Description of Life Cycle and Preimaginal Stages of *Alecton discoidalis* Laporte, 1833 under Laboratory Conditions

O. Madruga[1] and M. A. Branham[2]

[1]Vicedirección Curatorial, Museo Nacional de Historia Natural de Cuba, La Habana, Cuba

[2]Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611. USA

Abstract

Alecton discoidalis Laporte, 1833 is the most widespread species of the only firefly genus endemic to Cuba. It is commonly found in limestone landscapes across the western half of the country. Larvae of *A. discoidalis* were collected at Pan de Matanzas and reared through the adult stage under laboratory conditions. They were fed mainly terrestrial snails from the families Potamiidae and Helicinidae. *Alecton discoidalis* had between four and eight larval instars. Females had more larval instars than males due to the need to gain more body mass. Eggs, larvae and pupae are described and illustrated, with emphasis on the general body shape, as well as the larval head, antennae and mouthparts. Female of *A. discoidalis* is brachypterous.

INTRODUCTION

While the majority of research on fireflies (Coleoptera: Lampyridae) has been focused on their bioluminescent behavior, flash communication, mechanisms of sexual selection and chemical defensive behavior relatively few papers are devoted to aspects of their biology and ecology (Hess 1920, Buschman 1984, Rosa 2007).Information on the ecology and life history of organisms is paramount for design and maintenance of conservation programs. As fireflies spend most of their life as immatures, it is crucial to understand the ecology of this life stage.

Literature about Cuban fireflies is mainly restricted to taxonomic papers from the early 20th century (Olivier 1912, Leng & Mutchler 1922, Mutchler 1923a, Mutcher 1923b) and these are largely devoted to species descriptions reported from Cuba. More recent publications include species lists (Peck 2005, Branham 2006) and a short communication about the larval feeding habits of *Alecton discoidalis* (Madruga & Hernández 2010).

Alecton Laporte, 1833, with four described species is the only genus of the family Lampyridae endemic to Cuba. *Alecton discoidalis* Laporte, 1833, is its most common species of the genus and is distributed in the western half of the country. This species appears to be associated with limestone landscapes, which are very abundant in, but not limited to this region. Very little is known of its life history, although of all Cuban species of Lampyridae it probably has the most associated ecological information. This paper reports observations on the life cycle of *A. discoidalis* under laboratory conditions. The included description of the immature stages of this species is the first for this genus as well as for any species of firefly known from Cuba.

MATERIALS AND METHODS

Field Work

Specimens of *A. discoidalis* were collected throughout two nights in August 2010, at Pan de Matanzas (n = 3). This area is located in western Cuba (Fig. 1), and is characterized by outcrops of limestone, some degree of disturbance, and secondary vegetation. Larvae were collected near a path, relatively busy during day, while emitting glows of several seconds in duration during the evening (from approximately 20:00 to 22:00 hours). Individuals of different instars were collected in the leaf litter. Temperature and humidity of these collecting periods were 26–32 °C and 64–86%, respectively. Data was recorded with a digital Control Company thermohygrometer (error = 1°C and 1%RH).

Laboratory Conditions

Individuals were reared individually in 9cm diameter Petri dishes, with small pieces of cotton that were sprayed regularly with water to preserve humidity. Room temperature and humidity were monitored daily, and kept within 18–30°C and 33–90%, respectively. The humidity levels within the Petri dishes, while not measured, were less variable than the measured for the room, as the insides of the dishes were always wet. Larvae were fed mainly terrestrial snails from the families Potamiidae and Helicinidae (three different species of *Chondropoma* sp., *Helicina aspersa*, *Ustronia sloanei* were some of the molluscs offered and most commonly consumed). These snails were frequently observed in the larval habitat, but larvae also fed on other snails, such as the introduced *Praticolella griseola*.

Body Size Across Instars

Body length of larvae and pupae were measured from the anterior margin of pronotum to the posterior margin of the last abdominal segment with the larvae slightly curved.

