Mating behavior and description of immature stages of *Cyclocephala melanocephala* (Fabricius, 1775) (Coleoptera: Scarabaeidae: Dynastinae), identification key and remarks on known immatures of Cyclocephalini species

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

Mating behavior and description of immature stages of *Cyclocephala melanocephala* (Fabricius, 1775) (Coleoptera: Scarabaeidae: Dynastinae), identification key and remarks on known immatures of Cyclocephalini species. Some species of *Cyclocephala* Dejean, 1821 are regarded as rhizophagous crop pests and others as beneficial species. The objective of this work was to report the mating behavior and to describe the immature stages of *C. melanocephala*. The studies were developed at the Universidade Estadual de Mato Grosso do Sul in Cassiânão, Mato Grosso do Sul state, Brazil. Adults were collected with a light trap from September to December 2014 and 2015 to carry out studies of mating behavior, breeding, and descriptions of immature stages. Copulation lasted 10.4 ± 4.3 min and took place from 19:00 to 24:00 h. Some females refused males for mating and moved away from them. Regarding flight period, adults were collected in larger quantities from 20:00 to 23:00 h. Identification keys to immatures of three genera of Cyclocephalini, including several *Cyclocephala* species are presented.

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In the Cyclocephalini tribe (Coleoptera, Scarabaeidae, Dynastinae) about 500 described species are known and of these, more than 85% are represented by *Cyclocephala* Dejean, 1821 [Ratcliffe et al., 2013]. *Cyclocephala* occur from southeastern Canada to Argentina and the Antilles [Ratcliffe, 2003]. In Brazil 83 species are recorded [Morón, 2004].

Adults feed on plants and flowers, thus contributing to pollination. Some examples of the benefits to plants are described to some Araceae. Maia et al. (2013) in a studies conducted in Atlantic Forest of Pernambuco State, found that flowers of *Taccarum ulei* Engl. & K. Krause (Araceae) are pollinated exclusively by *C. earae* Höhne, 1923 and *C. ulata* Dechambre, 1980. In flowers of *Caladium bicolor* (Aiton) Vent. (Araceae) observed in Atlantic Rainforest of Pernambuco, Maia and Schlindwein (2006) found adults of *C. ulata*, an important pollinator, feeding and mating. Cyclocephala pollinators are also important to some Annonaceae. In Cerrado of Goiás State, Cavalcante et al. (2009) found that *C. atricapilla* Mannerheim, 1829,

* C. laterrica Höhne, 1923 and *C. octopunctata* Burmeister, 1847 are floral visitors and pollinators of *Annona crassiflora* Mart. Moreover, Costa et al. (2017) conducted studies in the Cerrado of Mato Grosso State and found that *C. atricapilla* is the main pollinator of *A. coriacea* Mart., while *C. octopunctata*, *C. ohausiana* Höhne, 1923 and *C. undata* (Olivier, 1789) are secondary pollinators. In addition to the *Annona* L. species cited above, Gottsberger (1989) noted *C. atricapilla* also as a pollinator of *A. dioica* A. St.-Hil. and *A. monticola* Mart., and *C. quatuordecimpunctata* Mannerheim, 1829 as a pollinator of *A. cornifoila* A. St.-Hil. and *A. tomentosa* R. E. Fr.

Species of Cyclocephala are also found in other plant families, like the example of Dieringer et al. (1998, 1999) who found adults of *C. caelestis* Delgado & Ratcliffe, 1990 feeding and pollinating flowers of *Magnolia tamaulipana* Vázquez (Magnoliaceae) in Mexico. In Mato Grosso do Sul State, Brazil, adults of *C. forsteri* Endrödi, 1963 were found feeding on inflorescences of *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. (Areaceae) (Oliveira and Ávila, 2011). In a recent study, Moore and Jameson (2013) related 80 species of Cyclocephala associated with flowers of various species.

The immature stages of *Cyclocephala* species remain in the soil and some species feed on organic matter, as *C. flavipennis* Arrow,
1914 observed in Rio Grande do Sul State by Salvadori and Pereira (2006) and C. paraguayensis Arrow, 1913 observed in the Pernambuco by Albuquerque et al. (2014).

However, in some species, larvae can feed on roots of crop plants and cause damage. Larvae of C. rumulata Burmeister, 1847 and C. fulgarata Burmeister, 1847 appear associated with onion crops (Allium fistulosum L., Amaryllidaceae) and grasses (Pennisetum clandestinum Hochst. & Chiov., Poaceae), observations made by Villegas et al. (2006) in Colombia. When larvae, Cyclocephala parallelula Casey, 1915 is considered an important pest of sugarcane (Saccharum officinarum L., Poaceae) in Florida, United States (Gordon and Anderson, 1981; Cherry, 1985), as well as C. rumulata in Mexico (Aragón-García and Morón, 2000). Also, C. forsteri and C. verticalis Burmeister, 1847 were reported damaging sugarcane crops in Mato Grosso do Sul (Coutinho et al., 2011). Cherman et al. (2014) reported C. modesta Burmeister, 1847, C. putrida Burmeister, 1847 and C. tucuma Brêthes, 1904 associated to the root system of several winter crops in Rio Grande do Sul. Santos and Ávila (2007) reported the occurrence of larvae of C. forsteri feeding on roots of soybean (Glycine max (L.) Merr, Fabaceae) in Mato Grosso do Sul. Depending on the circumstances, even species that are not usually considered economically important can damage some cultures, as C. flavipennis Arrow, 1914 (Salvadori and Pereira, 2006; Duchini et al., 2017).

