four days for immature larvae and six or seven days for last instar larvae (Table 1). Only one larva had a four-day 4th instar period, and two larvae had two-day and five-day 5th instar, respectively. There were differences in larval stage duration: 1st and 5th instar lasted longer than 2nd, 3rd, and 4th instar ($P < 0.001$), and the 6th instar lasted significantly longer than all the previous instars ($P \leq 0.003$). Most of the 40 larvae that completed their life cycle reached the 6th instar; only three of them reached the 7th instar and were not included in this analysis.

At the end of the larval phase, the larvae have a quiescent prepupal phase, which lasted about two days, and afterward they entered in the pupal stage. Pupal mortality was 15% of the 43 pupae considered. Adult emersion ($n = 35$) occurred at any time of the day, although most of it occurred in

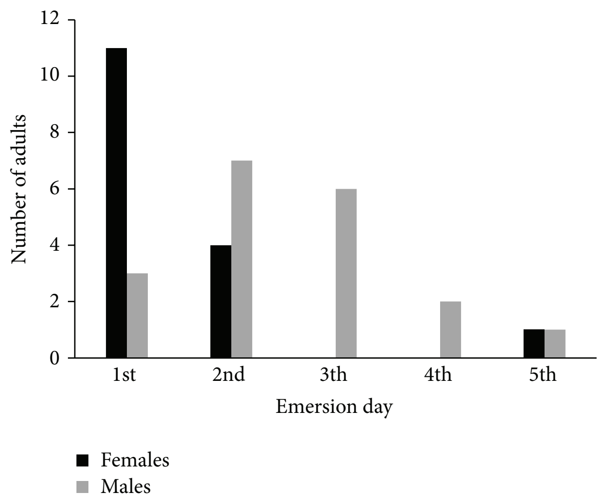

Figure 1: Number of *Cosmosoma auge* imagoes (both sexes) that emerged in each emersion day. Specimens were raised under laboratory conditions at Cojímar, Havana (September-October 2011).

the afternoon hours ($n = 29$). Pupal stage lasted eight to ten days. Females ($n = 16$) had a one day shorter pupal period than males ($n = 19$) ($P \leq 0.0032$). There were differences in emersion dynamics between sexes as a consequence of their differences in pupal period. The 68% of total females emerged in the first emersion day while males didn't reach the 50% of adults emerged in the second emersion day (Figure 1). These results suggest that *C. auge* presents protogyny. Adult lifespan was 15 days maximum ($n = 1$).

3.3. Immature Stages. Eggs are hemispherical and upright, with 0.87 ± 0.05 mm diameter and 0.62 ± 0.05 mm height. Eggs volume was 0.25 ± 0.028 mm^3. The base was not flat but concave. Egg color is ivory due to its content; all thought the transparent and shiny eggshell gives a pearly appearance (Figure 2(a)). There is no color change during development, though on eclosion day, the larva mandibles can be seen beneath the eggshell as two pale brown spots that are close together and mobile. Chorion was hexagonally and pentagonally reticulated (Figure 2(b)) except for the micropilar area, which was rosette-like, composed by five petaloid cells (Figure 2(c)). In the eclosion day, the only difference was the presence of two tiny, mobile, and pale brown spots (larva mandibulae) on the egg surface.

First instar larvae: minimum length 2.65 ± 0.25 mm ($n = 62$) and maximum length 3.75 ± 0.20 mm ($n = 34$), and head capsule width 0.40 ± 0.05 mm ($n = 53$). Body covered by chalazae and dorsal chalazae longer than lateral ones. Six stemmata in two rows, four dorsal stemmata forming a semicircle, and two ventral stemmata separated from dorsal ones by a distance equal to stemmata diameter. Prolegs with heteroideus crochets in a mesoseries, distal lobe with 10 big hooks in the middle and many little hooks in both sides. Body color was ivory, almost white, but A8, which was light yellow dorsally. Thorax and abdomen turn green when larvae feed because of their translucent body wall (Figure 3(a)). Head is yellow, but stemmata are black and distal edge of mandibles is reddish brown. Heads have white and tiny setae. Larvae had some two-tone dorsal setae (black base and white tip) interspersed with white regular setae. Rarely, these bicolored setae were predominant on larva's body, which looks grayish. All larval stages have bare venter.

