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Communication

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Observations on nesting activity, life cycle, and brood ball morphometry of the Bordered Dung Beetle *Onitićellus cinctus* (Fabricius, 1775) (Coleoptera: Scarabaeidae) under laboratory conditions

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Abstract: The nesting activity, life cycle, and brood ball morphometry of the dung beetle *Oniticellus cinctus* (Fabricius, 1775) (Coleoptera: Scarabaeidae) were studied under laboratory conditions for the first time in India. The females made a brood chamber within the dung mass provided, wherein they made brood balls to lay eggs. The life cycle includes egg, larva (three instars), pupa, and adult stages. The total duration for the development was about one month. The study found that there was a significant difference present in the brood ball diameter (except in the first and second instars) and brood ball weight (except in the second instar and pupa) of the six life cycle stages. It was also found that brood ball weight and diameter have a significant positive correlation as well as a linear relationship.

Keywords: Morphometry, nidification, scarabaeid beetle, Scarabaeinae, weight-diameter relationship.

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Author contribution: APS—collection of samples, laboratory work, and preparation of the manuscript; KD—laboratory work, data analysis, and preparation of the manuscript; SM—collection of samples and laboratory work; RM—designing the study, directing and supervising laboratory work and data analysis, and preparation of the manuscript; VPU—designing the study, directing and supervising laboratory work and data analysis, and preparation of the manuscript.

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INTRODUCTION

The coleopteran insects (beetles) belonging to the subfamilies Aphodiinae and Scarabaeinae under the family Scarabaeidae are commonly called dung beetles as they feed primarily on mammalian dung and also use it for providing nesting and food for their larvae. The beetles of the subfamily Scarabaeinae are well-represented insects in the tropical regions (Filgueiras et al. 2009). Both sexes of the adults were identified with the help of published taxonomic keys (Chandra & Gupta 2013).

Globally, some studies have been done to understand nidification of dung beetles. Klemperer (1982a,b,c, 1983a,b,c, 1984) studied the nesting behaviour of several species of dung beetles. Biscoe (1983) studied the effects of ovarian condition on the nesting behaviour of *Copris diversus* Waterhouse, 1891. Sato & Imamori (1987) studied the nesting behaviour of the African Ball-roller *Kheper platynotus* (Bates, 1888). Edwards & Aschenborn (1987) studied patterns of nesting and dung burial in *Onitis* dung beetles. Davis (1989) studied nesting of the Afrotropical *Oniticellus* and its evolutionary trend from soil to dung.

So far, there are no studies to understand the morphometry of brood balls (the round-shaped ball made up of dung constructed by the female to lay eggs within it) as well as the weight-diameter relationship of different life cycle stages of *Oniticellus cinctus* from India.

MATERIAL AND METHODS

The study was carried out from the first week of May to the end of the second week of June 2017 for a period of about six weeks. The adults (both males and females) of *Oniticellus cinctus* were collected from dung that was 2–3 days old using hand-sorting method and were transported to the laboratory of the zoology department, Alpine Institute of Management and Technology, Dehradun.

Five pairs of beetles (one male and one female in each pair) were chosen for the study. Five rearing trays, each of 40cm (L) × 30cm (W) × 15cm (H) size were set up and filled up to two-thirds with a mixture of soil and sand. The mixture was moistened with the requisite amount of water. For the maintenance of adequate temperature, humidity, and darkness, each rearing tray was covered with inverted earthen pots. One pair of adults (one male and one female) was released in each tray. Fresh cow dung was provided and the old dung replaced daily.

After about six days from the release of the adults in the rearing trays, the nest construction occurred. A total of 50 brood balls (10 from each pair in each tray) were selected for our study; the rest of the brood balls were removed from the tray. Regular observations were conducted once a day (at 08.00h) by opening the brood balls to observe the development of the individual from egg to adult stage. The opening in the brood balls was immediately sealed after observation with the help of fresh dung. The weight and diameter of the brood balls were taken on the final day of each developmental stage by Kerro laboratory analytical balance (accuracy 0.01gm) and Mitutoyo digital vernier calliper.

One-way ANOVA followed by post hoc Dunn's test was performed to find out the presence of a significant difference (if any) in the diameter and weight of brood balls between lifecycle stages. Pearson's product-moment correlation coefficient (r) was calculated to explore the strength of association between the diameter and weight of brood balls between lifecycle stages. Linear regression model between diameter and weight of brood balls in different life cycle stages was calculated. All the statistical analysis was performed using R version 3.3.1 (R Core Team 2016).

