

# Egg development and viability in the laboratory of Cyclocephala species (Coleoptera: Melolonthidae: Dynastinae)

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Thamyrys Bezerra De Souza, Larissa Simões Corrêa De Albuquerque, Luciana Iannuzzi, Fábio Correia Costa, Marc Gibernau, et al.. Egg development and viability in the laboratory of Cyclocephala species (Coleoptera: Melolonthidae: Dynastinae). Bulletin of Entomological Research, In press, 10.1017/S0007485322000384. hal-03867942

## HAL Id: hal-03867942 https://hal.science/hal-03867942

Submitted on 23 Nov 2022

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1	Egg development and viability in the laboratory of Cyclocephala species (Coleoptera:
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3	
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14	
15	Abstract
16	Cyclocephala species perform key functional roles in both natural and agricultural systems, such
17	as the cycling of organic matter and pollination, whereas also being known as destructive pests.
18	As such, the identification of biological parameters is crucial for defining strategies for their
19	conservation and efficient pest management. We daily monitored egg parameters (e.g. initial -
20	1st day after oviposition - and final - last day before hatching - egg masses, incubation durations,
21	egg mass increase, and viability) of three species of Cyclocephala native to the Brazilian Atlantic
22	Forest – a biodiversity hotspot. Our findings provide novel empirical evidence showing i) a
23	positive correlation between egg mass and incubation time, ii) idiosyncratic characteristics on
24	egg development, and iii) a negative involuntary effect of manipulation on egg development and
25	viability. Thus, for the successful breeding and rearing of Cyclocephala spp., one must be aware
26	of the importance of egg conditions according to each focal species. Our analyses present a
27	quantitative understanding of the egg phase and can assist in refining strategies for ovicidal

activity and pest management of *Cyclocephala* in agriculture systems. Moreover, they can
provide a basis for new studies related to captivity breeding, pollinator management, and
developmental biology for biodiversity conservation.

31

32 Keywords: breeding; Cyclocephalini; life cycle; masked chafer; rearing

33

### 34 Introduction

35 The cyclocephaline scarabs (Melolonthidae, Dynastinae, Cyclocephalini) comprise a fairly

36 diverse group of medium-sized beetles (ca. 10 - 35 mm) predominantly distributed in South and

37 Central Americas (Endrödi, 1985). With over 350 species, many of which yet to be described

38 (Moore et al., 2018), *Cyclocephala* encompasses more than 85% of the tribe's diversity and is

one of the most speciose genera among all of the Scarabaeoidea (Lawrence et al., 1999; Moore etal., 2018).

41

Many species of *Cyclocephala* have long gained special attention for being agricultural pests,
mainly during the larval stage (i.e. white grubs). Larvae of several species have been associated
with different cultures, such as agave (*Agave tequilana* var. Azul, Agavaceae; Garcia et al.,
2009), corn (*Zea mays* L., Poaceae; Gassen, 1993), onion (*Allium fistulosum* L., Alliaceae;
Villegas et al., 2008), soybean (*Glycine max* (L.) Merr., Fabaceae; Santos & Ávila, 2007),
sugarcane (*Saccharum officinarum* L., Poaceae; Cherry, 1958) among others (Richter, 1966;
Salvadori et al., 2004; Pardo-Locarno et al., 2005; Diez-Rodríguez et al., 2015). Furthermore,

49 different countries (e.g. USA, Brazil, Mexico, Colombia) experience difficulties with the damage

50 caused by the white grubs (Richter, 1966; Santos & Ávila, 2007; Villegas et al., 2008; Garcia et

51 al., 2009).

53 On the other hand, adult Cyclocephala scarabs are often anthophilous, although their role as 54 specialized pollinators has arguably been overlooked (Buchmann & Nabham, 1996). Schatz 55 (1990) has predicted that at least 900 Neotropical night-blooming angiosperm species belonging to nine extant families rely on these insects for their reproductive success, a testimony of their 56 57 relevance in natural ecosystems. Moreover, some commercially explored crops (e.g soursop, Annona muricata L.) are often so dependent on the presence of these specialized pollinators that 58 their decline is reflected in a considerable decrease in fruit yield (Cavalcante, 2000; Paulino-Neto 59 60 & Oliveira, 2006). Several factors such as scarce or excessive rainfall, low food availability, 61 unfavorable conditions for oviposition, and subsequent development of the soil-dwelling larvae 62 may negatively affect the abundance of Cyclocephala species (Gonçalves et al., 2020). 63 64 Despite recent advances in the studies demonstrating the importance of *Cyclocephala* to 65 agriculture and natural systems, the knowledge about the biological parameters of these insects is