Maximum Width was measured at the posterior lateral edges of the metanotum and Base of Pronotum at the posterior lateral corners of the pronotum. Measurements of eggs (two perpendicular diameters) and first instar larvae were taken with an ocular micrometer (error= 0.05) coupled to a Leitz WETZLAR stereoscope. Measurements of larvae from the remaining instars and pupae were made with a metric caliper (error= 0.1 cm). Structures (antenna, maxilla, and mandible) were cleared in lactic acid and arranged on slides with glycerin as the temporary mounting medium. Drawings were made using a Nikon Eclipse E600 compound microscope equipped with a drawing tube. The resulting sketches were converted to vector graphic files with CorelDraw X2. Photographs of the larval habitus and head were taken using a Microptics Digital Microscope Imaging System.

RESULTS AND DISCUSSION

Life Cycle

Eggs were obtained from a single pair of adults reared in the laboratory from larvae (Fig. 2). The female mated immediately upon emergence and laid 70 eggs five days later. Incubation lasted between 25 and 30 days, at which point a total of 62 eggs hatched. A previous attempt to culture this species involved a female reared in the laboratory and a male collected from the field. Although they copulated several times, the female produced only infertile eggs which were laid just prior to death.

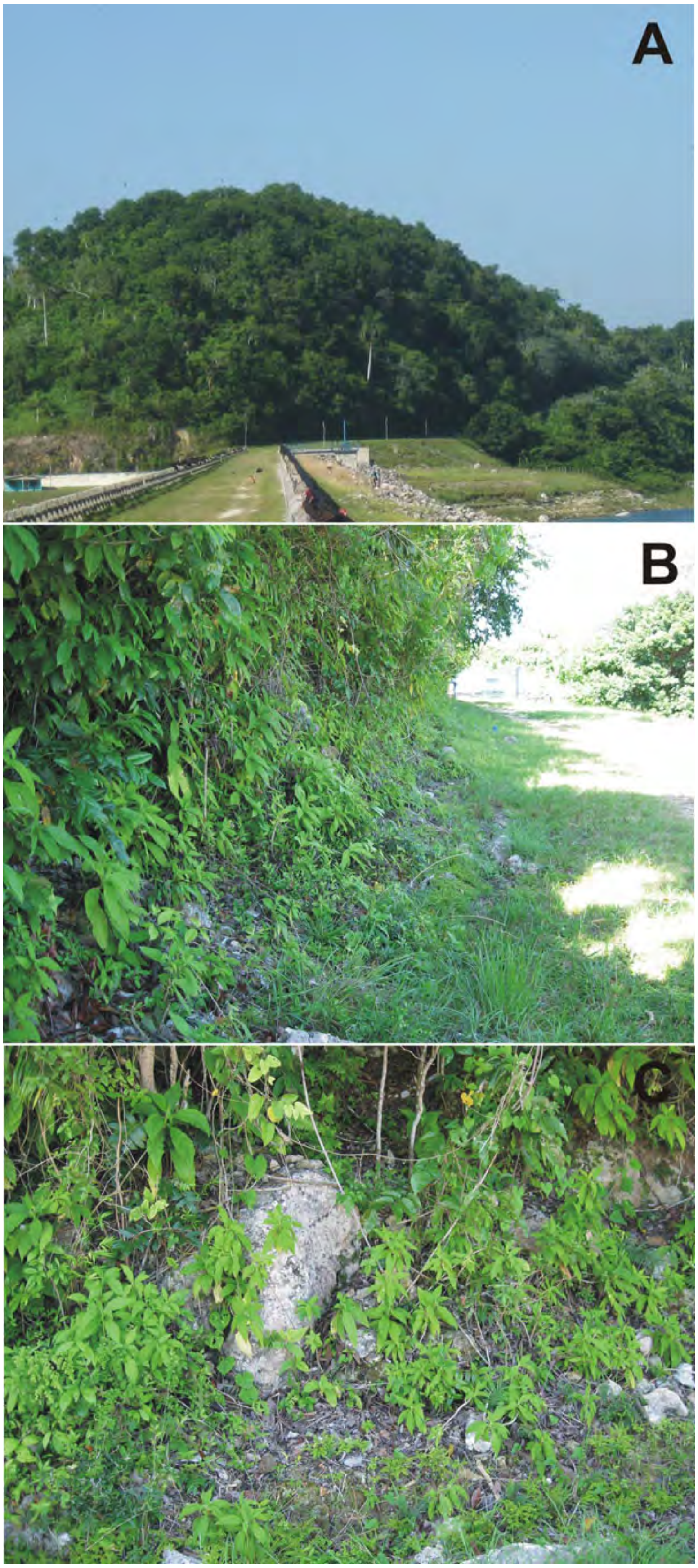

Figure 1. **A**: Elevation where collection took place, near of the Water Reservoir Caiguanabo, Pan de Matanzas, Matanzas Province, Cuba. **B**: Base of the elevation where larvae were found and trail to the Water Reservoir Caiguanabo. **C**: Microhabitat where larvae were capture.

Figure 2. **A:** Just emerged adults of *A. discoidalis*, brachipterous female and alated male. **B:** Eggs of *A. discoidalis* right before eclosion.

Total preimaginal period, including eggs, all larval instars and the pupal stage lasted between 149 and 265 days (n= 5). Larval period had a mean duration of 173.3 days, from October to June. *Alecton discoidalis* had between four and eight larval instars, depending on gender. Females underwent more larval instars than males. Consequently, they were considerably larger than males in the adult stage. This is likely a result of their roll in reproduction. Females need to accumulate more fat for high levels of egg production. Adult females of *A. discoidalis* are brachypterous. The original description (Laporte, 1833) does not mention the female at all, and Leng and Mutchler (1922) only describe the shape of antenna.