Second instar larvae: minimum length 4.10 ± 0.40 mm ($n = 43$) and maximum length 5.80 ± 0.65 mm ($n = 48$). Head capsule width was 0.50 ± 0.05 mm ($n = 45$). The main change in this instar is that chalazae are replaced by verrucae except the chalazae in the anterior edge of T1 which were oriented forward. Thorax segments T2 and T3 and abdominal segments were covered by verrucae. Verrucae with many white setae and two or three black and long setae. Legs and prolegs had white tiny setae. Setae in T2, T3, and A1 are longer than the other body's setae. There were no significant color changes from first instar (Figure 3(b)) in this or next stages.

Third instar larvae: minimum length 7.80 ± 0.60 mm ($n = 8$) and maximum length 8.35 ± 0.45 mm ($n = 18$). Head capsule width was 0.75 ± 0.05 mm ($n = 13$). There are five black chalazae in the anterior edge of T1 oriented forward. Body covered by verrucae, included T1. Verrucae had several black setae in T2, T3, and A1–A8.

Fourth instar larvae: minimum length 9.30 ± 1.45 mm ($n = 23$) and maximum length 11.65 ± 0.50 mm ($n = 26$). Head capsule width was 1.05 ± 0.05 mm ($n = 15$). Body covered by verrucae with white and black setae. Dorsal verrucae with some long setae and lateral verrucae with setae shorter than dorsal ones. Legs and prolegs covered by short, white, and thin setae. Oval spiracle in T1 and A1–A8. Head with some tiny setae.

Fifth instar larvae: maximum length 15.60 ± 0.90 mm ($n = 28$). Head capsule width was 1.45 ± 0.05 mm ($n = 21$). Body covered by verrucae with white and black setae. Legs and prolegs have white and tiny setae. Spiracles in T1 and A1 had medium size, A8 spiracle is big and conspicuous, the rest are tiny.

Sixth instar larvae: minimum length 15.40 ± 1.30 mm ($n = 35$) and maximum length 21.60 ± 2.00 mm ($n = 22$). Head capsule width was 1.95 ± 0.05 mm ($n = 16$). At the end of the larval stage they reached eight times their initial length at hatch and about 30% of this growth occurs during the last instar because of an increased growth rate (Figure 4). Mature larva's body was covered by verrucae with white setae, T2, T3, and A1 verrucae with some black setae. A1 and A7 had a pair of white tuft located laterally perpendicular to longitudinal body axis (Figure 3(c)). Most of the tuft setae are white but it had some black plumed setae. There are gray setae in A8 and A9. Also, in A8 there are two bright yellow spots dorsally, one in each side of heart.

In the prepupal phase, the mature larvae stopped feeding and searched for a place to pupate. Then, they remained quiet, emptied their gut, and turned bright yellow, especially setae (Figure 3(d)). Only white setae changed color, black setae remained the same. Afterward they shrank and detached setae from their bodies to construct cocoons mixing them with silk. The denuded larvae are light yellow and they keep that way until early pupal stage.