66 surprisingly limited. Studies dealing with the life cycle of *Cyclocephala* species are restricted to

67 less than 3% of the currently described species (Gavotto, 1964; Morón, 1977, 2004; Potter, 1983;

68 Mondino et al., 1997; Santos & Ávila, 2007; Rodrigues et al., 2010; Stechauner-Rohringer &

69 Pardo-Locarno, 2010; Coutinho et al., 2011; Nogueira et al., 2013; Albuquerque et al., 2014;

70 Souza et al., 2014, 2015; Saldanha et al., 2020), and there is still no information about egg

development along time nor on the influence of laboratory manipulation on their viability andduration.

73

The comprehension of egg development in *Cyclocephala* can be applied to i) refine strategies
directed to ovicidal activity and pest management (Canela et al., 2000; Abou-Taleb, 2010); ii)
improve pollinator management plans (Allen-Wardell et al., 1998; Peña et al., 2002); iii)
elucidate characters for systematic and phylogenetic studies (Hinton, 1981; Hansen, 2000); and

iv) improve current understanding on population biology (Fox & Czesak, 2000) and life-history
traits within the genus, since the eggs are a direct product of female activity and provide the
initial resources for the offspring (Fox et al., 1997; McIntyre & Gooding, 2000).

81

82 Here, we addressed these knowledge gaps about egg development in *Cyclocephala* by daily monitoring different parameters (e.g. egg mass, incubation durations, and egg viability) of three 83 species of the genus. We ask the following questions: i) How does time influence egg mass gain? 84 85 ii) Is the incubation duration variable follow a normal distribution pattern? iii) Does egg development vary among species? and iv) What influence does density and manipulation in the 86 87 laboratory exert on egg development? 88 89 We expect to find a positive correlation between egg mass and development time due to the 90 water absorption capacity of eggs (Potter, 1983). We also predict a normal distribution pattern in 91 egg development duration (hatching) and idiosyncratic responses, since studies suggest that the

92 development stages of embryogenesis have a specific duration (Campos-Ortega & Hartenstein,

93 1985; French, 1988). We expect a negative effect of minupulations (in the laboratory) due to the

highly sensible structure of eggs shell and their susceptibility to desiccation (Potter & Gordon,

95 1984).

96

97 Taken together, our analyses aims to provide a quantitative understanding on egg development of
98 *Cyclocephala* in laboratory conditions; Such/our results contribute to refine strategies related to
99 ovicidal activity and pest management, since the control of initial levels of infestation is
100 economically advantageous and desirable in insect pest management (Dent, 2000). Moreover,
101 ithis type of research can provide a basis for new studies related to breeding captive, pollinator
102 management and developmental biology for biodiversity conservation.

#### 104 Methods

105 Insects sampling

- 106 Male and female adults of three species of *Cyclocephala* were collected in their natural habitat:
- 107 Cyclocephala celata Dechambre, 1980; C. cearae Höhne, 1923; and C. paraguayensis Arrow,
- 108 1913. Field expeditions were conducted in February-April 2008, January-May 2009 and

109 February-April 2010 to a private Atlantic Forest reserve on the grounds of the Usina São José

110 S/A sugarcane company (USJ) in the municipality of Igarassu, Pernambuco, Northeastern Brazil

111 (7°49'S; 35°02'W; approx. 110 m.a.s.l.), with mean annual temperature and rainfall of 25 °C and

112 ca. 2000 mm, respectively (data from 2008 to 2010; Lamepe/Itep, 2012).

113

The Brazilian Atlantic Forest is one of the world's biodiversity hotspots (Myers et al., 2000), and has continuously experienced intense human disturbance and forest degradation ever since the early 1960s (Tabarelli et al., 2010; Melo et al., 2013; Filgueiras et al., 2016). Land use changes were particularly severe in large areas of previously continuous forests along the coast of the state of Pernambuco, in northeastern Brazil, which were converted to small fragments in different succession stages and embedded mainly within a mosaic of sugarcane monoculture matrix (Trindade et al., 2008).