Duration of first larval instar was quantified as a range as the eggs were not tracked individually. Therefore, the first larval instar lasted approximately 24 days as mean, with a variation between 13 and 38 days (n= 15). Larval mortality at this particular instar was exceptionally high. Second instar larvae showed more variation, with 13 to 48 days and a mean of 23 days (n= 9). Third and fourth instars had the same duration as mean, 32 days (n= 8). However, the latter had more variation than the first one, with ranges of 13- 64 days and 17- 57 days, respectively. One individual (male) pupated after four instars. Fifth larval instar lasted 34 days as mean, ranging between 19 and 49 days (n= 5). The rest of males pupated after five larval instars. No individual reared had only six larval instars, its duration was of 28 days and 48 days in the two individuals who reached it. Both of them survive and reached the seventh instar (38 days and 27 days respectively). The first one pupated at this point (female) but the second one moulted to eighth instar and died before another moult.

Larval mortality was very high when larvae were reared under laboratory conditions. This could have been related to fluctuating levels humidity, one of the most important factors related to successful insect rearing (Emlen & Nijhout, 2000). In this case, exceedingly high values of humidity could contribute to the growth pathogenic microorganisms. This is a problem for rearing firefly larvae as they appear more susceptible to fungal, mite and viral infection (McLean *et al.*, 1972).

When larvae prepared to change to pupal stage they curved their body and stayed immobile. This condition is called prepupa. In most cases it was possible to detect it. Prepupa showed a mean duration of 3.8 days (min= 2 days, max= 7 days, n= 12) while the pupal stage was 7 days (n= 16), varying between 6 and 10 days. These numbers include data from other individuals captured as larva in the field and reared until adult stage. In those cases, the numbers of larval instars was not possible to determine, but the prepupal and pupal stages could be easily identified. Duration of all inmature stages is shown in figure 3. Larval stage possesses the greater variation in time. This is the active immature stage, which depends on prey availability besides environmental factors that work on the rest of stages.

