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Morphology of immature stages and mating behavior in *Liogenys fusca* (Blanchard) (Coleoptera, Melolonthidae, Melolonthinae)

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**A B S T R A C T**

*L. fusca* is a rizophagous insect pest in various crops of economic importance in Brazil. Here we investigated the morphology of immature stages and mating behavior of this species. The redescriptions of the 3rd instar larvae of *L. fusca* in this work allows identification and registration of occurrence independently of adults, which occur sporadically in a certain period of the year. Male and female of *L. fusca* remained confined in the soil during the day and exited between 19:00 and 23:30 h. The populations occurred between 19:30 and 21:00 h, and were characterized by a typical behavioral sequence. Copulation durations in *L. fusca* lasted on average 512.23 s. Adults were observed feeding before the copulations on leaves and inflorescences of plant species belonging to the family Anacardiaceae, *Myracrodruon urundeuva*, *Schnius terebinthifolius*, *Astronium fraxinifolium* and *Aphananthe occidentale*.

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**Introduction**

Beetles of the genus *Liogenys* Guérin-Méneville, 1831 (Coleoptera, Melolonthidae) comprise an important group of rizophagous species of the subfamily Melolonthinae with 79 species already described (Evans and Smith, 2009). In Brazil, 28 *Liogenys* species are known (Evans and Smith, 2009) and most feed on various crops of economic importance, and, thus, are considered pests in many Brazilian states. *Liogenys fusca* Blanchard, 1850, has been reported in corn *Zea mays* L. (Poaceae) (Santos et al., 2008), soybean *Glycine max* (L.) (Fabaceae), sunflower *Helianthus annuus* L. (Asteraceae) (Rodrigues et al., 2011); sugarcane *Saccharum officinarum* L. (Poaceae) (Coutinho et al., 2011); as well as in winter grains (Poaceae) (Cherman et al., 2011). The geographic distribution of *Liogenys* in Brazil covers the states of Alagoas, Bahia, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná, Piauí, Rio Grande do Norte, Rio Grande do Sul, Rio de Janeiro and São Paulo (Moser, 1918, 1919; Frey, 1969; Morón, 2004; Rodrigues et al., 2008; Costa et al., 2009; Cherman et al., 2011).

*Liogenys suturalis* Blanchard, 1851 has been recorded in Mato Grosso do Sul state on *Z. mays* and *Triticum aestivum* L. (Poaceae), and *Avena byzantina* C. Koch (Poaceae) (Santos and Ávila, 2009). *Liogenys bidenticeps* Moser, 1919 was reported in *Z. mays*, *G. max* in Mato Grosso do Sul (Rodrigues et al., 2011), and in winter grains in Rio Grande do Sul state, as well as *L. obesa* Burmeister, 1855 and *L. sinuaticeps* Moser, 1918 (Cherman et al., 2011).

For all species, there are scarce reports about their biocology and behavior. There are only rarer descriptions of the immature stages for *L. fusca* Blanchard, 1850 (Cherman et al., 2011; Coutinho et al., 2011), *L. bidenticeps* Moser, 1919, *L. sinuaticeps* Moser, 1918 and *L. obesa* Burmeister, 1855 (Cherman et al., 2011).

*Liogenys fusca* is a univoltine species with life cycle between 255–348 days, and adults mate on the onset of the rainy season between August and December in the southern-central region of Brazil (Rodrigues et al., 2008). The purpose of this study was to investigate the morphology of immature stages and mating behavior of *L. fusca*.

**Material and methods**

Adults of *L. fusca* were collected with a light trap between October and December 2011, at the experimental farm of the Universidade Estadual de Mato Grosso do Sul (UEMS) in the municipality of Aquidauana, Mato Grosso do Sul state (Lat 20°28’16” and Long 55°47’14”), Brazil. Couples were formed and kept in plastic containers of 4000 mL, containing soil and seedlings of *Brachiaria decumbens* Stapf cv. Basilisk (Poaceae). The trays were covered

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with voile fabric to prevent the insects from escaping. The containers were checked visually every day for location and collection of eggs and removal of dead insects. The eggs were transferred to Petri dishes containing lightly moistened sifted soil, and were kept in a temperature-controlled chamber in the laboratory (26 ± 1°C, 60 ± 10% RH, and scotophase). The Petri dishes were observed on a daily basis and after eclosion, larvae were transferred and individualized in plastic containers of 500 mL containing soil and seedlings of B. decumbens (26 ± 1°C and 12 h photophase) (Rodrigues et al., 2011).