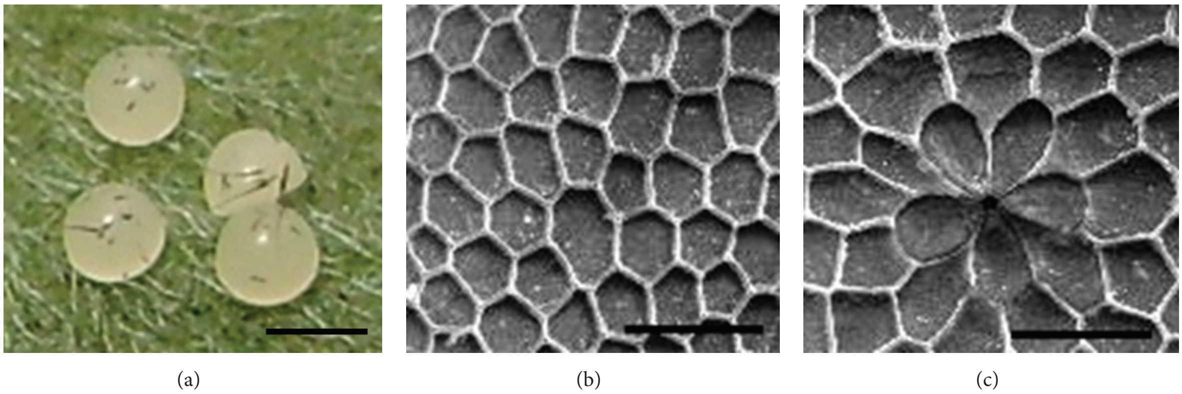

Figure 2: *Cosmosoma auge* eggs. General view (a), chorion surface detail (b), micropilar area (c). Scale bar represents 1 mm (a) and 500 μm ((b) and (c)).

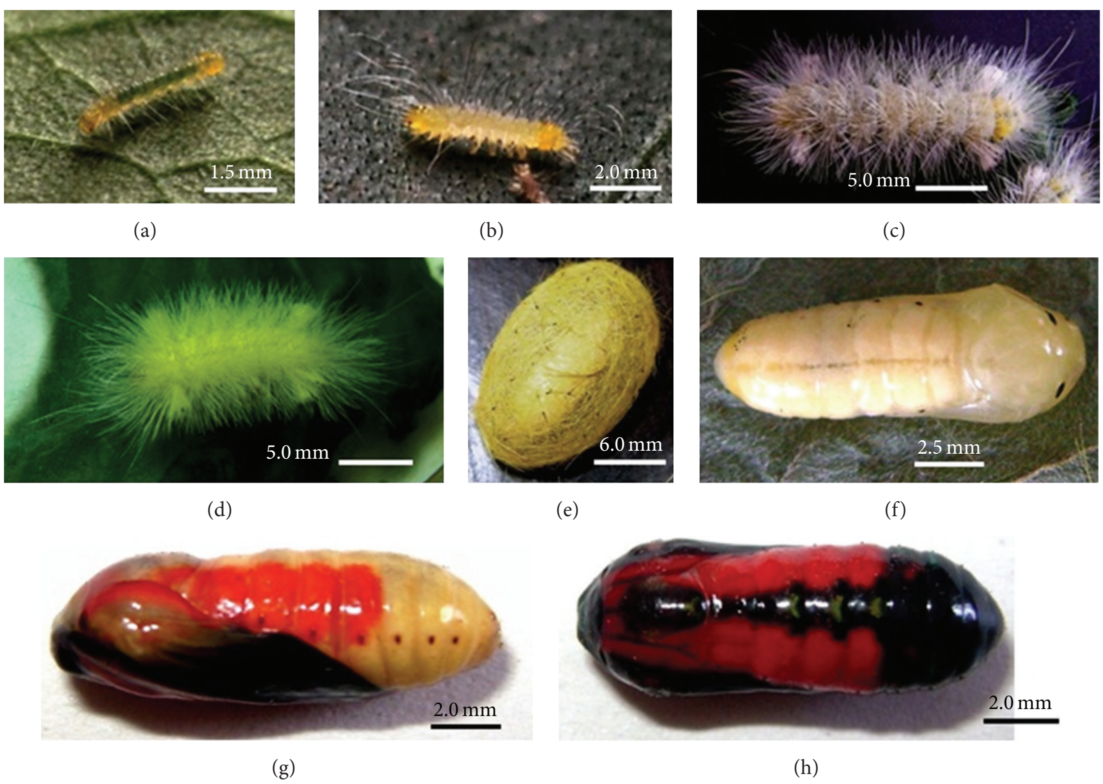

Figure 3: Some immature stages and cocoon of *Cosmosoma auge*. First instar larva (a), second instar larva (b), last instar larva (c), prepupa (d), cocoon with pupa inside (e), recently molted pupa, dorsal view (f), pupa 12 hours before emersion, lateral view (g), and pupa just before emersion, dorsal view (h).

