



Description of mating behavior, life cycle, and antennal sensilla of *Cyclocephala putrida* Burmeister, 1847 (Coleoptera, Scarabaeidae, Dynastinae)

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Abstract: The genus *Cyclocephala* is common in Brazil (Coleoptera, Scarabaeidae, Dynastinae). The adults of some species are important pollinators, and the larvae develop in the soil, feed on organic matter, and contribute to nutrient cycle, but immatures of some species feed on plant roots, and some were registered causing damage in crops. The mating process of some phytophagous scarab beetles has a chemical recognition step, and the antenna is the main structure involved in the detection of odorants associated with insect communication. In the present study the mating behavior, life cycle, and antennal sensilla of *C. putrida* are described. The study was conducted at the Universidade Estadual de Mato Grosso do Sul, Cassilândia, Brazil. Adults were collected by a light trap installed from January 2016 to December 2017 and were taken to the laboratory for studies. Adults swarms are brief and were registered from January to February, and specimens were mostly collected at 20:00 to 22:00h. Chemical recognition may occur at least during one of the mating steps, during which the couple kept their antennae moving and the lamellae open, while females select males. In laboratory, the mating process lasted 7.5 minutes on average. The antennae of females have about 3399 sensilla and males about 4229 sensilla. Sensilla placodea types I, II, and III are the most abundant, and sensilla ampullacea, basicônica, and coelocônica are also present. The embryonic period lasted 16.0 days; first, second and third instars lasted 16.0, 48.3, and 165.3 days, respectively. The pupal period lasted 24.0 days. The period between egg deposition and adult emergency is about 271.5 days.

Keywords: Chemical Communication, Chemical Receptors, Cyclocephalini, Neotropical, Swarming Flights.

Descrição do comportamento de cópula, ciclo de vida e sensilas antenais de *Cyclocephala putrida* Burmeister, 1847 (Coleoptera, Scarabaeidae, Dynastinae)

Resumo: O gênero *Cyclocephala* é comum no Brasil (Coleoptera, Scarabaeidae, Dynastinae). Os adultos de algumas espécies são importantes polinizadores, e a larva desenvolve-se no solo, alimenta-se de matéria orgânica e contribui para a ciclagem de nutrientes, mas imaturos de algumas espécies alimentam-se de raízes de plantas, e alguns são registrados causando danos em plantas cultivadas. O processo de cópula de algumas espécies de Scarabaeidae fitófagos, apresentam reconhecimento químico, e nas antenas aparecem várias estruturas responsáveis pela detecção dos odores envolvidos na comunicação. No presente trabalho o comportamento de cópula, ciclo de vida e sensilas antenais de *Cyclocephala putrida* são descritos. O estudo foi conduzido na Universidade Estadual de Mato Grosso do Sul, Cassilândia, Brasil. Adultos foram coletados com armadilha luminosa instalada de Janeiro de 2016 a Dezembro de 2017, e levados para laboratório para estudos. Os adultos revoam por curto período de janeiro a fevereiro, e os espécimes foram coletados em maior quantidade das 20:00 às 22:00 h. O reconhecimento químico possivelmente ocorre durante as etapas que envolvem o comportamento de cópula, no qual o casal mantém as antenas em movimento e as lamelas abertas e as fêmeas selecionam os machos. Em laboratório a cópula dura 7,5 minutos em média. As antenas das fêmeas possuem 3399 sensilas e os machos 4229 sensilas, e as sensilas placódeas dos tipos I, II e III, foram as mais abundantes e sensilas ampuláceas, basicônicas e coelocônicas também estão presentes. O estágio de ovo durou 16,0 dias; o primeiro, segundo e terceiro instar duraram 16,0, 48,3 e 165,3 dias, respectivamente. O período pupal durou 24,0 dias. O período entre deposição dos ovos e emergência dos adultos é de 271,5 dias em média.

Palavras-chave: Comunicação Química, Receptores Químicos, Cyclocephalini, Neotropical, Período De Voo.

Introduction

The genus *Cyclocephala* Dejean, 1821 (Coleoptera, Scarabaeidae, Dynastinae, Cyclocephalini) is distributed through the Americas, and about 123 species have been recorded in Brazil (Grossi & Vaz-de-Mello 2019).