Ten larvae of the 3rd instar were killed in boiling water and preserved in alcohol 70% in May 2012, and were later described using the nomenclature proposed by Costa et al. (1988), Morón and Salvadori (2006), and Neita-Moreno et al. (2012). The observations and drawings of the morphological aspects were carried out in stereomicroscope SV-06 Zeiss, with a camera lucida attached. The mouth parts were mounted on slides containing Hoyer liquid. We observed the preparations using a Nikon E200 microscope, with a camera lucida attached. Adults and immature stages of L. fusca were deposited in the Entomological Collection of the Universidade Estadual de Mato Grosso do Sul, in Aquidauana – MS, Brazil, and Entomological Colección, Institute of Ecology (IEXA), Xalapa, Veracruz, Mexico.

Adults of L. fusca were obtained initially in a light trap in September, October and November 2012, at the experimental farm of UEMS in Aquidauana, MS, Brazil. Females and males were selected on a daily basis through the observation of tarsomeres on the first pair of legs, which are wider in males (Rodrigues et al., 2008). Subsequently, the insects were individualized and kept in containers of 1000 mL (9 cm high × 15 cm diameter), closed with voile fabric, containing 500 mL of lightly moistened sandy soil.

Individuals emerged to the soil surface from 18:00 h the next day. Fifty couples of insects were formed and arranged in a new container of 4000 mL, containing soil and seedlings of B. decumbens and sealed with voile fabric. Observations on mating behavior follow Facundo et al. (1999) for the oriental beetle Exomala orientalis (Waterhouse, 1875). Mating behavior was recorded using a Sony® model DCR-SX21 STD camera.

During the flight of adults in September, October and November 2012, field observations were carried out at the farm of the Universidade Estadual de Mato Grosso do Sul, Aquidauana, Brazil, from 19:00 h during the night, to monitor the activity of adults of L. fusca. To visualize and record the behavior of males and females, we used a camera Sony®, model DCR-SX21 STD.

Results

Description of immature

Liogenys fusca Blanchard, 1850, 3rd larval instar (Figs. 1 and 2). Average length of 36.1 mm (range 31.0–39.0 mm). Light gray color; as it achieves the pre-pupa phase, white color with short hair covering the body.

Head. Hypognathous, sclerotized and lightly reticulate (Fig. 2A). Average width of cephalic capsule of 3.7 mm (3.4–4.0 mm). Visible coronal and frontal suture. Frontal suture slightly sinuous in its anterior third and reaching the base of the antennal insertion. Stemmata absent. Epicranium with 3–4 short setae on each side of the epicranial suture, and with 11 setae near each antennal insertion. Frontal region with 2 frontal outer setae, 4 posterior frontal setae on each side, and 6 anterior frontal setae. Anterior angle with a seta on each side. Post-clypeus with 2 external clypeal setae on each side, and 2 anterior central setae. Length of labrum similar to the clypeus, but wider and with sinuous anterior margin. Labrum surface with 9 setae, 2 posterior labral setae on the right side, 1 on the left side and 3 posterior lateral setae on each side of medium size.

Epipharynx (Fig. 2B). Right acroparia with 8 setae; left acroparia with 7 setae; corypha with 14 setae; haptomerum with zygum, epizigum and a row of 7 spine-like setae, helus absent. Right acroparia with 10 setae and left with 12 setae; gymnoparia present; chaetoparia well-developed, with more robust setae from the edges to the center, with 73 setae on the right side and 84 setae on the left side; plegmatia present; pedium longer than wider; laetotorma shorter than deixiotorama; phoba present on the right side; nesium present.