Cyclocephala melanocephala (Fabricius, 1775) occurs throughout most of the New World, as United States (Ratcliffe, 1992; Bauernfeind, 2001), Mexico (Ratcliffe, 1992) and Brazil (Camargo and Amabile, 2001; Nogueira et al., 2013; Taira et al., 2014). Adults are typically found feeding on inflorescence of sunflower plants (Helianthus annuus L., Asteraceae) (Camargo and Amabile, 2001). Taira et al. (2014) found in Mato Grosso do Sul, adults of this species causing damage to shoots of young plants of rubber trees (Hevea brasiliensis (Willd. ex A. Juss.) Müll. Arg., Euphorbiaceae). Regarding the biology of C. melanocephala, Nogueira et al. (2013) observed that the egg–adult cycle was completed in 113 days on average and more than one generation is possibly formed per year.

Despite the species richness of Cyclocephala, immature descriptions are scarce (Morón et al., 2014). Thus, studies were conducted to verify the mating behavior and descriptions of immature of C. melanocephala.

Material and methods

Mating behavior of adults

Studies on mating behavior were conducted on the experimental farm of Universidade Estadual do Mato Grosso do Sul (UEMS) in Cassilândia, Mato Grosso do Sul State (MS). To collect adults, a light trap (model “Luiz de Queiroz”, Silveira Neto and Silveira, 1969) was installed daily alongside the pasture area (Urochloa decumbens Stapf cv. Basílik, Poaceae) from October to December 2014.

Adults collected were taken to the entomology laboratory of UEMS, separated by sex (first pair of legs in males are dilated) (Figs. 1–4) and separated individually in 1000 mL plastic containers with half of its volume filled with soil from pasture area (500 mL). The containers were closed on top with voile fabric. At night fall, the containers were monitored to observe the flight moment.
After that, twenty-three couples were sorted from the adults that emerged from the soil and flew off. Each male and female was gathered in couples and put in 500 mL containers for mating behavior observations. The observation room was kept dark according to the methodology from Facundo et al. (1999). To visualize and record the behavior of males and females, a Sony® camcorder model DCR-SX21 STD was used.

To study adult flight hours in the field, a light trap was installed from 18:00 h until 06:00 h of the next day, from October 30 to November 2, 2014. At 60-min intervals, the trap was inspected, and insects captured were collected. The flight schedule data were transformed into $\sqrt{x + T}$ and submitted to the analysis of variance (ANOVA). The means were grouped and compared by the Scott-Knott test ($p<0.05$) using SISVAR software (Ferreira, 2011). Data on average temperature (°C), precipitation (mm) and solar radiation ($\text{kJ/m}^2$) in Cassiãndia, were obtained from the Instituto National de Meteorologia (INMET).

Description of immatures

The described larvae were obtained from adults reared in the laboratory. From September to December 2015, adults of C. melanocephala were collected at the experimental farm of the UEMS, with light trap. The adults were carried to the entomology laboratory and formed couples, which remained in 1000 mL plastic containers containing soil and Brachiaria decumbens Stapf (Poaceae) seedlings, and the containers were covered with voil fabric.

The vessels were inspected every day to find eggs and to remove the dead insects. The eggs were kept in Petri dishes, containing sieved and moistened soil, and kept in an air-conditioned room in the laboratory (26 ± 2 °C and scotophase). The Petri dishes were observed at intervals of two days, and the hatched larvae were transferred and individualized in 500 mL plastic containers containing soil and B. decumbens seedlings (26 ± 2 °C and 12 h photophase) (Rodrigues et al., 2014).

From May to August 2016, third instar larvae and pupae were killed in boiling water and preserved in 70% alcohol. The observations and drawings of the morphological aspects of the larva and pupa were carried out in Stereomicroscope Motic, Zeiss Stemi SV 6 stereomicroscope or Zeiss Axioscop microscope, both with light camera coupled. The detached structures of the larval body (e.g. mouth parts and legs) were slide mounted in Hoyers’ (Johnson and Triplehorn, 2005). The adults of C. melanocephala were deposited in the UEMS entomology collection in Cassiãndia; the immature was deposited in the collection of the Museu de Zoolo gia da Universidade de São Paulo, São Paulo (MZSP). Adobe Photoshop CS6 software was used for image processing and drawing of the plates.