Several adults were registered as flower visitors, with potential to be pollinators, and the visited plants are usually used as feeding and mating sites (Moore & Jameson 2013). Adults of *Cyclocephala forsteri* Endrödi, 1963 have been associated with flowers of *Acrocomia aculeata* Lodd. ex Mart. (“macaúba” Areaceae) (Oliveira & Ávila 2011), while adults of *Cyclocephala melanocephala* (Fabricius, 1775) have been found in flowers of *Cereus peruvianus* (L.) Mill. (Cactaceae) (Silva & Sazima 1995), sunflowers (“girassol”, *Helianthus annuus* L., Asteraceae) (Camargo & Amabile 2001), and passion-fruit flowers (“maracujazeiro”, *Passiflora edulis* Sims, Passifloraceae) (Dias & Rodrigues 2018). *Cyclocephala literata* Burmeister, 1847 consumes flowers of *Magnolia ovata* Spreng. (Magnoliaceae) and uses the plant as a mating site (Gottsberger et al. 2012). *Cyclocephala celata* Dechambre, 1980 and *Cyclocephala cearae* Höhne, 1923 have been associated with *Taccarum ulei* Engl. & K. Krause (Araceae) plants and their adults were observed feeding and copulating on its flowers (Maia et al. 2013). It is known that some phytophagous scarab beetles locate plants by detecting volatile odorants using the sensilla on their antennae (*Anomala cuprea* Motschulsky, 1854; Larsson et al. 2001), and the same may occur for Cyclocephalini beetles (Barbosa & Rodrigues 2016; Maia et al. 2013).

Some Scarabaeidae species release sexual pheromones to chemical recognition, and females select males before copulation (e.g. Facundo et al. 1999, Rodrigues et al. 2014). As well as plant volatiles, insect structures involved in detecting pheromones are antennal sensilla, which are mainly distributed on the surface of lamellae (e.g. Leal & Mochizuki 1993, Kim & Leal 2000).

Before copulation, females return to the soil for egg deposition, and their biological cycle varies with species. The time between egg deposition and adult emergence is about 229 days for *C. verticalis* Burmeister, 1847 (Rodrigues et al. 2010), about 164 days for *C. celata* (Souza et al. 2014), and about 108 days for *C. distincta* Burmeister, 1847 (Souza et al. 2015).

The larvae of *Cyclocephala* use soil with organic matter as food, therefore being important for nutrient cycling such as *C. paraguayensis* Arrow, 1913 (Albuquerque et al. 2014). On the other hand, these larvae may consume plant roots, and some species can cause damage to crops such as *C. forsteri* which has been reported as potential pest to soybean crops (“soja”, *Glycine max* (L.) Merr, Fabaceae) (Santos & Ávila 2007), or such as *Cyclocephala flavipennis* Arrow, 1914, which has been registered damaging pastures (Duchini et al. 2017).

Although studies have been conducted on *Cyclocephala putrida* Burmeister, 1847 biology in Cassilândia, state of Mato Grosso do Sul (Brazil), little information is known for this species. Cherman et al. (2014) found larvae of the species in cultivated and non-cultivated areas in the state of Rio Grande do Sul (Brazil), and Bonivardo et al. (2015) reported immatures associated with pasture and maize (“milho”, *Zea mays* L., Poaceae) in Argentina.

Regarding the above-mentioned biological and economic importance of the genus *Cyclocephala*, the present work aims to describe the mating behavior, the antennal sensilla, and life cycle of *C. putrida*.

Material and Methods

The study was conducted at the Universidade Estadual de Mato Grosso do Sul (UEMS), Campus of Cassilândia, MS (19° 06' 48" S; 51° 44' 03" W). Sampling and rearing procedures, as well as analysis strategies, were modifications of Rodrigues et al. (2008, 2010).

Adults of *C. putrida* were collected using a light trap model “Luiz de Queiroz” from January 2016 to December 2017. The trap was installed in a pasture area (*Urochloa decumbens* Stapf cv Basilisk, Poaceae) and was turned on daily at 18:00 and turned off at 6:00 of the next day. The insects were collected every 60 minutes, and collected adults were sexed by observing male dilated protarsomeres (Figure 1), and couples were placed in 500 mL plastic containers with half-filled with the same soil from where adults were collected. The adults were reared with slices of bananas (fruit of *Musa* sp., Musaceae) as food source (see Ferreira et al. 2016). Each couple was observed from February 2016 to February 2017 to behavior observations.