Pupae: obtect and enclosed in a thin, walled, and bright yellow ellipsoidal cocoon (Figure 3(e)). Pupae length is 12.45 ± 0.65 mm (n = 43) and width is 4.60 ± 0.25 mm (n = 42). Abdominal segments with punctures moderately dense on dorsal part. Few setae dispersed on the lateral part close to the spiracles and also on dorsal part. Ventrally, the wing tips reach the A4 segment. The cremaster is weak and consisting of three rows of translucent hook-like setae only visible with a magnification of 40x. Pupae color is shiny pale yellow, head and thorax are translucent yellow but abdomen was whitish yellow. Newly formed pupae have two little dark spots on anterior mesonotum, which correspond with thoracic spiracles (Figure 3(f)). Eyes (only a line) and spiracles are brown, which quickly became black. After four days, eyes became brown oval areas and in the 5th day, they became black. In the 7th day, joints are light brown. In the 8th day, head and thorax as well as two stripes on the abdominal dorsum from A1 to A4 turn orange red (Figure 3(g)); wings and the four rear segments as well as a middorsal stripe on the thorax and abdomen between the red stripes are dark

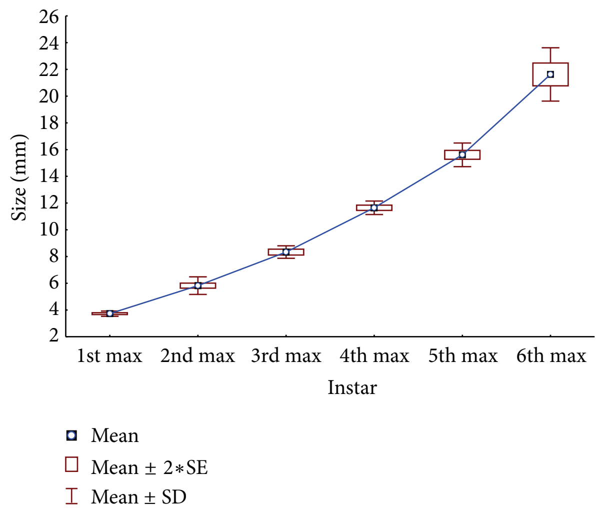

FIGURE 4: Growth curve of *Cosmosoma auge* larvae raised at Cojímar, Havana, under laboratory conditions (September-October 2011). In each stage lengths were taken in the premolt phase.

gray. Just before emersion these gray zones turn black with dark blue iridescence (Figure 3(h)). Pupae show adult's color and looked shiny at that point. After that, it turned opaque because pupae skin separates from imago within it.

4. Discussion

Mikania micrantha is the first host plant reported for *C. auge* in Cuba and belongs to the same genus of *M. scandens*, host plant reported for North American *C. myrodora* [9]. It is known that Lepidoptera phytophagous larvae had a strong specificity for a host plant family or even genus [10]. As Thompson and Pellmyr [11] state, females are capable of discrimination among plant species, host plant genotypes, and host plants in different microhabitats. The record of *Cecropia peltata* as *C. auge*'s host plant could be a misidentification of larvae feeding on it. Also, it could be a result of taxonomic problems in *C. auge*, in case of being a complex group cryptic species.