The terminology used follows Böving (1936) and Lawrence (1991) with some modifications by Sousa et al. (2018). The terms helus (tooth or fixed and rigid cuticular process) and phoba (a group of flexible fixed cuticular processes) were used for both epi- and hypopharynx. The epipharynx area subdivisions (corypha, hap- tomerus, paria, pedium and haptolachus) are in italic to make it easier to find. Head chaetotaxy follows Ritcher (1966) and Sawada (1991) as summarized by Sousa et al. (2018). Mandibles incisor usually have 3 defined teeth (S1, S2, S3; distal to proximal), and S2–S3 separation is noted by the incisor notch (Figs. 17, 22). Even when S2 is reduced, the notch is easy to find and defines the S2 and S3 area. Besides these three teeth, a proximal most incisor tooth may occur (S4) between S3 and molar (on mandible inner concave margin).

Hair-like setae are termed as (modification of Šípek et al., 2008): minute, when its length is at most three times longer than the diameter of associated puncture (barely distinct under magnification less than 40×); short or long when its length is at least four times longer than the diameter of associated puncture (easily visible under magnification of 20×). Long and short setae are only differentiated when conspicuous relative differences occur (e.g. rastet setae, Figs. 43, 44), otherwise (i.e. wide setal length variation) the opposition minute/long formerly defined is used (e.g. cranial setae, Fig. 7). The larvae size could affect the visibility of minute and long setae, but differences in relative size and spatial distribution help to separate both setae group.

Head chaetotaxy was widely used as diagnosis in scarab immaturity works, while thoracic and abdominal chaetotaxy (except from rastet) were not or partially described (e.g. Jameson and Morón, 2001 described the dorsal lobes setation). To explore the body setae as an identification tool, the thoracic and abdominal setation were described and illustrated (Fig. 14). This uncommon approach adds new data and encourage future works to investigate the entire body chaetotaxy. Chaetotaxy were given to each body lobe. Terms like scutum, scutellum, pretorium, sternum, and derived terms are avoided because the homology between larval lobes and adult sclerites are doubtful, and the ventral body surface has sternal and pleural elements in Coleoptera (Kobayashi et al., 2013). The resulting lobes terminology is as follows (Fig. 14): thorax dor- sum: anterior, medial, and posterior tergal lobes; thorax lateral: anterior and posterior pleural lobes; thorax venter: anteromedial and posterior ventral lobes; abdomen dorsum: anterior, medial, lateral and posterior tergal lobes; abdomen lateral: anterior and posterior tergal lobes, and spiracle lobe; abdomen venter: anterior, medial, and posterior lobes. When this lobe division is indistinct (e.g. Scarabaeoidea prontotum have 1–3 lobes and abdomen segment IX usually has an undivided dorsum and a simple pleural lobe, Ritcher, 1986), a general position name is given to the region if necessary. The chaetotaxy to prothoracic lateral sclerite is also given and the lobes names are not applied to abdominal segment X, because its already has a particular terminology (raster and anal lobes).

The proposed identification keys and comparative table (Table 1) included herein use new data and information available in the bibliography (Albuquerque et al., 2014; Bran et al., 2006; García et al., 2009; Johnson, 1941; King, 1984; Morelli, 1991; Morelli and Alzugaray, 1994; Morón et al., 2014; Neita-Moreno and Yepes, 2011; Pereira and Salvadori, 2006; Remedi-de-Gavotto, 1964; Ritcher, 1966; Souza et al., 2014a,b; Vinciini et al., 2000).

Results

Cyclocephala melanocephala (Fabricius, 1775)

Third instar larva (Figs. 5–44). Body (Fig. 5) length: 19–26 mm; grayish or yellowish white, head and respiratory plates yellowish brown; surface densely setose, setae yellowish brown to brown. Head (Figs. 6, 7, 13) width: 2.8–3 mm; epicranial and epistomal sutures distinct; stemmata very small; antenmere some-what cylindrical and with 3 punctures: cranium, Clypeus and labrum (Figs. 7, 8) with many homogeneously distributed punctures, except in labral anterior area. Each half of cranium and clypeus with (Fig. 7): 4–3 long and 2–3 min dorsoepicranial setae (des) in a row and 1 min seta internally positioned, 2 long and numerous minute posteroepicranial setae (pes), 2–3 long anteroepi- cranal setae (aes), 5–8 long exeroepicranial setae (ees), 2–3 long and 1 min posterofrontal setae (pfs), 1 long externofrontal setae (efs), 2–3 long anterofrontal angle setae (aas), 2 long anterofrontal setae (afs), 2 long externoclypeal setae (ecs), 1 long anteroclypeal seta (acs). Labrum (Fig. 8): each half with 2–3 long and 1 min posterolabral setae (plfs), 1 long mediolabral seta (mls), 4–5 long laterolabral setae (ils), 2 long anterolabral setae (als). Antenna with 4 antennomeres (Figs. 9–12): 1 short (length/larger dorsal
Table 1  
Chaetotaxy of the known third instars of Cyclocephala.