Paired light organs where both functional and present on abdominal segment VIII in all larval instars, as well the prepupal and pupal stages. Bioluminescence across these immature stages was emitted in the form of glows of various durations at random intervals, but glows were more numerous and more bright in response to being disturbed. The larvae were both diurnally and nocturnally active. *Alecton discoidalis* possess light organs as adults, although they seem to be degenerated. At least the males of this species are diurnally active in the field.

<u>Description of Preimaginal Stages</u>

Eggs

Diameter 1.45 mm (n= 19) as mean. Spherical, orange-yellow becoming more transparent near to hatching, without microsculpture (see Fig. 2B).

Larval Stage

Figure 4 shows three body measurements taken to larvae at different instars during their growth. Maximum Width and Base of Pronotum showed less variability than body length. These two are taken in the same segment and are independent of position of larva, so they can be measured with more accuracy than body length. In spite of that, the latter give us a better idea of larval size. Body length of last instar

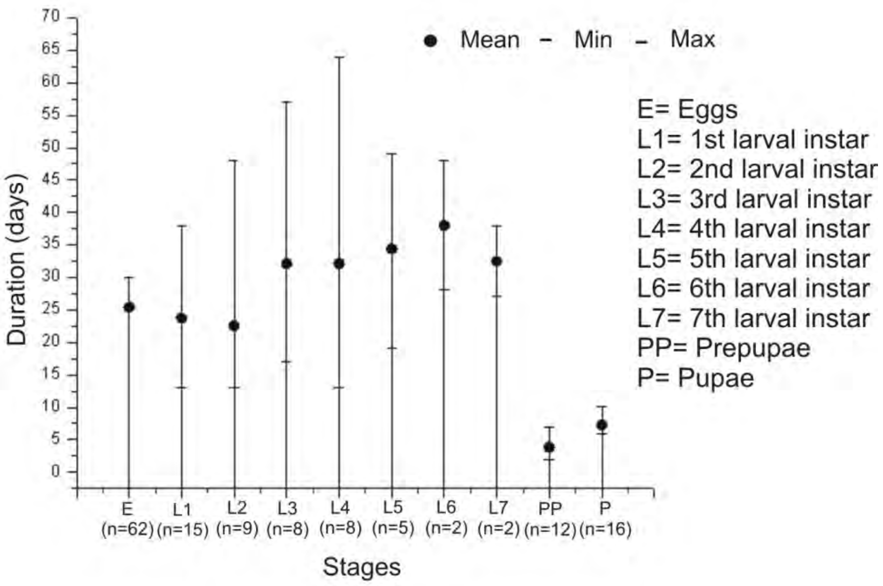

Figure 3. Duration of immature stages of *Alecton discoidalis*.

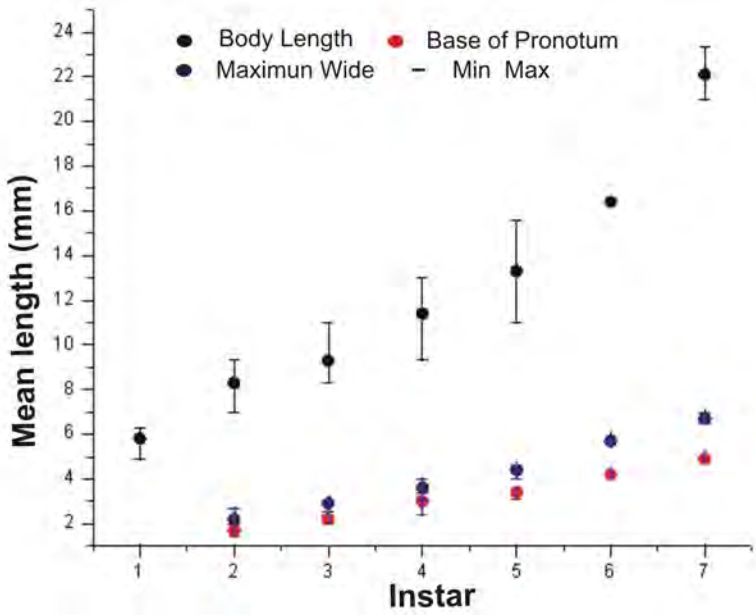

Figure 4. Measurements taken across larval instars of *Alecton discoidalis*.