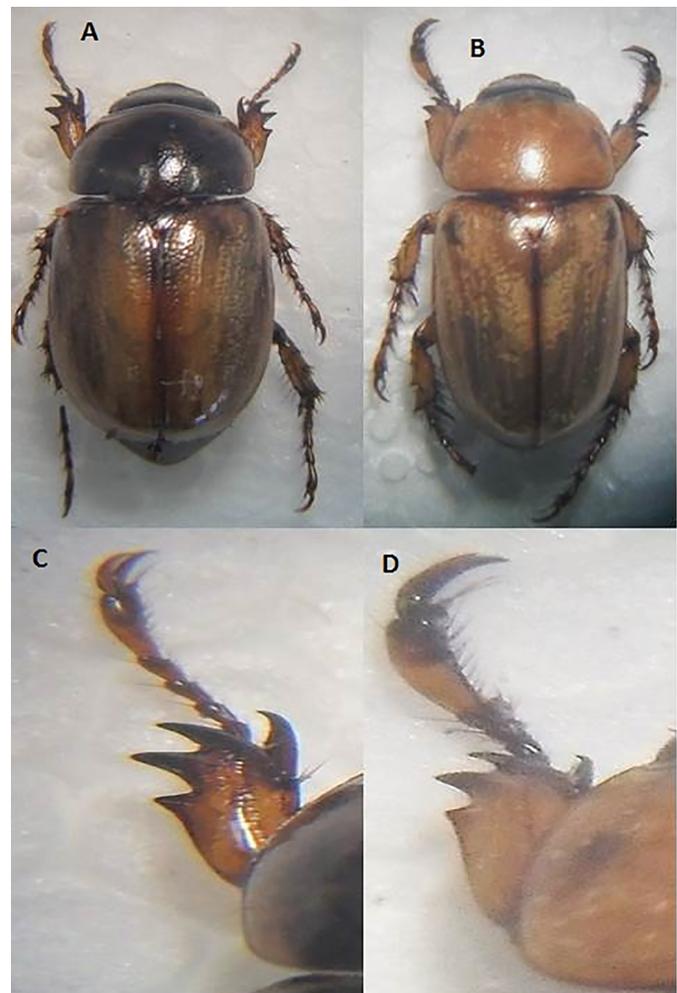


Figure 1. *Cyclocephala putrida* Burmeister, 1847. A-B) male and female habitus, dorsal; C - D) male and female protarsus.

The containers were checked weekly when the soil was sieved to egg separation. After removed, the eggs were individualized and placed in other 250 mL plastic containers one-third filled whit soil, which were maintained in a climatized chamber (26 ± 1 °C and 12 hours scotophase).

Each newly hatched larva was transferred to a 250 mL plastic container with soil and *U. decumbens* seedlings. Cranium width and body total length were weekly measured (Figure 2).

Flight activity was observed from February 2 to 27, and data were transformed into $\sqrt{x+1}$ and submitted to analysis of variance (ANOVA). Means were grouped and compared by the Scott-Knott test ($p < 0.05$) using SISVAR software. Data on average temperature (°C), precipitation (mm), and solar radiation (kJ/m²) in Cassilândia, were obtained from the Instituto Nacional de Meteorologia (INMET).

The study of antennal sensilla was based on antennal clava of 10 dissected specimens. Antennal segments were formerly treated in a 10% potassium hydroxide solution at 80°C for 60 minutes. The pieces were then successively washed in distilled water, 70% ethanol, 80% ethanol, and 90% ethanol, respectively (Romero-Lópes et al. 2010). Images were taken by a scanning electron microscope (model JSM 5410) at the Laboratório de Microscopia Eletrônica of the Universidade Estadual Paulista, campus of Jaboticabal, state of São Paulo, Brazil.

Part of dissected lamellae was mounted on slides with Hoyer’s solution (Johnson & Triplehorn 2005) and observed under a Nikon microscope model E2000. Sensilla terminology follows Keil (1999). Meinecke (1975) was used as an alternative sensilla terminology (in brackets) to easily comparison of terms. The insects were deposited in the UEMS entomological collection, campus of Cassilândia.

Results

Mating behavior. Four hundred and fourteen (414) adults were collected in 2016, 132 in January and 282 in February, which indicates a short swarming period. From 18:00 to 19:00 h the brightness decreased from 131.8 kJ/m² to 0, beetles flew from dusk till dawn (from 18:00 to 6:00 of the next day), and swarm activity was high at nightfall (from 20:00 to 22:00 h). Temperature ranged from 28.5 °C to 23 °C during the flight (from 18:00 to 6:00 of the next day; Figure 3).



Figure 2. *Cyclocephala putrida* Burmeister, 1847. A) Eggs, B) Larvae in containers with soil and seedlings of *Urochloa decumbens*. C) measurement of larvae.