<table>
<thead>
<tr>
<th>Species</th>
<th>Parietals</th>
<th>Frons</th>
<th>Clypeus</th>
<th>Labrum</th>
<th>Raster</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>des pes</td>
<td>aes</td>
<td>ees</td>
<td>pfs</td>
<td>efs</td>
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<tr>
<td>C. barrera</td>
<td>2–3 3</td>
<td>–</td>
<td>–</td>
<td>3–4</td>
<td>2</td>
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<tr>
<td>C. borealis</td>
<td>2–3 –</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>1</td>
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<tr>
<td>C. celata</td>
<td>4 –</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C. comata</td>
<td>3–4 2–3</td>
<td>–</td>
<td>–</td>
<td>2–3</td>
<td>0</td>
</tr>
<tr>
<td>C. disticta</td>
<td>3–4 5–6</td>
<td>–</td>
<td>–</td>
<td>4–5</td>
<td>1</td>
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<td>C. fasciolata</td>
<td>3 2</td>
<td>2–3</td>
<td>–</td>
<td>1–2</td>
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<tr>
<td>C. flavipennis</td>
<td>–</td>
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<tr>
<td>C. fulgurata</td>
<td>3–4 5–6</td>
<td>2–3</td>
<td>–</td>
<td>~3</td>
<td>1</td>
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<tr>
<td>C. gregaria</td>
<td>1 1</td>
<td>2–3</td>
<td>–3</td>
<td>2</td>
<td>1</td>
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<tr>
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<td>3 2</td>
<td>2–3</td>
<td>–</td>
<td>1</td>
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</tr>
<tr>
<td>C. longula (C. abrupta)</td>
<td>7–8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>C. lumulata</td>
<td>2–4 5–6</td>
<td>–</td>
<td>–</td>
<td>2–3</td>
<td>5</td>
</tr>
<tr>
<td>C. lurida (C. immaculata)</td>
<td>3 3</td>
<td>–2</td>
<td>–</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>C. melangeopaca</td>
<td>3–4 2</td>
<td>2–3</td>
<td>5</td>
<td>8</td>
<td>2–3</td>
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<tr>
<td>C. modesta</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>C. paraguayensis</td>
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<td>C. pasadenae</td>
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<td>–</td>
<td>2–4</td>
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<td>–</td>
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<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>C. signaticolius</td>
<td>3 2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3</td>
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<tr>
<td>C. sinalae</td>
<td>3–4 3</td>
<td>–</td>
<td>~3</td>
<td>~4–5</td>
<td>2–3</td>
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<tr>
<td>C. testacea</td>
<td>4</td>
<td>5</td>
<td>1–6</td>
<td>~8</td>
<td>–</td>
</tr>
</tbody>
</table>

The chaetotaxy is given for one side of the structure, except for ventral anal lobe (al). *"unapplied data (i.e., when palidia is absent, it is impossible define the presegmental setae). ~"about. Cyclocephala lumulata: general chaetotaxy by Bran et al. (2006). **redescription of Morlin et al. (2014) recorded only 1 pes; † first characterization by King (1984). aas, anterofrontal angle setae; acs, anteroventral setae; aes, anteroepisternal setae; afs, anterolateral setae; al, ventral anal lobe setae; als, lateral anal setae; des, dorsoepisternal setae; ecs, erectocerebral setae; ees, eusternoparietal setae; efs, eufemosternal setae; pa, palpidium setae (palpi); pes, posteroepisternal setae; pfs, posteroventral setae; pls, posteroalidral setae; pr, tegulum presegmental setae; tg, tegillum setae (including the presegmental ones).

Figs. 5–6. Cyclocephala melancopaca, third instar larva; 5, lateral; 6, head, dorsal. 110, abdominal tergite X; usb, U-shaped sclerotized bar. Scale = 1 mm.
Figs. 7–12. *Cyclocephala melaniceps*, third instar larva: 7. cranium and clypeus, dorsal; 8. labrum, dorsal (epipharyngeal ventral setae omitted); 9–12, left antenna (dorsal, internal, external, ventral; external and internal sides with apex detail; some sensilla numbered to easily the correspondence; sensillum h can be absent). Chaetotaxy (italic) on the text. Scale = 0.5 mm (antennal details with magnification four times bigger than antennae).