measured 11.4- 13.3 mm as mean for males (4 and 5 instars), while 21.0- 23.3 mm was the range for females (7 instar). Body dorsoventraly flattened, last thoracic segment and two first abdominal tergites wider than the rest of the body (Fig 5 A, B). Tergites granulose, dark brown in thoracic segments and in abdominal tergites III-IX; abdominal tergites I and II cream colored (white to yellowish). Body surface covered by small hyaline or brown setae, located in the light or dark regions, respectively. Brown setae grouped like stars or asterisks, their concentration determine the observed coloration (various intensities of brown).

Head: Subquadrate (Fig. 5C), partially retracted into prothorax, joined by a membranous neck and not fused ventrally (Fig. 5D). Labrum and clypeus fused, centrally divided in two plates. Frontal arms of epicraneal suture posterior to frontoclypeus, with lateral stemmata in proximal portion, below antennal base.Dorsolateral region of frontal arms and near antennal base, dark brown colored.

Antenna: Three-segmented, located on lateroapical ridge of head. Basal segment partially sclerotized with four long setae. Second segment more sclerotized with four long setae; globular sensorium present on distal surface beside third antennal segment. Third segment minute with a few short setae (Fig. 5E).

Maxilla: Cardo subquadrate. Stipes large and elongate with a few long setae and short setae on lateral ridge (Fig. 5F). Palpi 4-segmented. Galea palpiform, 2-segmented with one spiniform, long setae on top and three others small setae on the surface. Lacinia covered by long and dense pubescence with some branched setae.

Mandible: Symmetrical, falcate, strongly sclerotized, with inner longitudinal channel opening subapically on outer margin, above branched setae (Fig 5G). Prostheca covering a large portion of the mesalaspect of the mandible; penicillus present on both extremes of prostheca; outer margin with dense and short pubescence in the middle region; dorsal surface of mandible bearing a row of short spines on the external ridge of the inner channel; basal third with long and dense setae covering the inner channel; base of mandible with an inner membranous lobe covered by a dense and short pubescence in the margin.

Labium: Closely attached to maxilla, narrow. Prementum heart-shaped. Palpi two-segmented; basal segment broad, with a long setae in the inner side apically; second segment smaller and conical.

Thorax: Pronotum almost triangular, sagittal line extends to apical edge, coloration dark brown (darker into the center) and yellow in lateral borders (Fig 5A). Several depressions in the tegument, visible as lighter colored spots (more visible in darker regions) with a non-uniform distribution. Prosternum trapeziform, moderately sclerotized.Mesonotum and metanotum subquadrate,broader than long, subdivided by sagittal line with lateral margins paler, tegument with several depressions of paler brown with a non-uniform distribution. Margins of thoracic terga slightly curved upwards. Meso and metasternum subdivided into basisternum and sternellum. One pair of spiracles present on mesopleuron.

Legs: 5-segmented. Coxa large; trochanter smaller; femur twice the size of trochanter; tibia with several spines in the inner margin; tarsungulus strongly sclerotized, remains to an apical spine. All segments cover by several small setae.

Abdomen: With 9 dorsally visible segments (Fig 5B). Tergites subdivided by a fine, light brown sagittal line, two protuberances present on the mid of posterior margin on each side of sagittal line (Fig 5A).Tergites 1 and 2 similar in shape and pale yellow colored. Tergites 3 and 4 of variable coloration among specimens, margins dark brown and mid region lighter colored, with several depressions which look like lighter punctures in dark zones, non-uniform distributed, but with a symmetric pattern in relation with sagittal line; lateral margins join to posterior forming an acute angle that protrude outward. Last tergite (9) with one spine at each angle, visible on ventral view. Sternites 1-7 similar in shape, subcuadrated, pale yellow colored, except for sublateral margins which are dark brown. Sternite 8 trapezoid formed and pale yellow colored, with four spines at base. Sternite 9 similar to 8 in shape, one third of its size, brown colored in the middle with two spines at base.