Antennae joined with epicranium through a cylindrical projection (Fig. 2C). Antennomere I short; II long with a dorsal seta; III with a small ventrodistal projection; IV with a large dorsal sensory area and two ventral sensory areas.

Mandibles strongly sclerotized with black and yellowish areas on medium portions and asymmetrical shape. Right mandible with 2 incisor teeth (S1–2) (Fig. 2G). Scrobes with 11 setae. Molar with 11 dorsal setae. Dorsal surface with a short seta and a long seta near the base of S3. Brustia with about 40 setae. Concave preartis. Ventral surface without defined estridulatory area. Postartis and ventral process present. Five basolateral setae. Molar area with three lobes.

Left mandible with black color and yellowish central areas (Fig. 2F), and with 3 incisor teeth. Scrobes with 10 setae. Molar dorsal area with 8 setae. Dorsal surface with a short seta and a long seta near the base of S3. Brustia with about 38 setae. Ventral surface without defined estridulatory area. Nine basolateral setae. Postartis and ventral process present. Well-developed molar lobe.

Maxillae with galea and lacinia separated by visible suture (Fig. 2D). Galea with apical uncus and lacinia with 4 apical unci and several thick, long dorsal and ventral setae. Stipes with numerous dorsal setae on the dorsal region, maxillary striulatory area with a row of 12–13 acute denticles. Four segmented maxillary palps.

Hyopharynx with asymmetrical sclerome and with the right process developed (Fig. 2D). Posterior right margin with 21 long,
thin setae, posterior left margin with 30 long, thin setae and 2 short, thin setae. Central lobe of glossa with 51 short, thick setae and 2 short, thin setae. Lateral right lobe with 4 setae. Lateral left lobe with 6 short, thick setae. Labial palp with palpmere II narrower and shorter than I, with apical small setae.

Thorax. Pronotum with a dorsal lobe, meso- and metanotum with 3 dorsal lobes (Fig. 1). Prothoracic spiracle 0.30 mm long and 0.25 mm wide (Fig. 2H). Respiratory plate with about 12 holes in the middle region. Irregular spiracles. Anterior and middle legs are slightly smaller than the posterior legs. Coxa, trochanter, femur, tibia and tarsus with stout setae. Each tarsus with an apical and a medial setae.

Abdomen. Abdominal spiracle I 0.25 mm × 0.18 mm, II 0.30 mm × 0.20 mm, III 0.28 mm × 0.20 mm, IV 0.25 mm × 0.25 mm, V 0.30 mm × 0.25 mm, VI 0.30 mm × 0.20 mm, VII 0.20 mm × 0.15 mm, and VIII 0.20 mm × 0.15 mm long by wide. Raster with palidalis bearing 2–4 large spine-like setae and 2–4 small spine-like on each side (Fig. 2E). Lower anal lip with short setae and thin and long setae. Area around the spiracles with several setae of different sizes.

Pupa (Fig. 3). Pupa color ochre. Well-defined eyes, inner margin with a groove. Antennae, maxillae and pedipalps easily visible. Length 15.24 mm (range 14.3–16.7 mm) and width 7.44 mm. Flat head. Clypeal posterior margin proeminent. Pronotum with small longitudinal posterior groove. Mesonotum with sinuous posterior margin. Abdomen with eight pairs of spiracles visible dorsally. Suture of ec dysis visible dorsally on thorax. Narrow elytra, curved ventrally around the body and with visible grooves. Tergites I–VIII convex. Spiracle I elongated and prominent, not covered by the wings, spiracles II–IV circular and prominent with visible peritremes, spiracles V–VIII oval and slightly prominent. Abdominal segment IX with urogomphi.

Mating behavior

During the day, male and female of L. fusca are maintained in underground burrows. In the evening they exposes their antennae surface, with the antennal lamellae opened and in movements in different directions for 64.9 ± 4.75 (10–170) min (n = 100). Beetles leave the soil between 19:00 and 23:30 h. For a mean of 4.2 ± 0.13 (2–6) h (n = 100), adults alternated burrow activity and exit of the soil, walking on surface, with short flights and many mating events.