setae. Left molar with 9 dorsoproximal setae in a row, 3 ventro-
proximal setae in a tuft; 2 anterior chisel-like teeth transversally
positioned to each other, a dorsal and a ventral tooth, 2 trans-
versal shallow carinae between the dorsal and ventral teeth; acia with
apex rounded and bearing about 6 setae; calx semicircular; brustia
with 12 setae. Maxillae (Figs. 23–25). Galea and lacinia separated
by suture; galea with an uncus; lacinia with 3 unci; mala without
conspicuous setae row. Stipe with stridulatory area bearing 13
obtuse teeth and a distal truncate process. Palp with 4 palpomeres:
1 with an externoproximal sensillum and a minute externodistal
seta; II with an externodorsal sensillum and 3 ventral sensilla; III
with an external seta, a ventral seta and 2 ventral sensilla; IV with
3 external sensilla and an intermodal minute seta, distal sen-
sorial area bearing about 13 sensilla. Hypopharynx (Figs. 16, 24) with
asymmetrical sclerite, right and left lateral with about 14 setae (7
long, 7 min), lateromedial left area with a row of about 22 stout
setae, lateromedial right area with a group of phobae; right ante-
rior area with a prominent tooth, posterior area with a prominent
semispherical process. Posterior preoral area: each side of dorsal
area (posterior to epipharynx, Fig. 15) with a sensillum; each side
of ventral area (posterior to hypopharynx, Fig. 24) with 2 sensilla,
right area with an anterior stout seta, left area with a row of about 23
stout setae. Labium (Figs. 16, 24, 25). Submentum with a posterior
sclerite bearing 1 setae and 4–5 sensilla on each side, anterior area
with 2 setae and a sensillum on each side. Mentum with a glabrous
sclerite medially interrupted, anterior area with 7–8 setae and 1–4
sensilla on each side. Prementum with a sclerite bearing 6–8 setae
distributed around palpi insertion; ligula (Figs. 16, 24) with 3 large
medial setae on each side, 16 small posterior tooth-like setae, a
small medial tubercle-like process, a posterior transversal sclerite,
posterior area bearing asperites. Palp with 2 palpomeres: I with a
minute ventroproximal seta; II with a ventrodistal sensillum, distal
sensory area with about 13 sensilla. Thorax (Figs. 5, 14). Protho-
rax with a tergal lobe bearing 40–50 thin setae (setae similar to
Fig. 44d), anterior pleural lobe with 4–5 thin setae, posterior pleu-
ral lobe with 9–17, prothoracic lateral sclerite with 6–8 thin setae,
anteromedial ventral lobe with 46–54, posterior ventral lobe bare.
Meso- and metathorax with anterior tergal lobe bearing 15–19 thin
setae, medial tergal lobe with 44–60 thin setae, posterior tergal lobe
with 16–20 thin setae, anterior pleural lobe with 4–7 thin setae,
posterio pleural lobe with 2–4 thin setae, anteromedial ventral lobe
with 34–40 thin setae, posterior ventral lobe with 2 setae. Legs (Figs.
26–28). Pro-, meso- and metafemur intermedistal area with a small
and acute tubercle bearing a distal seta, meso- and metafemur with an
externodorsal macula; pretarsus with 2 lateral setae and an acuminate
apex, propretarsus longer than meso- and meso-longer than the metapretarsus (Figs. 29–31). Tho-
racic spiracle (Figs. 32–34) with 11–14 perforations in dorsal radius
(DR), 6–8 in lateral radius (LR), 13–16 perforations in ventral radius
(VR); perforations oblong or slightly ameboid shaped; bulla slightly
larger than the distance between respiratory plate arms. Abdomen
(Figs. 5, 14): Abdominal segment I with anterior tergal lobe bearing
14–16 thin setae (setae similar to Fig. 44d), medial tergal lobe with
26–34 thin setae and 16–18 stout setae (setae similar to Fig. 44c),
posterior tergal lobe with 16–22 thin setae and 22–26 stout setae,
lateral tergal lobe 3–6 thin setae, spiracle lobe with 6–8 thin setae,
posterior pleural lobe with 1–2 thin setae, posterior pleural lobe
with 6–12 thin setae, anterior ventral lobe with 13–18 thin setae,
medial ventral lobe with 10–15 thin setae, posterior ventral lobe
bare. Abdominal segment II–VI with anterior tergal lobe bearing
6–10 thin setae and 15–20 stout setae, medial tergal lobe with
24–30 stout setae and 44–58 stout setae, posterior tergal lobe with
10–14 thin setae and 43–46 stout setae, other lobes with similar
setation as abdominal segment I. Abdominal segment VII with a
tergal lobe bearing an anterior group of 6–9 thin setae and 14–20
stout setae, a medial group of 22–28 thin setae, a posterior group of
14–20 thin setae, spiracle and pleural lobes with similar setation as
abdominal segment I–VI, anterior ventral lobe with 8–11 thin setae,
medial ventral lobe with 5–7 thin setae, posterior ventral lobe bare. Abdominal segment VIII similar to VII, but tergal lobe with 25–28 thin setae. Abdominal segment IX with a tergal lobe with an anterior group of 10–15 thin setae and a posterolateral group of 21–24 thin setae, a pleural lobe with 14–17 thin setae, anterior ventral lobe bare, posterior ventral lobe with 4 medial thin setae and 2–4 lateral thin setae. Segment X (Figs. 5, 14, 43) with a curved anal opening, tergite with an anterior U-shaped sclerotized thin bar, a group of about 170–180 anterior thin setae and a posterior group of 46–61 stout setae; ventral anal lobe with 10–15 thin setae and 28–34 hamate setae. Spiracles (Figs. 35–42): I smaller than other ones and with 6–8 perforations in DR and LR, and 13–15 in VR; II–VI similar to each other and with 9–13 perforations in DR, 8–11 in LR, 12–16 in VR; VII–VIII similar to each other, wider than other ones, number of perforations of VII similar to II–VI, VIII with 11–13 perforations in DR, 9–11 in LR, 16–20 in VR; bulla I strongly narrower than the distances between respiratory plate arms, II–VII slightly narrower than the distances between respiratory plate arms, VII as wide as the distances between respiratory plate arms. Each side of the raster (Fig. 43) with: tegillum with 1–3 acute setae (Fig. 44b) and 8–11 hamate setae (Fig. 44d), of which 1–2 hamate setae are pre-posterior setae (anterior to the palidia); barbula indistinct; palidium with 3–4 short bifurcate setae (Fig. 44a); septula irregular shaped and barely distinct.
Figs. 15–16. Cyclocephala melanocephala, third instar larva; 15, epipharynx; 16, cibarium. aca, anterior most acanthoparia seta; acr, lateroposterior acroparia seta; crp, right part of crepis; lip, ligular tubercle-like process; ppa, posterior preoral area. Scale = 0.5 mm.