121

122 Within the private Atlantic Forest we actively searched inside inflorescences of *Philodendron* 

123 acutatum Schott, Caladium bicolor (Aiton) Vent. and Taccarum ulei Engl. & K. Krause

124 (Araceae), known hosts of flower-visiting C. celata and C. cearae (Maia & Schlindwein, 2006;

125 Maia et al., 2010). Since most species of cyclocephaline scarabs are attracted to light sources at

- night (references), light traps were also installed during early evening hours, from 17h30 to
- 127 21h00. A 250W mercury vapor light bulb was disposed in front of a 2.5 x 2.0 m sheet of white

128 cloth, stretched along the border of wooded areas. Settling beetles are then manually recovered
129 (Janzen, 1983). Individuals of *C. paraguayensis* are frequently sampled with this method
130 (Albuquerque et al., 2014, 2016).

131

## 132 Captivity breeding and rearing

133 Collected male and female beetles were accommodated in transparent plastic containers with

134 perforated lids (45 x 45 x 30 cm) and a layer of ca. 15 cm of topsoil extracted from the collection

sites. We distributed 20-50 beetles per container (1:1 gender ratio) and provided them with a diet

136 of fresh sliced apples and plantains, substituted every 2 days.

137

The rearing containers were kept under permanent shade inside a greenhouse where temperatures oscillated roughly between 22 – 30 °C. On a daily basis we examined the soil substrate for newly oviposited eggs, which were recovered and transferred individually to new containers. The methodology of "manipulated" and "non-manipulated" eggs follows Albuquerque et. al. (2014). The oviposition substrate (soil) was kept moist (what frequency?) with the aid of a hand water sprayer, thus avoiding desiccation of the eggs.

144

### 145 Egg mass measurement

146 Eggs from *C. cearae*, *C. celata*, and *C. paraguayensis* were individually weighted on a daily

147 basis, from oviposition until hatching. This procedure was carried out with a 4-digit electronic

scale (AE260 DeltaRange®, Mettler-Toledo, USA) and a precision scale of 0.0001g.

149

## 150 Statistical analyses

151 Eggs from each of the three *Cyclocephala* species were grouped according to the date of

152 oviposition (assumed as the date of recovery from the substrate) and monitored daily to

document the initial and final egg masses, incubation duration, egg mass increase, and viability.
We calculated egg mass increase as the ratio between final and initial egg mass, and egg viability
as the percentage ratio between the number of hatched larvae from the initial egg batches in each
container. We used linear regression analyses to assess the relationship between egg mass
increase and incubation duration. We preliminary assessed the distribution suitability and then
model fit through residual analysis. Furthermore, to evaluate the normality of incubation
durations, we applied the Shapiro-Wilk normality test for each studied species.

160

161 To evaluate if egg development parameters (i.e. initial and final egg mass, incubation duration, 162 egg mass increase ratio, and viability) varies among species, we applied a comparative analysis 163 using Kruskal-Wallis tests, because the data did not follow a normal distribution. When 164 significant relationships (p < 0.05) were found we used a post hoc pairwise test for multiple 165 comparisons of mean rank sums (Nemenyi test) to identify differences between species. 166

To understand if oviposition density (number of oviposited eggs by area  $-250 \text{ cm}^3$ ) influences egg development (i.e. initial and final egg mass, incubation duration, egg mass increase, and viability), we adopted a comparative analysis using ANOVA or Kruskal-Wallis tests according to the distribution of data for each species. Finally, to assess the influence of laboratory manipulation on egg development parameters (i.e. incubation duration and viability), we separated batches of eggs from *C. celata* (n = 312) and *C. paraguayensis* (n = 237) that were not handled and performed a Mann-Whitney test.

174

175 We performed analyses in R software (R Core Team, 2020) using the following packages for

176 computing: (1) Shapiro-Wilk, Kruskal-Wallis, Mann-Whitney, ANOVA, linear regression –

177 stats; (2) Kruskal Nemenyi test – PMCMR; (3) figures – ggplot2.