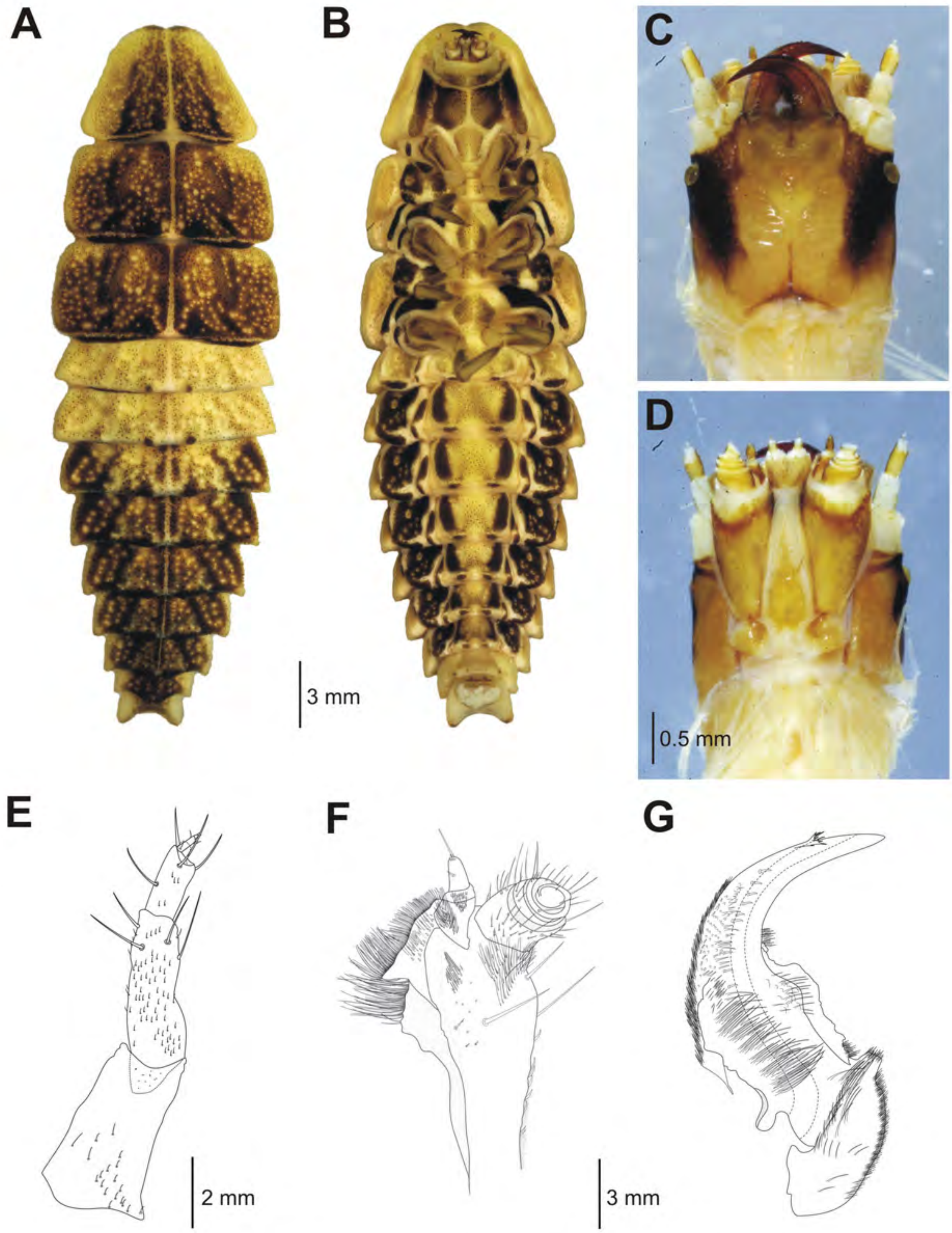

Figure 5. *Alecton discoidalis* last instar larva. **A**, **B**: dorsal and ventral habitus. **C**, **D**: dorsal and ventral view of the larva head. **E**: right antenna (dorsal view). **F**: right maxilla (ventral view). **G**: left mandible (dorsal view).

Pleural areas of segments I to VII subdivided; upper plates bearing spiracles (1- 7); lower plates small and narrow. Eighth pleurite complete, carrying eighth pair of spiracles and paired photic organs. Segment X tiny, with three small spines at base, bearing eversible larval holdfast organ.

Pupa

Female. Total length between 12.1- 13.5 mm. Pupa adecticous, exarate; cream to pale orange in color, older pupa become transparent some hours before eclosion (Fig 6A). Slightly curved, ventrally concave. Head: Completely covered by pronotum in dorsal view. Eyes small on sides of head. Antennae inserted in front of eyes, almost filiform, extending in length to sides of metathorax. Thorax: Pronotum large, semicircular, marginate at apex. One pair of spiracles on pleuron of mesothorax. Meso and metanotum shorter than pronotum, subrectangular, carrying wing pads on sides. Legs completely visible on ventral and lateral views. Abdomen: Segments wider than long. Tergites 1- 8 similar in shape but becoming narrower after tergite 3, subrectangular, with posterolateral corners forming an acute angle which projects outward. Segment IX small, partially visible in dorsal view, with conspicuous acute projections on each posterolateral corner. Eight pair of spiracles on abdominal segments I-VIII.

Male. Total length between 9.9- 14.2 mm. Pupa adecticous, exarate; bright orange colored, older pupa become transparent some hours before eclosion (Fig 6B). Slightly curved, ventrally concave. Head: Completely covered by pronotum in dorsal view. Eyes of median size, bigger than those of female. Antennae inserted in front of eyes, thicker than those of female, extending in length over elytra until their apical third. Thorax: Pronotum large, semicircular, marginated at anterolateral borders. One pair of spiracles on pleuron of mesothorax covered by wings. Meso and metanotum shorter than pronotum, subrectangular, carrying wing pads on sides. Legs visible on ventral and lateral views, except third pair with only metatarsus visible. Abdomen: Segments wider than long. Tergites 1- 8 similar in shape but becoming narrower after tergite 3, subrectangular, with posterolateral corners forming an acute angle which projects out of body margins. Segment 9