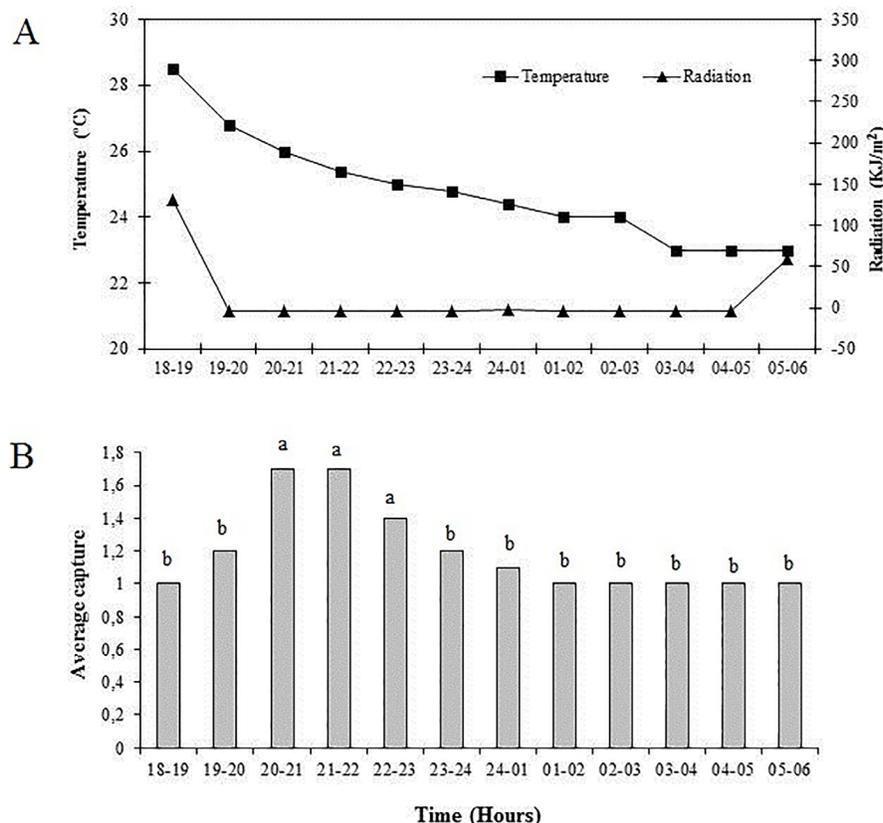


Figure 3. Radiation (kJ/m²) and average temperature (°C) obtained from the weather station (INMET) in Cassilândia, MS, Brazil, from 2 to 27 February 2016. A) Adults of *Cyclocephala putrida* collected with a light trap in the same period. B) Bars with the same letter are not statistically different ($P < 0.05$, Scott-Knott test).

Adults remained buried in the soil during the day and started flying from 18:00 onwards. After leaving the soil, the beetles flew for 12.6 ± 1.86 min (11-15) when landed on plants for feeding and mating. Then, they started walking while their antennae kept moving and lamellae remained open. Afterwards, some couples were formed (Figure 4) and mated, with antennae movement being maintained during all stages.

Six of the 30 couples formed mated. At the first stage, males walked over females from behind ($n=5$) or side ($n=1$) and repeatedly touched female pygidium or apex of elytra with their antennae and protarsi. After, males climbed on females dorsum ($n = 6$) using their protarsal claws to seize females, remaining in such position for 0.55 ± 0.22 min (0.39-1.0). Sometimes, female refused the male for mating ($n=4$) and walked away from it (Figure 4). If accepted by the female ($n=6$), the male gripped the female elytra, using all six legs. Then, rhythmical movements were observed in the male abdomen while its aedeagus was exposed, and the copulation began.

The copula lasted 7.5 ± 1.87 min (6-10) and occurred from 00:00 to 01:00 h. During the process, females stood still ($n=6$) and copulate only once. After copulation, males climbed off females and retracted their aedeagus, and then the pair quickly separated from each other. This final step lasted 7 ± 0.31 sec (5-12).

Antennal sensilla (Table 1). The entire outer surface of the antennal clava is covered by sensilla chaetica (long and short setae) and sensilla trichodea (minute setae, Figure 5), which were identified mainly as tactile (mechanoreceptors) but also as gustatory (contact chemoreceptors) sensilla (Keil 1999). Whereas the inner surface of clava (the lamellar side in contact with another lamella when the clava is close) was observed covered by sensilla ampullacea, basiconica, coeloconica, and placodea.

Sensilla ampullacea were identified as pores on the surface of the lamella. In Hymenoptera (Insecta), antennal pores were identified as CO_2 receptors (Keil 1999); therefore, their function in scarab beetle need to be checked. Sensilla basiconica were identified as type I (Meinecke 1975: L4) and resembles minute setae enclosed in a depressed pit, with the apex projected over pit limits. Two types of sensilla coeloconica were observed, and both are ovoid and enclosed into a depressed pit, the type I (Meinecke 1975: L1) had a pointed apex and the type II (Meinecke 1975: L2) had blunt apex.