#### 178 Results

In captivity, we observed that adult beetles of all studied species were most active during early
evening hours, although neither feeding nor copulation was entirely restricted to this period.
However, during most of the daytime, they preferably remained deeply buried in the soil layer,
moving around over the surface only sporadically. Females of the three studied species always
laid their eggs individually and enclosed them inside an egg chamber, formed by spherical
clumps of soil. Freshly laid eggs of all species exhibited a milky white coloration and were ovalshaped, but each species presented idiosyncratic features in egg development.

Linear regression analyses revealed strong support for the influence of time (day) – during 186 187 incubation phase – on the gain of mass (mg) in eggs of *Cyclocephala* (Fig. 1). We found a 188 significant positive relationship between egg mass and time, for C. cearae, C. celata, and C. paraguayensis. According to the Shapiro-Wilk normality test, incubation durations of C. 189 paraguayensis followed a normal distribution (Fig. 2), implying that the embryonic development 190 of this species is somewhat standardized. The highest hatching rate of C. paraguayensis was on 191 day 13. On the other hand, C. cearae and C. celata did not follow a normal distribution in the 192 193 incubation durations (Fig. S1).

We found that different features of egg development are strongly species-specific (Fig. 3; Table S1). The initial and final egg masses were significantly different among the three studied species (F = ?,  $p \le 0.0023$ ). The initial and final egg masses were higher in *C. cearae* (Median - Mdn =

197 2.7 and 8.0 mg, respectively), medium in *C. celata* (1.7 and 5.0 mg) and small in *C*.

198 *paraguayensis* (1.0 and 2.4 mg). During embryonic development, the eggs of all three studied

199 species bloated and their shells became translucent. The eggs of *C. paraguayensis* presented a

200 lower rate of mass gain (Mdn = 2.0) compared to C. cearae (Mdn = 2.9) and C. celata (Mdn =

3.1); however, no differences were observed between the latter. We also found that the period of
egg incubation (days) was shorter in *C. celata* (Mdn = 12), while *C. cearae* (Mdn = 22) and *C. paraguayensis* (Mdn = 17) showed no significant differences between them. Finally, egg
development viability in laboratory of *C. cearae* (38%) was higher when compared with *C. paraguayensis* eggs (14%).

Egg development parameters (i.e., initial and final masses, incubation durations, mass increase,
and viability) were not affected by oviposition density in the three species studied (Table 1). On
the other hand, egg development was negatively affected by manipulation in the laboratory (Fig.
4). We found that manipulated eggs had lower viability rate and longer incubation durations
(25% and 18 days, respectively) than non-manipulated eggs (58% and 14 days).

211

### 212 Discussion

213 Female *Cyclocephala* of the three studied species laid their eggs individually and inside an egg 214 chambers, consisting of spherical clumps of loosely compacted soil. The construction of 215 individualized soil chambers for the laid eggs is commonly recorded among studies with 216 different groups of Scarabaeoidea, including Cyclocephala spp. (Morelli, 1991; McMonigle, 2006; Lai & Hsin-Ping, 2008; Rodrigues et al., 2010; Nogueira et al., 2013; Souza et al., 2013, 217 218 2015, Albuquerque et al., 2014). These chambers protect the egg from desiccation and are likely built by the gravid females (Nogueira et al., 2013). Moisture is a key factor during 219 embryogenesis since it contributes to egg mass gain and development of the larva (Potter, 1983). 220 221 Structures in the serosal layer of the eggshell, called *hydropyles*, promote active uptake of water when the embryo is undergoing rapid growth (Hinton, 1981). 222

223 Previous research has demonstrated that Cyclocephala lurida Bland, 1863 (=Cyclocephala

*immaculata*) eggs absorbed water and gained mass more intensely during the first ten days of

development (Potter, 1983). Eggs of some pest species of *Cyclocephala* enlarge significantly

during embryogenesis, increasing in weight and volume up to threefold (Potter, 1998). Similar to

the results obtained to *C. cearae*, *C. celata* and *C. paraguayensis*.

Although C. paraguayensis showed a normal distribution for the incubation duration, the lack of 228 229 standards regarding the embryonic development period might indicate that this parameter is less 230 strict for C. cearae and C. celata. It could also be interpreted as a delicate mechanism for sequential larvae eclosion for mated females that lay all their eggs at a single oviposition event. 231 232 However, the rule among Melolonthidae females is to oviposit progressively over the course of a few days (McMonigle, 2006; Lai & Hsin-Ping, 2008). The manipulation of eggs at an early 233 234 developmental stage may have influenced their incubation and viability, as already observed in a 235 previous study with Cyclocephala paraguayensis (Albuquerque et al., 2014). Additionally, the 236 destruction of the protective soil chamber might have disrupted normal embryonic development 237 due to stress.