RESULTS

The life cycle of *Oniticellus cinctus* includes egg, three larval (first, second, and third instar) stages, pupal stage, and adult.

The body of the adult (Image 1A) is dorsoventrally compressed and oblong and the colour is shiny black. The head is shining, smooth, and without any carina (elevation or ridge of the cuticle). The antennae are 8-segmented. The scutellum is visible. The pronotum is smooth and a deeply impressed median longitudinal line is present upon its posterior half. The elytra (external and sclerotized forewings) are deeply striated and each elytron has a pale yellow external border. Fore tibia of the male have small inner teeth with inner spur while that of the female have broad inner teeth and no spur.

The female mangled the dung gradually to prepare a lopsided (one side lower or smaller than the other) chamber initially. Finally, a hollow chamber (called brood chamber) of around 6–10 cm in width, 4–8 cm in height, and 5–7 cm in depth was constructed to store brood balls within the provided dung mass (Image 2). The females used prothoracic legs (Image

Image 1. *Oniticellus cinctus*: A—Adult male and female | B—Prothoracic leg of adult male and female.

Image 2. Brood chamber of *Oniticellus cinctus*: A—Initial brood balls | B—Final brood balls.

Image 3. Egg of *Oniticellus cinctus* within the brood ball.

1B) for the construction of the brood chamber or nest. Approximately 12–20 brood balls were constructed by each female and one egg was laid within each brood ball. The female beetle was present in the chamber during the entire period of the brood development. It also applied fresh dung on the brood ball during the entire period of the brood development and made the next nest after the development was completed.

Eggs were cylindrical and white or creamy and only one egg was present in each brood ball (Image 3). Egg development was completed and the larva hatched within 3–5 days (mean = 4.2 days, SD = 0.75; Fig. 1).

Three larval stages, namely first instar larva (Image 4), second instar larva (Image 5), and third instar larva (Image 6), were observed. The developmental time for the first, second, and third instars was 1–3 days (mean = 2.32 days, SD = 0.68), 1–3 days (mean = 2.24 days, SD = 0.74), and 10–16 days (mean = 13.52 days, SD = 1.52), respectively (Fig. 1). Larvae were C-shaped with a projecting hump, light grey; the head was somewhat light orange. Four segmented antennae and two segmented legs were present. Maxilla with galea and lacinia were distinctly separated in the larva.

The pupa (Image 7) was whitish and appeared pointed from the posterior portion. A large and blunt pronotal projection extended over a posterior portion of the head. Pupa development was completed within 4–8 days (mean = 5.58 days, SD = 1.2; Fig. 1).

The adult remained in the brood ball for 1–3 days (mean = 2.24 days, SD = 0.72; Fig. 1), after which it emerged. The total duration of the development was about one month (mean = 30.08 days, SD = 5.35).

Mean weight and diameter of the brood balls on the final day of egg development (freshly-hatched larva) were 0.27g (SD = 0.11; Fig. 2) and 6.25mm (SD = 1.24; Fig. 3), respectively. Mean weight and diameter of the brood balls on the final day of the first instar larval development was 0.50g (SD = 0.15; Fig. 2) and 9.55mm (SD = 1.48; Fig. 3), respectively. Mean weight and diameter of the brood balls on the final day of second instar larva development was 1.14g (SD = 0.30; Fig. 2) and 10.046mm (SD = 1.02; Fig. 3), respectively. Mean weight and diameter of the brood balls on the final day of third instar larval development was 1.83g (SD = 0.31; Fig. 2) and 12.012mm (SD = 1.47; Fig. 3), respectively. Mean weight and diameter of the brood ball on the final day of pupa development was 1.11g (SD = 0.23; Fig. 2) and 15.018mm (SD = 0.66; Fig. 3), respectively. Mean weight and diameter of the brood balls where freshly developed adults rested was 0.66g (SD = 0.26; Fig. 2) and 15.294mm (SD = 0.71; Fig. 3), respectively.