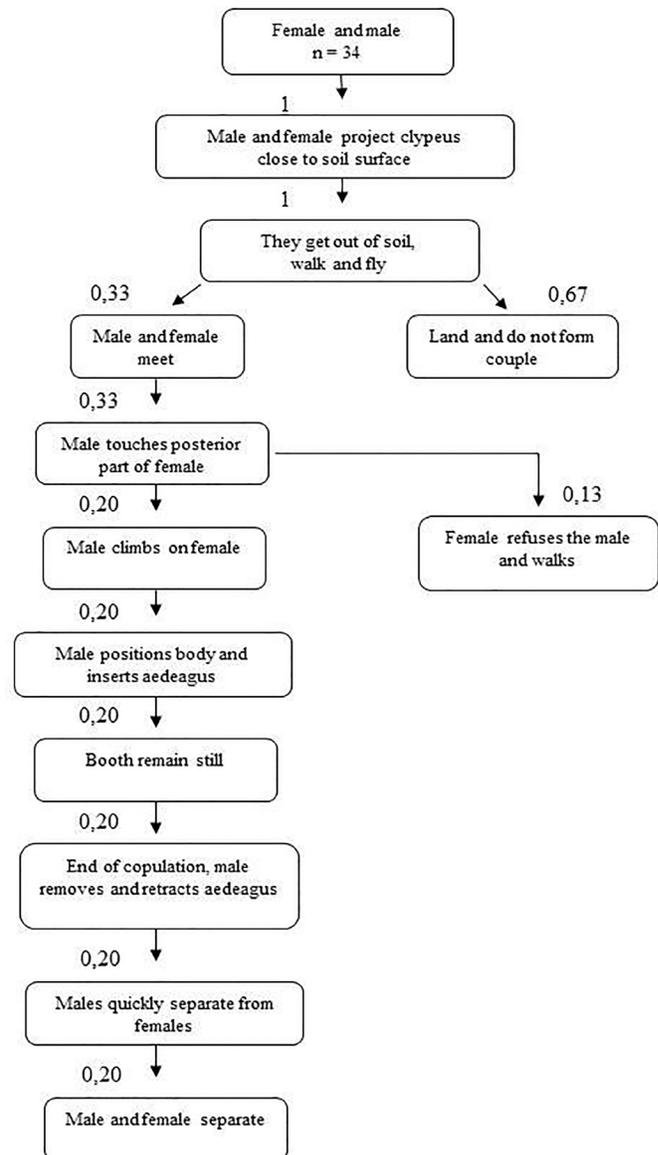


Figure 4. Ethogram of the mating behaviour of *Cyclocephala putrida*, Burmeister, 1847 in the laboratory.

Table 1. Sensilla of antennal lamella of *Cyclocephala putrida*.

Sensillum	proximal lamella		medial lamella		Distal lamella		Total
	external (proximal)	internal (distal)	external (distal)	internal (proximal)	external (distal)	internal (proximal)	
Female							
Placodea	0	943	645	833	0	663	3084
Coeloconica	0	100	78	59	0	52	289
Basiconica	0	11	6	6	0	3	26
Total	0	1054	729	898	0	718	3399
Males							
Placodea	0	1004	960	986	0	1066	4016
Coeloconica	0	66	33	11	0	80	190
Basiconica	0	7	4	4	0	8	23
Total	0	1077	997	1001	0	1154	4229