Egg development varies among different species of *Cyclocephala*. Previous studies suggest that
embryonic development stages have a specific duration (Campos-Ortega & Hartenstein, 1985;
French, 1988) and theroretical models of ontogenetic growth indicate that the metabolic rate of
individual organisms may be related to their body size and temperature (van der Meer, 2006).
Also, other factors may also influence egg development, such as environmental fluctuation and
genetic variation within a population (Johnson et al., 2007).

244

245 Our results demonstrate that the investigated egg development parameters were not affected by 246 oviposition density in any of the studied species. From natural conditions, the density of 247 *Cyclocephala* eggs in the soil varies between 11.37 and 20.31 eggs/ $m^2$ , and the reduction in egg 248 density over time is related to the hatching of the larvae (Mondino et al., 1997). From data 249 obtained in laboratory conditions indicate that gravid females lay variable sets of eggs in one or more oviposition events (13-20 eggs per female; Gavotto, 1964; Mondino et al., 1997; Nogueira 250 251 et al., 2013; Souza et al., 2015). If that observation is extrapolated to natural conditions, egg 252 development in clusters cluestered development of eggs together is common to Cyclocephala and this pattern may be related to abiotic factors since females prefer periods of greater rainfall 253 to carry out oviposition and ensure soils with a higher moisture content (Mondino et al., 1997), 254 255 favorable to the development of eggs (Potter, 1983).

256

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265

266 We found that egg viability and incubation duration were negatively affected by manipulation,

267 reinforcing the importance of soil chambers built by gravid females to protect their eggs

268 (Triplehorn & Johnson, 2011; Nogueira et al., 2013) and the negative influence of environmental

stress on egg development (Potter & Gordon, 1984). The shells of freshly oviposited eggs are

270 thin and highly susceptible to desiccation (Potter & Gordon, 1984) and the larvae hatched from 271 eggs subjected to stress are significantly smaller in size and sometimes unable to disengage from 272 the chorion membrane (Potter & Gordon, 1984). Thus, it is crucial to control egg conditions for 273 the successful breeding and rearing of *Cyclocephala* spp. This information on egg susceptibility 274 is particularly important because it can be applied to strategies on pest management during the initial ontogenic stage of targeted species (e.g., Burgess, 2009; Ferreira et al., 2011) or in 275 reinforcing egg care for pollinating and/or threatened species (e.g., Klusener et al., 2018; 276 277 Hanberry et al., 2020).

278

## 279 Conclusion

Our research assessed the egg development of three distinct *Cyclocephala* species from the Brazilian Atlantic forest hotspot (Myers et al., 2000). We found a positive correlation between egg mass and incubation duration, and each species presented idiosyncratic traits in egg development. Efforts towards the understanding of *Cyclocephala* embryogenesis already seem worthwhile from a developmental biology perspective and such investigations should become a standard on life cycle descriptions, helping for conservation and management strategies in agriculture or natural systems.

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- 527 Figure 1. Influence of time interval (days) on egg weight (mg) for three Cyclocephala species: (a) Cyclocephala
- 528 cearae, (b) Cyclocephala celata, and (c) C. para-guayensis. The black solid line is the linear regression of time
- 529 interval vs. egg weight for all individuals measured (black circles).





- 534 Figure 2. Histogram for the incubation duration of (a) Cyclocephala cearae and (b) C. celata. Black dotted line
- 535 represents the curve of the normal function.



- 540 Figure 3. Boxplots of initial and final egg weights, egg weight gain, egg incubation duration, and egg viability for
- 541 the three studied Cyclocephala species.
- 542
- 543 Table 1. Influence of egg density (number by batch) on initial and final mass (mg), mass gain, incubation duration
- 544 (days), and viability (%) for three species of *Cyclocephala*.
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- 549 Figure 4. Boxplots showing the influence of manipulation in the laboratory on viability (%) and incubation (days)
- 550 eggs of (a) C. celata and (b) C. paraguayensis. The letters represent a significant difference between individuals
- 551 manipulated and unmanipulated.