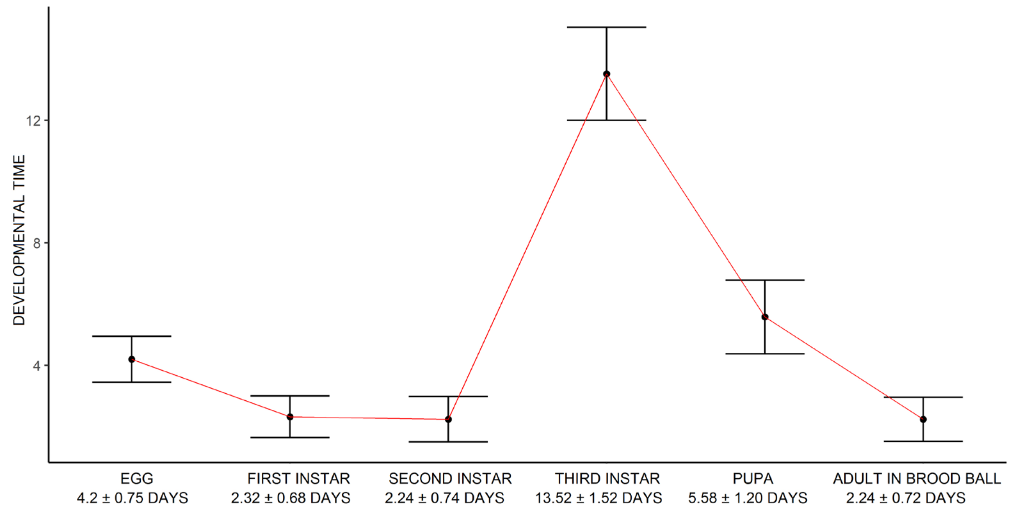

Figure 1. Comparative account of the mean developmental time of different life cycle stages of *Onitičellus cinctus*.

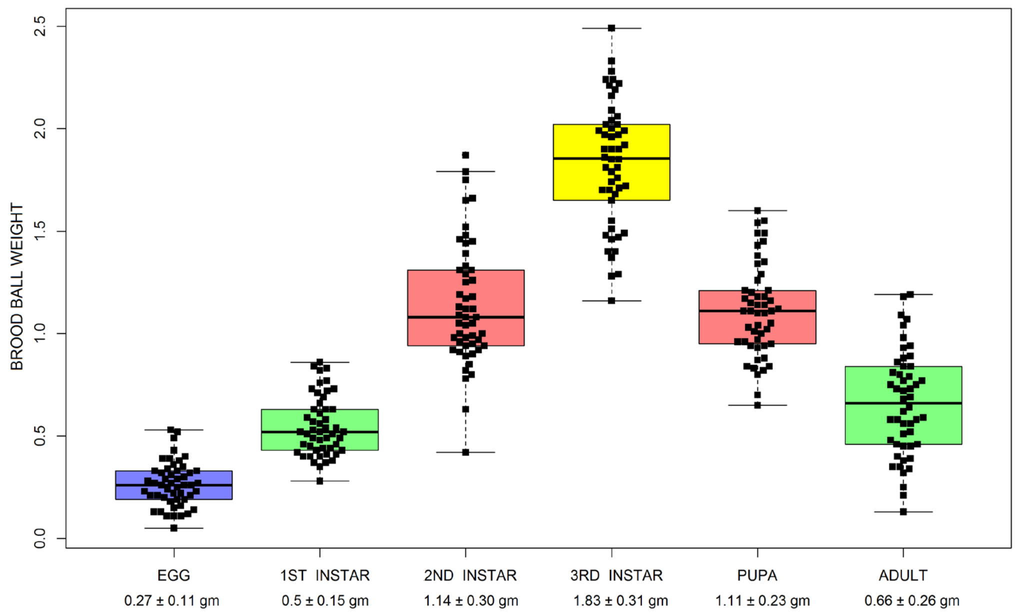

Figure 2. Comparative account of brood ball weight of different life cycle stages of *Oniticellus cinctus*. Life cycle stages marked by similar colour had no significant difference in the mean weight of the brood balls (post hoc Dunn's test, p > 0.05).

The result of one-way ANOVA showed that there was a significant difference present in the brood ball weight of the six life cycle stages ($F = 279.24$, $df = 5,294$; $p < 0.05$). The result of post-hoc Dunn's test suggested that there was no significant difference (at $\alpha = 0.05$) present in the brood ball weight of the second instar and pupa (z score = -0.066, $p = 0.474$; Fig. 2).

The result of one-way ANOVA showed that there was a significant difference present in the brood ball diameter of the six life cycle stages ($F = 458.84$, $df = 5,294$; $p < 0.05$). Result of post-hoc Dunn's test suggested that there was no significant difference (at $\alpha = 0.05$) present in the brood ball diameter of first instar and second instar (z score = -0.843, $p = 0.1995$) and of pupa and adult (z score = -0.594, $p = 0.276$; Fig. 3).

Pearson product-moment correlation between diameter and weight of brood balls in different life cycle stages was found to be significant ($p < 0.05$) and positive (Fig. 4). It was found that the weight of the brood balls of different life cycle stages had a simple linear relationship with the diameter of the brood balls (Fig. 4).