Figs. 17–22. Cyclocephala melanocephala, third instar larva; 17–19, right mandible (ventral, internal, dorsal); left mandible (dorsal, internal, ventral). Scale = 0.5 mm.
Remarks. Larvae of *C. melanocephala* and *C. paraguayensis* are easily distinguished from other known Cyclocephalini larvae by the presence of palidia with bifurcate setae (Fig. 24a). The body chaetotaxy (Table 1) is useful as supplementary data to the species identification.

For *C. modesta* and *C. putrida*, only the raster is known. Morelli (1991) provided images of these species, raster and attributed the original data to an unpublished thesis: “L. Alvarado. 1980. Sistemática y bionomía de coleópteros que en estados inmaduros viven en el suelo. Universidad Nacional de la Plata. Facultad de Ciencias Naturales y Museo, Argentina”. Larvae of *C. modesta* have a peculiar raster (not seen in other Cyclocephalini) with palidia long and posteriorly divergent. Otherwise, larvae of *C. putrida* cannot be distinguished from other Cyclocephala larvae as the raster do not show any specified pattern. More data about both species are needed to clarify the larvae taxonomy.

Third instar larvae of *C. flavipennis* and *C. signaticollis* Burmeister, 1847 are easily differentiated from other Cyclocephalini larvae by ventral anal lobe ornamentation, both have posteromedial hamate setae distinctly bigger than anterolateral setae. Immature of *C. signaticollis* was described by Remedi-de-Gavotto (1964) and redescribed by Morelli (1991), and larvae of *C. flavipennis* were first time characterized by Pereira and Salvadori (2006). Until now, it is impossible to separate both species and more studies are needed to solve this problem.

The dorsolateral macula of meso- and metafemur was not noted in other Scarabaeoidea larvae. A similar modified area was described in *Bubas bubalus* (Olivier, 1811) posterior leg.
Figs. 26–42. *Cyclocephala melanocephala*, third instar larva; 26–28, right legs and pleurites (anterior, medial, posterior); 29–31, right pro-, meso- and metapretarsus, dorsal; 32–33, detail of perforations of mesothoracic spiracle (dorsal arm, medial area); 34–42, mesothoracic spiracle and abdominal spiracles I–VIII. Scale, Fig. 26 = 0.3 mm; Figs. 29, 34 = 0.1 mm; Fig. 32 = 0.05 mm.

Figs. 43–44. *Cyclocephala melanocephala*, third instar larva; 43, raster; 44, setae detail; a, pali; b, hamate setae; c, short setae; d, long setae. Scale, Fig. 43 = 1 mm; Fig. 44 = 0.5 mm.
Key to known third instar larvae of known Cyclodaphiini (minute setae are omitted in the key)

1' - Antennennore IV with one or more than 2 dorsal sensorial spots; zygum as a cross-bar or beak-like with 1 or more than 2 teeth; abdominal tergites VIII–IX with or without small stout setae. ........................................................................ Dynastinae other than Cyclodaphiini
2' - Antennennore IV with 2 dorsal sensorial spots; zygum beak-like with 0 or 2 teeth; abdominal tergites VIII–IX without small stout setae. ................................................................. Cyclodaphiini

3(1) - Head without anterofrontal setae (afs); zygum toothless and with posterior margin crenulate or straight; left mandible with fourth scissor tooth (S4) present ........................................................................................................ 3
4' - Each head side with 0–2 anterofrontal setae (afs); zygum toothed; left mandible with or without S4. .............................................................................................................................. Cyclodaphiinae Dejean, 1821 (part)
5' - Each head side with 1–2 post-antefrontal setae (afs); perforations of thoracic spiracle oblong, if perforations slightly irregular shaped, then them with less than 4 sinuositites. .................................................................................................................. 6
6' - Each head side with 3–4 anterofrontal angle setae (as); haptolachus left side with less than 15 setae. .............................................................................................................................. D. dubius (Olivier, 1789)
7(2) - Palpia present, sometimes pali irregularly distributed and sepulta barely distinct, but pali evenly differentiated from tegillar setae. .............................................................................. 8
8 - Palpia absent. .................................................................................................................................................................................................................................................. 11
9' - Each palpium with more than 30 setae. ................................................................................................................................. C. testacea Burmeister, 1847
10 - Each palpium with less than 10 setae. ........................................................................................................................................................................................................................................ 9