The finding of wild small clutches and solitary eggs, as in Florida's *C. myrodora* [2], suggests that *C. auge* has a solitary larval strategy in concordance with Uvarov [12] definition. Wild larvae feeding pattern also reinforces this hypothesis. Larval growth under different density conditions was studied for this species (León-Finalé and Barro unpublished data) and results agree with the hypothesis of larva solitary strategy in *C. auge*. According to Stamp [13], species with solitary larva reduced the probabilities of been found by parasitoids and predators, and also reduced food and pupation sites competition. Matsumoto [14] states that grouped larvae are at risk of suffering starvation due to host plant defoliation if plant is small or isolated. *Mikania micrantha* is a vine with limited leaves number and *C. auge* larvae are voracious in later stages. A large number of individuals could potentially defoliate a single plant quickly. Occasionally, *M. micrantha* grows grouped and there is potentially more food available for larvae. Females could detect this unusual availability of larval food [11] and laid larger clusters of eggs (eight or ten), as we detect in Sierra del Rosario population. All these arguments make a study to define *C. auge* larval strategy necessary.

Eggs shape, surface relief, and color were similar to Florida's *C. myrodora* eggs [2, 5]. Eggs are fairly uniform in structure throughout the subfamily [7]. Dyar [2] found that *C. myrodora* had an egg period length of eight days but we found an egg period of five days. This difference could a result of differences in abiotic parameters, as temperature. Time and size traits are affected by temperature, photoperiod, humidity, diet, density, and host quality [15]. Also this could be a product of inherent differences between *C. auge* and *C. myrodora*.

As a whole, morphology of immature stages of *C. auge* is similar to general arctiid pattern [7, 8] and is very constant along non-first instar larvae in this species, without major changes in color or morphology. As in *Phoenicoprocta capistrata* [16], the first instar is only covered by chalazae bearing one long filiform seta and the intermediate instars are covered by verrucae. The last instar (6th) was morphologically recognizable from the previous ones for the presence of one pair of tufts in A1 and A7. The drastic color change in the prepupal period could be a result of accumulation of colored substances in setae, in case of *C. auge* having transparent and hollow setae. The larvae features are basically the same of *C. myrodora* [2] but we observed six instars in almost all the cases instead of seven, also the head widths of all instars were larger in this work, but the seventh stage mature larvae in Dyar [2] have a wider head capsule than sixth stage mature larvae of *C. auge*.

In *C. auge*, as in other Lepidoptera species [17–20], last instar is where maximal growth and reserve accumulation occurred, because it was the only instar that lasted more than four days and the larvae grew around 30% of their total length. In this case, the last instar could be a critical period determinant of adult size, fecundity, and lifespan. The adult size could be determinant in sexual selection [21], mate frequency [22], and number of eggs laid by females [23].

Life cycle of *C. auge* was similar to but more dynamic than other Arctiinae species, probably as a result of an intense feeding behaviour [24, 25], because larvae were feeding during day and night [7]. Besides *C. auge*, there are differences in pupal period between sexes in other Arctiinae species as *Empyreuma pugione, Dyauxes ancilla,* and *Phoenicoprocta capistrata* [16, 26, 27]. In those, as in *C. auge,* males emerge after females. Differences in emersion rate could be a result of sex specific needs in gonadal maturation or a strategy to avoid inbreeding [28]. Also, *C. auge* time lapse between the start of females and males' sexual receptivity could be even longer if males consume pyrrolizidine alkaloids after the emersion as *C. myrodora* males do [9].

5. Conclusions

Cosmosoma auge life cycle was quite similar to that described for other Arctiinae species but was much more dynamic probably as a result of intense feeding behavior. Larvae are very constant in color and shape but pupae, on the other hand, are brightly colored and variable, on the contrary of most other arctiid moths.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors would like to thank Elier Fonseca and Dania Saladrigas (Universidad de La Habana) for their help in maintenance of *Cosmosoma auge* cultures in laboratory, to Jorge L. Guerra and Sierra del Rosario Biosphere Reserve staff for their help in the field collect, to Ramona Oviedo for host plant identification, to Dr. William Conner for answering some questions about taxonomic status of *C. auge* and finally to Casablanca Meteorological Station for providing meteorological data.

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