On several occasions, females remained motionless, and we observed a rhythmical motion of the tarsomers of the posterior legs, which were kept suspended in the air and in movement toward the abdomen. This behavior had variable duration and was interrupted when another insect approached or walked around the female. The movement of tarsomers had an average duration of 30.7 ± 3.60 (11–60) s (n = 15). Immediately after, they rub the femur and tibia in the abdomen, with an average duration of 22.1 ± 5.56 (5–95) min
(n = 15). Of the 50 couples formed in the laboratory, 13 of them copulated, 10 showed attempts of copulation, and the other 27 did not copulate.

Copulations were characterized by a typical behavioral sequence for *L. fusca* (Fig. 4). After finding the female, the male touched with its anterior legs the elytra or pygidium, and if accepted, it climbed on the female and held it with all legs. At this point, the female could walk a little, moving laterally, and then remained motionless. During this period, the male moved the antennae slowly, keeping the lamellae scoures open. In this position, the couple remained on average 59.0 ± 7.56 (38–120) s (n = 13), when the male positioned its body backward, exposed the aedeagus and initiated copulation. A little before or right at the beginning of copulation, the female could, eventually, perform lateral movements with the body to the left and to the right, also moving the male. During copulation, male and female usually remained motionless, except for occasional rub movements of posterior legs of the female on its own abdomen. Copulation durations in *L. fusca* ranging 126–960 s (n = 13), and lasted on average 512.23 ± 60 s.

For the closure of copulation, the male slowly removed the aedeagus, during an average time of 66.5 ± 4.86 (40–95) s (n = 13). During this process, the male moved away from the female slowly, formerly releasing the anterior legs, and then and first pairs of legs, respectively. After the male became detached completely from the female, the aedeagus remained exposed and was slowly retracted. At least male continued walking around the female, climbing on and down her, and this behavior lasted on average 127.6 ± 9.87 (60–180) s (n = 13).

On various occasions, frustrated copulation attempts were observed (n = 10). In these cases, males tried to climb on females, which moved away quickly in an attempt to detach. If they failed, females fell sideways on their body and moved their legs removing the males. Detachment from an unwanted male took on average 358.1 ± 52.98 (120–600) s (n = 10). On these occasions, the male could extend the aedeagus (n = 3) or remain with the aedeagus retracted (n = 7).

On containers with more than a couple, it was regular that females or males approaching and climbing on the couple in copula, and this resulted in some unsuccessful of copulations. In this sense, on a number of occasions, a crowd between males and females was observed. In the field, *L. fusca* copulation occurred between the 19:30 and 21:00 h. Males and females were observed feeding before the copulations on leaves of mastic trees (*Anacardium*), *Myracte- dron urundeuva* Allem. (n = 20); *Aroeirinha, Schinus terebinthifolius* Raddi (n = 15); *Concao do Campo, Astronium fraxinifolium* Schott (n = 25); and inflorescences of cashew, *Anacardium occidentale* L. (n = 27). The four plant species measure 4–8 m tall, and except for *A. occidentale*, all other species are of common occurrence in the Brazilian Cerrado and Pantanal (Pott and Pott, 1994).

In all plants, couples in copulation were observed after feeding, especially in basal and middle part of the plants. In *A. occidentale*, after adults landed on the branches or leaves, they walked toward the inflorescences to feed, and subsequently performed the copulations (n = 12). In other plant species, after landing on the branches or leaves, they began feeding on leaves and then performed one or more copulations (n = 9). After copulation, male and female parted and walked on the leaves, branches or flowers respectively, or flew off (n = 21).
Discussion

Larvae of *L. fusca* feed on roots of various plant species, including crops of agricultural importance, and this description allows their identification and registration of occurrence. This may be useful considering that adults occur sporadically in a certain period of the year. September to December, in the southern central region of Brazil, and generally emerge and remain inactive during the day, fly at night and are found far from the areas where they emerged (Rodrigues et al., 2008; Costa et al., 2009). The features presented by *L. fusca* are very similar to those of other species of the subfamily Melolonthinae. In accordance to Morón (2004), these larvae are characterized by the absence or significant reduction of stridulatory area of the maxillae, asymmetrical labrum, clithra absent, estridulatory maxilla area without previous process, lacinia with three unci, dorset of the last antennomere with a sensory stai, anal bottom labrum with a groove or sagittal cleft, raster usually with pali arranged in rows and falsiform tarsungulus with two arrows.