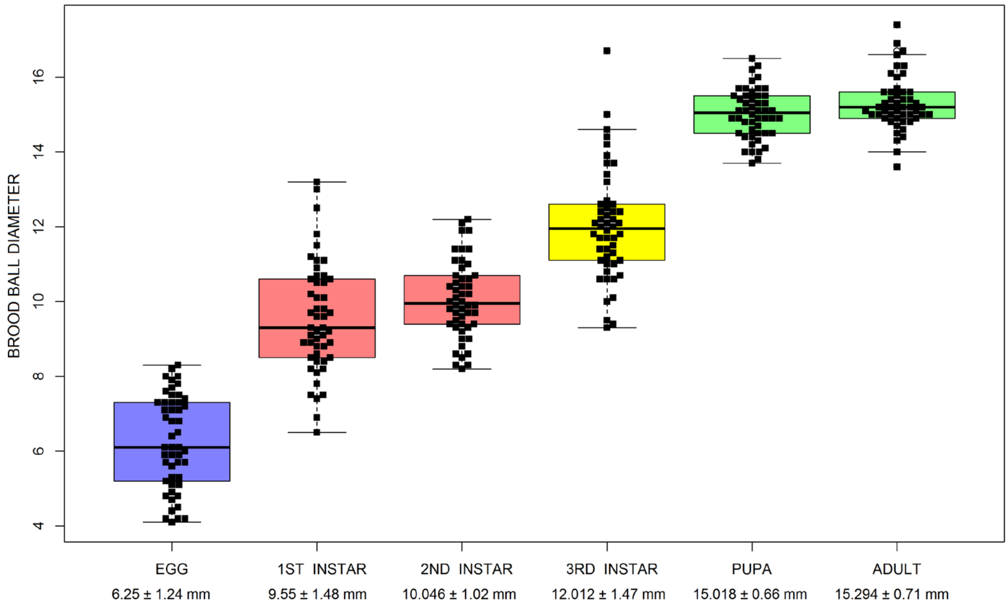

Figure 3. Comparative account of brood ball diameter of different life cycle stages of *Oniticellus cinctus*. Life cycle stages marked by similar colour had no significant difference in the mean diameter of the brood balls (post hoc Dunn's test, $p > 0.05$).

Image 4. First instar larva of *Oniticellus cinctus* within the brood ball.

Image 5. Second instar larva of *Oniticellus cinctus* within the brood ball.

DISCUSSION

Three groups of dung beetles are distinguished based on their behaviour in creating a brood mass, namely teleocoprids, paracoprids, and endocoprids (Ridsdill-Smith 2003). Teleocoprid dung beetles make balls of dung and roll the dung ball away from the dung pat and bury it in soil. Paracoprid dung beetles dig a tunnel in the soil under the dung pat, carry small piece of dung down that tunnel, and pack in to the end as a compacted brood mass. Endocoprid dung beetles construct brood balls in cavities within the dung pat (Ridsdill-Smith 2003). *Oniticellus cinctus*, which was chosen for the study, is an endocoprid dung beetle. This genus belongs to the variation 1 of Group 1 nidification category (Halffter & Matthews 1966) because the female prepares a small