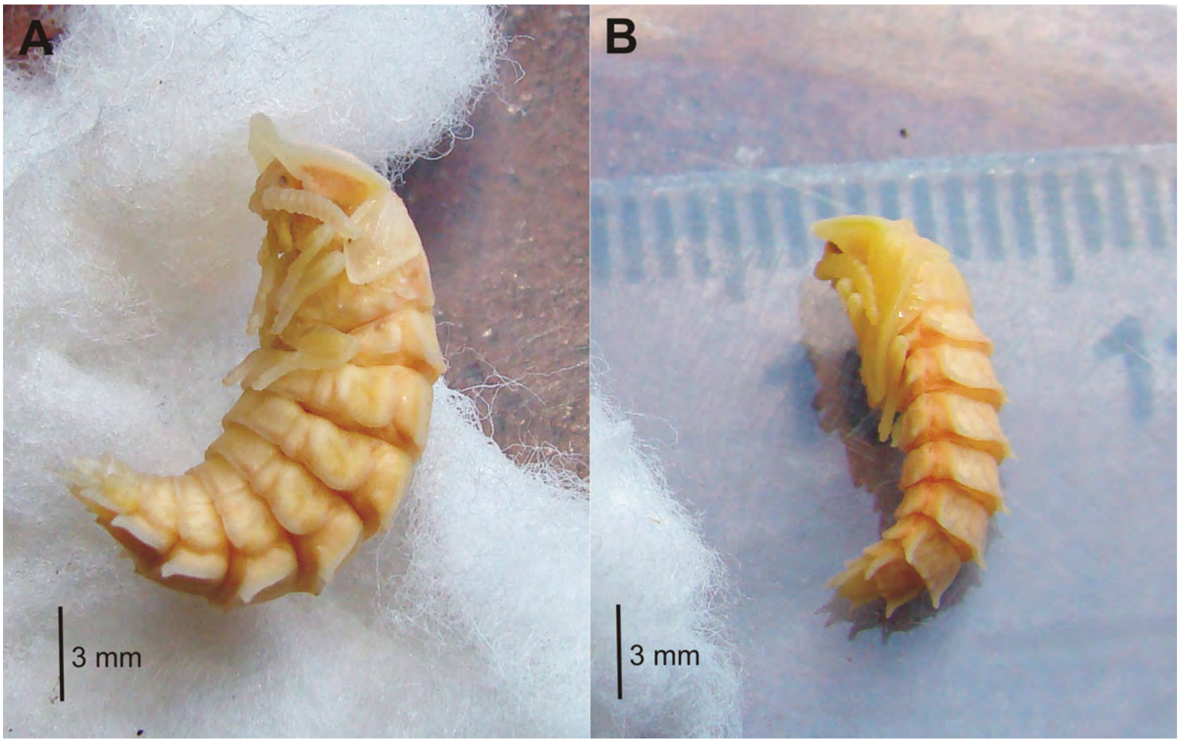

Figure 6. *Alecton discoidalis* pupae. **A**: female. **B**: male.

small, partially visible in dorsal view, with conspicuous acute projections on each posterolateral corners. Eight pair of spiracles on abdominal segments I- VIII.

Conclusion

Larval instar number is variable within Lampyridae and can also vary within individual species. Bushman (1977) found that *Pyractomena lucifera* underwent between 4-9 instars under different photoperiod regimes. Higher instar numbers appear to correlate with shorter photoperiods, perhaps as an adaptation for persisting in the larval stage through the winter months. Archangelsky and Branham (1998) reported that both males and females of *Pyractomena borealis* underwent seven preimaginal instars (egg + 5 larval instars + pupa) under a fixed photoperiod. *Luciola substriata* (Fu *et al.* 2005) and *Luciola leii* (Fu *et al.* 2006) underwent 6 larval instars, presumably both sexes, and a constant photoperiod of 12L: 12D was recorded for *L. leii*. Our research concluded that male *Alecton discoidalis* underwent between 4 and 5 larval instars, while females underwent at least an additional instar and in some cases more than one. Due to the high mortality of larva at first instars, sample size at the end of this period was very small. Presumably, there is missed information on life cycle of this species, but at least we can approach to it.

The pattern of females undergoing an additional larval instar when compared to males of the same species was also found in *Lampyris noctiluca* (Naisse 1966) and *Pyractomena lucifera* (Buschman 1977). It is interesting to note that *L. noctiluca* females are brachypterous, but *Pyractomena* females are alate, so these differences in female morphology did not appear to yield differences in instar number. While the females of both *L. noctiluca* and *A. discoidalis* are brachypterous, *A. discoidalis* females are also physogastric and considerably larger than their males. Hence, additional preimaginal instars in the females of this species may likely be necessary for females to attain this larger size, which presumably corresponds to increased egg production.

Particularly the firefly fauna of Cuba has been very poorly studied. Most of described species remains without other data than their taxonomic status. More ecological studies are needed to have a better idea of their role in ecosystems or even their conservation status. Nevertheless, those studies must be sustained on reliable species recognition and association between immature and adult stages. Taxonomic and morphologic studies are still needed for Cuban fireflies.

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