12(11) - Ventral anal lobe with 7–9 postemeral hamate setae twice larger than other hamate setae (cf. Remedi-de-Gaveto, 1964; Fig. 6b; Pereira and Salvador, 2006; Fig. 6b). ........................................................................ C. flavipes Arroyo, 1914 and C. significolus Burmeister, 1847 (see remarks)
13 - Ventral anal lobe with medial setae not or slightly bigger than lateral setae OR setae progressively larger to medial area, but setae enlargement never abrupt... 13

Pupa (Figs. 45–51). Body (Figs. 45–47) length 15.2–15.4 mm; thorax width 7.1–7.5 mm; whitish, integument macroscopically smooth and glabrous but covered by a thin and short microscopic pubescence, which gives a velvety appearance to the surface, this pubescence slightly longer in abdomen laterals. Head (Fig. 48). Vertex hidden under pronotum from dorsal view. Epistomal suture distinct laterally and indistinct medially. Canthus small. Labrum short and transversal, positioned between the mandibles. Maxillary palps prominent. Labium slightly rounded. Antenna with two defined regions: scape–pedicel and funicle–clava. Thorax. Pronotum wider than long, greater width at the posterior margin, lateral margins rounded. Prosternum with visible posterior process between pro- and mesocoae, process acute in males (Fig. 49) and rounded in females (Fig. 50); precocx area hidden by the head in ventral view. Mesonotum as long as pronotum and longer than metanotum. Elytra curved ventrally around the body and almost smooth. Pro–, meso– and metacoxa contiguous; profemur-tibia slightly exposed in dorsal view; mesosomere-tibia superposed to wings in ventral view and hidden in dorsal view; protibia with three external tubercle-like teeth; mesotibial spurs tubercle-like; metatibial spurs indistinct; male protarsus slightly larger than female tarsus. Mesothoracic spiracle present in a cavity between the pronotum, elytron and anterior and medial legs. Abdomen. Five dinoeiform organs present between tergites I–II, II–III, III–IV, IV–V, V–VI; tergites II–V with a barely distinct transversal carina. Abdominal spiracles I–IV well developed and with peritreme, I hidden under the wings, II–IV slightly prominent, V–VII as cuticular invagination, VIII slightly larger than V–VII. Tergite IX ventrally folded and distally setose; urogomphi absent. Female terminalia. Sternite IX with genital ampulla formed by a small concavity; tergite X ventrally exposed. Male terminalia (Fig. 51) with proximal genital ampulla medially strongly constricted;
Figs. 45–47. *Cyclocephala melanocephala*, female pupa (dorsal, ventral, lateral). Scale = 5 mm.

Figs. 48–51. *Cyclocephala melanocephala*, pupa; 48, head, frontal; 49–50, prosternal posterior process (male, female); 51, male terminalia, ventral. Scale = 2 mm.
posterior genital ampullae larger than long and with a distal impressed line; sternite X slightly exposed.

Remarks. Pupa of C. melanocephala and C. paraguayensis are easily distinguished from other known Cyclocephalini pupae (see the key below) by the presence of 5 dioneiform organs, other cyclocephaline pupae have 4 (C. signaticollis) or 6 organs. Morón et al. (2014) characterized pupae of C. jalapensis with 6 dioneiform organs, more data are needed to separate this species from others.

The larvae and pupae of C. borealis, 1911 were first described and illustrated by Johnson (1941), but more morphological data are needed to separate its pupae from others.

Besides the Cyclocephala species, other two species of Dyscinetus Harold, 1869 have their pupae described. Dyscinetus rugifrons (Burmeister, 1847) pupa was described by Vincini et al. (2000), and D. dubius pupa (Olivier, 1789) was described by Neita-Moreno and Yepes (2011). Apparently, pupae of Cyclocephala have tergite IX ventral fold hidden most of sternite IX in lateral view (Fig. 47), while Dyscinetus pupae have a short tergite IX ventral fold and most of sternite IX is exposed in lateral view (cf. Neita-Moreno and Yepes, 2011: Fig. h). More data are needed to confirm these differences and to propose an adequate diagnosis.


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Key to known pupae of Cyclocephala

1. Abdomen with 4 dioneiform organs between III–IV, IV–V, V–VI, VI–VII................................................................. C. signaticollis Burmeister, 1847
2. Abdomen with more than 4 dioneiform organs, organs present between I–II and II–III ........................................... 2
4. (2) – Abdominal tergite VII anterior margin about 2,5 times wider than the posterior most dioneiform organ (between VI–VII)............................................ C. paraguayensis Arrow, 1913
5. (4) – Abdominal tergite VII ventral fold forming a posterior tubercle-like process bearing some relatively long setae (cf. Souza et al., 2014a) (Figs. 16–18)............................................................ C. testacea Burmeister, 1847
6. (5) – Profemur-tibia articulation area hidden in dorsal view ......................................................................................... C. fulgarata Burmeister, 1847
7. (6) – Profemur-tibia articulation area visible in dorsal view as a small area next to pronotum posterior angle and elytral humeral area (similar to Fig. 45).................. C. testacea Burmeister, 1847
8. (7) – Pubescent of abdominal tergite VII ventral fold almost hidden in dorsal view, male anterior genital ampulla slightly constricted medially..................................... C. gregaria Heyne & Taschenberg, 1908
9. (8) – Pubescent of abdominal tergite VII ventral fold evident in dorsal view, male anterior genital ampulla strongly constricted medially................................................. C. limulata Burmeister, 1847

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Mating behavior of adults

Several stages of mating behavior were observed for the 23 couples mated in the laboratory. Adults remained in the soil during the day. At nightfall, males (n = 18) and females (n = 13) expressed a portion of the clypeus near the soil surface and moved the antennal lamellae in different directions for 5.2 ± 2.1 min (range 3–11) before they left the soil and started the flight. However, some males (n = 5) and females (n = 10) arrived near the soil surface, left quickly, and began to fly (Fig. 52).