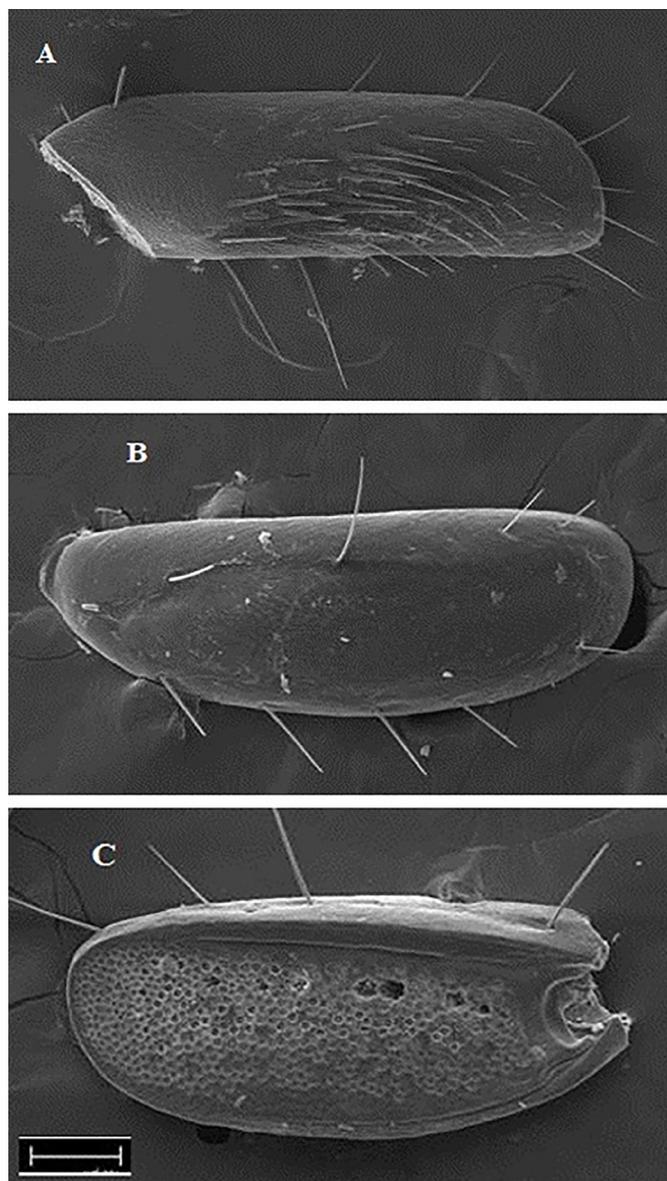


Figure 5. *Cyclocephala putrida*, Burmeister, 1847, antennae. A) proximal lamella, external (proximal side), B) distal lamella, external (distal side), C) medial lamella, external (distal side). Scale: 100 micrometers.

Three types of sensilla placodea are present: type I (Meinecke 1975: G2) is a plate surrounded by a ditch, the surface is irregularly reticulate and has mean diameter of 7.01 μm (3.3-10.0); type II (Meinecke 1975: G1) is similar to type I but with a smooth surface and mean diameter of 6.18 μm (3.89-10.0); type III (Meinecke 1975: J4) is almost smooth and the peripheral ditch is absent, mean diameter of 9.64 μm (8.33-10.0) (Figure 6).

Table 2. Stages and instar development to *Cyclocephala putrida*.

stage or instar	mean duration (days)	variation (days)	number of repetitions	viability (percent)
egg	16.0 + 1.03	15-18	36	100
first-instar larva	16.0 + 1.03	15-18	29	80.5
second-instar larva	48.3 + 3.56	45-54	18	62
third-instar larva	165.3 + 4.81	150-180	18	100
pupae	24.0 + 1.56	20-28	16	88.9
egg to adult	271.5 + 19.28	245-298	15	41.6

Females have a total of 3399 sensilla in lamellae, of which 3084 (90.7%) are sensilla placodea, 289 (8.5%) are sensilla coeloconica, and 26 (0.8%) are sensilla basiconica. Males have a total of 4229 sensilla, of which 4016 (95.0%) are sensilla placodea, 190 (4.5%) sensilla coeloconica, and 23 (0.5%) sensilla basiconica. Both sexes have a sparse amount of sensilla ampullacea in inner clava surface.

Life cycle. In the laboratory, 22 eggs were obtained in February and 14 eggs in March. The eggs showed 100% viability (Table 2). The mean duration of egg stage was 16.0 days, first-instar larvae 16.0 days, second-instar larvae 48.3 days, third-instar larvae 165.3 days, and pupae 24 days. The first and second instars showed 80.5 and 60% viability, respectively. The initial development stages had lower viability, as they are the most sensitive during the larval phase. The mean period between egg deposition and adult emergence was 271.5 days, and viability was 41.6%. Cranium width of first-instar larvae was 1.3 mm, of second-instar larvae was 2.4 mm, and of third-instar was 4.17 mm. The body length of first-instar larvae was 4.4 mm, of second-instar larvae was 7 mm, and of third-instar larvae was 15.91 mm. The third-instar larvae had a light grey colour that changed to white when pre-pupal stages started. Pre-pupae stop eating and make pupa chamber, possibly with aid of saliva or moist soil, manipulated with mouth appendages.