About the behavior of *L. fusca*, males, when near or on top of females, kept moving their antennae, probably detecting volatile chemical compounds released by females. In other species of Melolonthidae, males have a number of sensilla on the antennae, which are directly related with the detection of the sexual pheromone released by the female, as for example, in *Phyllophaga obsoleta* (Blanchard, 1850) (Romero-López et al., 2004), and *P. anxia* (LeConte, 1850) (Ochieng et al., 2002).

In this study, we observed that females of *L. fusca* rub the posterior legs on abdomen, suggesting a calling behavior to males. A similar behavior was observed in *P. capillata* (Blanchard, 1850) in which the females raised the posterior legs to release a sex pheromone (Oliveira, 2007). Females of *P. cuyabana* (Moser, 1918), besides raising their posterior legs, expose the terminal segments of the abdomen that contains a yellow glandular region, which is possibly associated with sexual pheromone release (Oliveira and García, 2003). According to these authors, copulation in this species lasts an average of 83.9 min. Females of *L. suturalis* possibly also release a sex pheromone to attract males, and copulations, when they occur, last on average 9.8 min (Santos and Ávila, 2009).

Females of *Holtificria paralella* (Motschulsky, 1854), attach themselves to leaves or branches of host plants as they leave the soil and expose an abdominal gland, indicating the call behavior (Leal et al., 1993).

Adults of *L. fusca* were found feeding and mating in plants of Anacardiaceae, suggesting that these plants have an important role in the reproduction of this species. Plants of mastic *M. urundeuva, S. terebinthifolius* and *A. fraxinifolium*, are native to the site where this study was carried out and, thus, represent an important natural source of food and reproduction of adults of *L. fusca*, whereas cashew plants, *A. occidentale*, were introduced and, therefore, represent an alternative food source.

The presence of a feeding and mating site appears to be a common feature for adults during their reproductive stage in many species of Melolonthidae. Males and females of *Hylomorpha elegans* (Burmeister, 1844) fly and find themselves on leaves of *Nothofaga obliqua* (Mirb.) Oerst. (Nothofagaceae), where they feed and mate (Quiroz et al., 2007). The same occurs for *P. capillata*, on leaves of *Euphorbia heterophylla* L. (Euphorbiaceae) and *Sonchus oleraceus* L. (Asteraceae) (Oliveira, 2007). However, *Meadrodactylus lineatus* Chevrolat, 1834 and *M. mexicanus* Burmeister, 1855 (Scarabaeidae), adults feed and mate on flowers of *Hibiscus rosa sinensis* L. (Malvaceae) (Morón, 1996).

Males of *L. fusca*, throughout the mating period, remained stuck on the female and detached itself only at the end of this process. However, males of some species of Melolonthidae such as *P. capillata* and *Dasypedia ishigakienis* (Nijimma & Kinoshita, 1927), after finding the female, the male climbs on its back, introduces the aedeagus and lifts its legs, getting suspended in the air, attached only by the genital organ (Arakaki et al., 2004; Oliveira, 2007). At the end of copulation, the male of *L. fusca* remains near the female for 127.6 ± 9.87 s, suggesting a guard behavior, as previously stated by Rodrigues et al. (2008).

On several occasions throughout the study with *L. fusca*, clusters of males and females are formed on the couples copulation. This same pattern of behavior has also been reported for other species such as *Examola orientalis*, as said by Facundo et al. (1999), and *Phyllophaga cuyabana* (Moser, 1918), according to García et al. (2003).

Conflicts of interest

The authors declare no conflicts of interest.

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