When they left the soil, adults flew actively on average for 8 ± 3.5 min (range 4–14) and then began to walk on the soil with the antennae erected and the lamellae open. Fourteen out of the couples mated showed multiple stages of mating behavior, while nine couples showed no mating behavior. At the first stage of mating, the male approaches the female from behind (n = 9) touching the end of the elytra or pygidium of the female with the antennae and protarsi or touches the female on her side (n = 5). When the male touches the female, possibly chemical recognition between both is occurring; however, evidence of a pheromone release was not observed. At the next stage, the male climbed on the female (n = 14), holding her with his claws and remained in that position for 2.3 ± 1 min (range 1–4). Sometimes, the females refused the males for mating (n = 4), those walked away from the male and began to fly.

When the male was accepted by the female (n = 10), it placed its body to reach the female’s pygidium. Next, rhythmic movements of the male abdomen were observed while it exposed the aedeagus and began the copulation. Copulation lasted on average 10.4 ± 4.3 min (range 5–16). After copulation, the male (n = 10) retracted the aedeagus in 8.5 and remained on the female an average of 18.0 ± 6 min (range 10–28); afterward the male climbed off the female and they separated from each other. Females copulated in the laboratory only between 19:00 and 00:00 h; two copulations from 19:00 to 20:00 h, four from 20:00 to 21:00 h, two from 21:00 to 22:00 h, one from 22:00 to 23:00 h, and one from 23:00 to 24:00 h.

Adults were collected with a light trap from 18:00 to 04:00 h the next day (Fig. 3). After 18:00 h, brightness of 1072 kJ/m² began to decrease and at 20:00 h, brightness was 0 kJ/m², coinciding with the beginning of the flight activity. Adults were collected in greater quantity from 20:00 to 23:00 h. The average temperature during the flight observations ranged from 32.7 °C to 21.1 °C between 18:00 and 06:00 h, respectively (Fig. 53).

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Discussion

The steps related to the mating behavior of C. melanoccephala are described for the first time. Some information is known about mating behavior of some Cyclocephala species. Souza et al. (2014a) studied the biological aspects of C. disticta Burmeister, 1847 and found that copulation occurred from 18:00 to 20:00 h. For C. celata, Souza et al. (2014b) reported that adults mate day and night. Adults of C. verticalis kept in the laboratory mate with an average duration of about 12–19.2 min (Barbosa and Rodrigues, 2016; Rodrigues et al., 2010). For C. limulata, copulation lasted 15–20 min, and the females may mate more than once (Stechauner-Rohringer and Pardo-Locarno, 2010).

Even though there was a contact with the antennae and first pair of legs when males get closer to females, no evidence of sexual pheromone release has been observed for them. In adults of Exomalda orientalis (Waterhouse, 1875) (Rutelinae), Facundo et al. (1999) found that females release sexual pheromone and attract males for mating. Although further studies should be carried out to evaluate this, we suggest that in C. melanoccephala recognition and chemical communication among adults might exist, since several females did not accept some males for mating. Fávila (1988)
Female and male \( n = 23 \)

Leave the soil and fly actively

Land and form a couple

Male touches the front part of female

Female accepts male for copulation

Male bends the abdomen and expose edeagous

Copulation starts with introduction of edeagous

Female remains still

Copulation finishes and male retracts edeagous

Male remains on female after copulation

Male and female detach from each other

Female rejects male and detaches

Male touches the back part of female

Male climbs on female

Male and female detach from each other

Fig. 52. Ethogram of mating behavior of *Cyclocephala melanocephala* \( n = 23 \) couples in the laboratory.

demonstrated that the non-acceptance of *Canthon cyanellus cyanellus* LeConte, 1859 (Scarabaeinae) females by some males for mating was related to differences in sexual maturity of both sexes. Such behavior was also observed in *Anomala testaceipennis* Blanchard, 1851 (Rutelinae) by Rodrigues et al. (2014).

After copulation, males of *C. melanocephala* remained on females probably to prevent another male from copulating with her. Arakaki et al. (2004) observed that in copulations of *Dasylepida ishigakiensis* (Niijima & Kinoshita, 1927) (Melolonthinae) the male remained on guard over the female after copulation to avoid the approach of other males. In *Liogenys bidenticeps* Moser, 1919 (Melolonthinae), males remained for 4 h on average on the female after copulation, as a protection against other males (Rodrigues et al., 2014).

**Conflicts of interest**

The authors declare no conflicts of interest.