Discussion

Adults of *C. putrida* occurred in the field from January to February, as with *C. paraguayensis* found by Riehs (2006) in South of Brazil, but different from *C. melanocephala*, *C. tucumana*, *C. verticalis*, and others, which occur at beginning of rainy season, in September and October (Rodrigues et al. 2010, Nogueira et al. 2013). The occurrence patterns of some *Cyclocephala* species may be a strategy to avoid competition, but this hypothesis needs to be checked further.

Field observation showed that *C. putrida* start to flight at dusk, as other *Cyclocephala* species, as *C. verticalis* (Rodrigues et al. 2010, Barbosa & Rodrigues 2016), and other phytophagous Scarabaeidae, as *Anomala testaceipennis* Burmeister, 1856 (Rutelinae) (Rodrigues et al. 2014).

In the laboratory, adults started swarming at 00:00 h and copula started at 1:00 h. Adults of *C. verticalis*, when coupled in the laboratory, started copulating regardless of the day (Rodrigues et al. 2010).

Mating of *C. putrida* encompassed several stages, including coming out of the soil, start walking, swarming, forming couples, selecting females, and copulating. This process is quite similar to that observed in other *Cyclocephala* species such as *C. melanocephala*, *C. testaceipennis*, and *C. verticalis* (Rodrigues et al. 2014, 2018, Barbosa & Rodrigues 2016). Female selection in Scarabaeidae may be related to the recognition of male sexual maturity as in *Canthon cyanellus cyanellus* (LeConte, 1859) (Fávila 1988) and *C. verticalis* (Barbosa & Rodrigues 2016), and is might be intermediated by chemical communication (see Rodrigues et al. 2019).

At the beginning of mating behavior, adults showed active movements of antennae and lamellae, probably for detection of pheromones released by other specimens. Some studies with Scarabaeidae have found that lamellar sensilla are involved in the detection of sexual pheromones, and these sensilla are more abundant in males than in females (Romero-López et al. 2004, Tanaka et al. 2006, Mutis et al. 2014). Also, males of *C. putrida* had more sensilla than females. Sensilla placodea was predominant in males and females of this species, while sensilla coeloconica and sensilla basiconica occurred in smaller quantities. Such dominance of sensilla placodea was also found in other Dynastinae species such as *Oryctes rhinoceros* (Linnaeus, 1758) (Renou et al. 1998), and in other phytophagous subfamilies such as *Anomala iconstans* Burmeister, 1844 (Rutelinae, Rodrigues et al. 2019). Larsson et al. (2001) studied the antennal sensilla of *Anomala cuprea* (Hope, 1839) and concluded that the outer sensilla placodea are responsible for detecting sexual pheromones while the inner ones are responsible for detecting plant volatiles.

The predominance of sensilla placodea may be related to important roles in detection food resources and adult info-chemicals (sexual or aggregative).

Concerning the life cycle of *C. putrida*, the period between egg deposition and adult emergence was on average of 271.5 days, so it could be considered a long life beetle if compared to other *Cyclocephala* species. Long cycles were also noticed for *C. melanocephala* (113 days) (Nogueira et al. 2013), *C. paraguayensis* (171 days) (Albuquerque et al. 2014), *C. tucumana* (211.2 days) (Nogueira et al. 2013), *C. verticalis* (228.6 days) (Rodrigues et al. 2010) (Table 3). The embryonic phase of *C. putrida* had a similar length to that other *Cyclocephala* species; however, the first instar lasted 16 days, a period shorter than in the other species (Table 3). The second and third instars of *C. putrida* lasted, in days, shorter than did *C. tucumana*, which lasted 66.1 and 176.8 days, respectively (Table 3). And its pupal phase was also shorter than *C. celata* (Table 3).