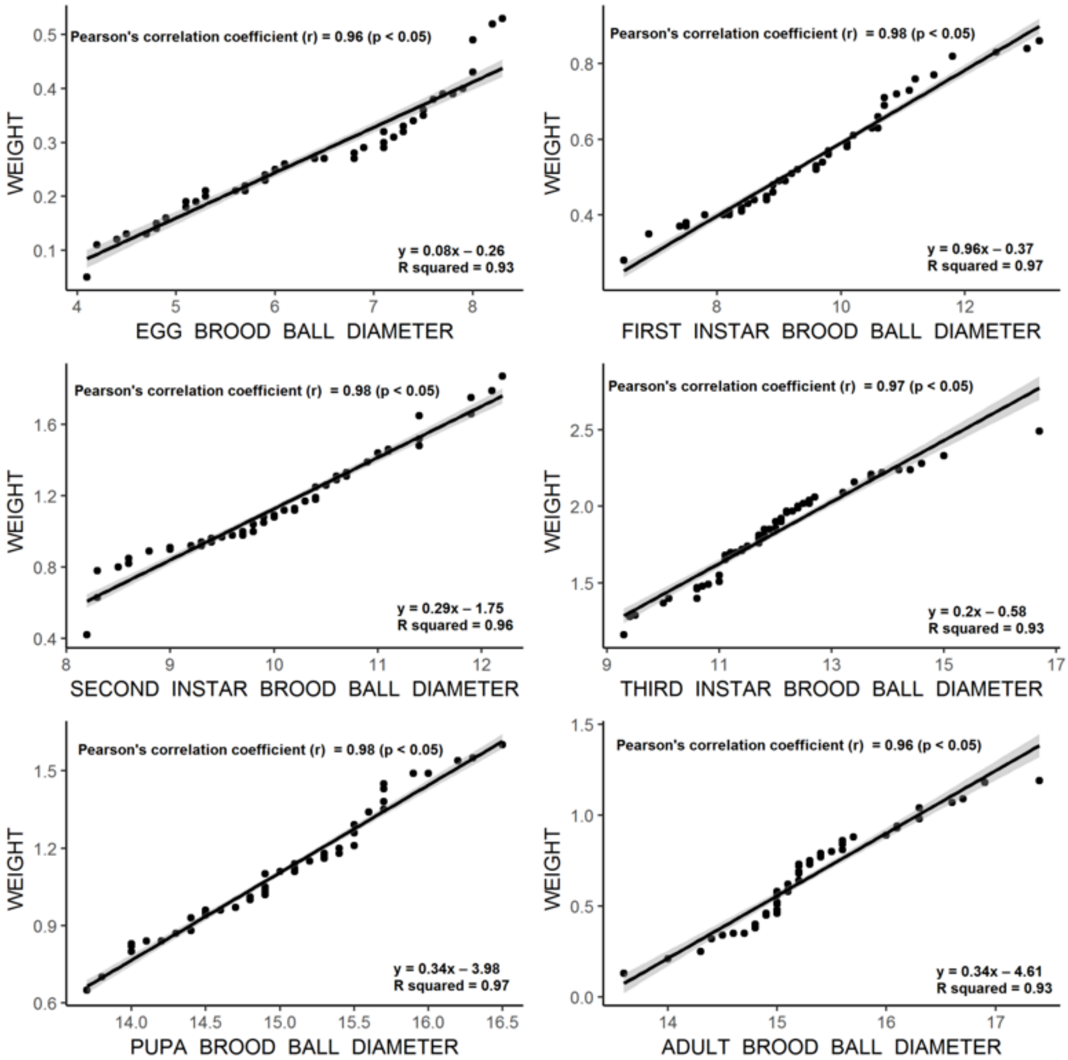

Figure 4. Pearson's product moment correlation and linear regression model between weight and diameter of brood balls in different life cycle stages of *Oniticellus cinctus*.

Image 6. Third instar larva of *Oniticellus cinctus* within the brood ball.

Image 7. Pupa of *Oniticellus cinctus* within the brood ball.

dung mass and lays one egg in each under the food source, i.e., the dung.

The present study found that the life cycle of *Oniticellus cinctus* is completed within six weeks with egg, three larval stages (first, second, and third instar), pupa and adult stages, of which duration of third instar larva is maximum.

By performing one-way ANOVA, it was found that the mean weight and mean diameter of the brood balls of different life cycle stages had significant differences; however, as it is an omnibus test, it did not specify which stage of the life cycle had different mean weight and mean diameter of the brood balls. Post hoc Dunn's test was performed to overcome this issue. It was found that there was no significant difference in brood ball diameter of first instar and second instar and of pupa and adult and there was no significant difference in brood ball weight of second instar and pupa and of first instar and adult. As correlation and simple linear regression models are two ways of exploring a potential linear relationship between the values of the two traits (Puth et al. 2014), these methods were applied to find the relationship between diameter of the brood balls of different life cycle stages of *Oniticellus cinctus*; it was found that weight and diameter of brood balls had significant ($p < 0.05$) positive correlation and they fit the simple linear model.

Previously only Klemperer (1983b) had studied the effect of the brood on parental care and oviposition of this dung beetle species. The present study had similarities with the study by Klemperer (1983b) in terms of morphometry of brood balls and developmental times for different life cycle stages. The present study reported the use of prothoracic legs by female to built brood chamber or nest. Klemperer (1983b) found that often a male adult was present in the nest when several beetles were present in the experimental setup. But the present study did not observe such thing, most probably because of only one pair of adult beetles (one male and one female) was released in each rearing tray for the study.

It is necessary to study the nidification of dung beetles of all three behavioural categories (teleocoprids, paracoprids, and endocoprids) in both laboratory and field conditions, especially the field-level nidification and brood ball morphometry studies in different seasons and habitats.

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Article

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Short Communications

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