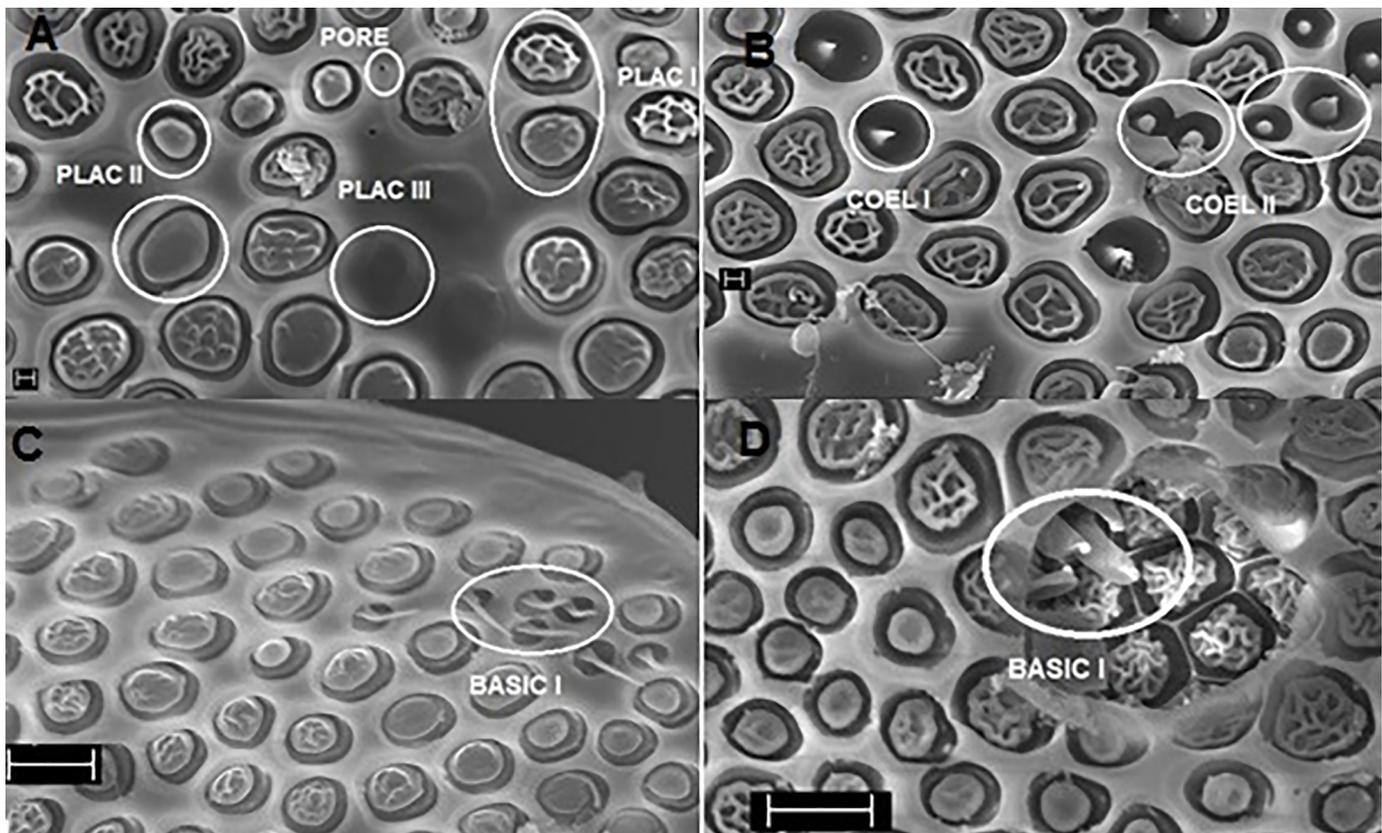


Figure 6. *Cyclocephala putrida*, Burmeister, 1847, antennal sensilla. A) Sensilla placodea type I, II, and III (PLAC I-III), and sensilla ampullacea (PORE). B) Sensilla coeloconica type I and II (COEL I-II). C-D) Sensilla basiconica type I (BASIC I). A-B) Scale 2 micrometers. C-D) Scale: 10 micrometers.

Table 3. Biological data of *Cyclocephala* species.

<i>Cyclocephala</i> species	Duration (days)						reference
	Egg	Fist instar	Second instar	Third instr	Pupae	Egg to adult	
<i>C. melanocephala</i>	17.8	22.3	21.0	38.3	13.3	113.0	Nogueira et al. 2013
<i>C. paraguayensis</i>	18.7	42.2	20.0	75.3	11.4	171.0	Albuquerque et al. 2014
<i>C. tucumana</i>	14.7	47.7	66.1	176.8	15.0	211.2	Nogueira et al. 2013
<i>C. verticalis</i>	17.2	22.0	23.7	150.8	14.8	228.6	Rodrigues et al. 2010
<i>C. celata</i>	13.9	22.0	26.2	68.3	38.0	164.0	Souza et al. 2014

Regarding the immatures, *C. putrida* larvae fed soil organic matter, so the species is not a potential pest to cultivated plants. White grubs had similar behavior compared to other *Cyclocephala* species. Its active third instar is yellowish-white, while prepupa is white and builds a pupal chamber, just as observed in *C. celata* and *C. distincta* (Souza et al. 2014, 2015).

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Author Contribution

Fabiola Gonzaga Saldanha: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Sérgio Roberto Rodrigues: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Juarez Fuhrmann: Contribution in the analysis and interpretation of data, also assisting in article revision, adding intellectual content.

Ricardo Aparecido Amaro: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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