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Karyotypic Analysis of *Oniticellus cinctus* (Coleoptera: Scarabaeidae: Scarabaeinae) from Kurukshetra, Haryana, India

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Abstract
This research investigated the chromosomal structure and dynamics of *Oniticellus cinctus*, a species from the Scarabaeinae subfamily within the Scarabaeidae family. The analysis utilized traditional Giemsa staining for morphological characterization, silver staining to detect nucleolar organizer regions (NORs), and C-banding to visualize constitutive heterochromatin. The karyotype analysis revealed a modal chromosome number of 2n=20 and a meioformula of 9AA+Xyp. Consistent with the typical characteristics of the Scarabaeinae subfamily, the chromosomes of *Oniticellus cinctus* revealed primarily metacentric and submetacentric. Silver staining indicated dense spots during the early pachytene stage of prophase I, corresponding to nucleolar organizer regions. The findings also explored a strong association between the chiasma frequency and terminalization coefficient in *Oniticellus cinctus* and other members of the Scarabaeinae subfamily. Overall, the study highlighted the conservative nature of this subfamily and emphasized the significance of chromosomal structure, centromere position, heterochromatin distribution, and NOR localization in understanding evolutionary relationships among beetles and other insects.

Keywords: *Oniticellus cinctus*, karyotype, cytogenetics, Scarabaeniae, Dung Beetles

Introduction

The order Coleoptera, through numerous speciation events, has emerged as the most species-rich group among insects, boasting the highest species diversity. As early as 1968, Arnett recorded 350,000 Coleoptera species. By 1978, Smith & Virkki had compiled chromosomal data for 2,160 species, subspecies, and parthenotes across 45 Coleoptera families, based on research conducted up to 1975. Over the past three decades, however, there has been a significant expansion in our understanding of chromosome cytology within this order. Despite its remarkable species diversity, cytogenetic studies employing specific banding techniques remain relatively scarce. Among the vast number of species, only about 390 (approximately 1.57%) have been analyzed, primarily using traditional staining methods, as reported by Arcanjo *et al* in 2009 [1]. The Scarabaeidae family, in particular, exhibits relatively conserved karyotypes, with more than half of the species displaying a diploid number of 2n=20, an Xyp sex determination mechanism and biarmed chromosomes; a chromosomal arrangement considered primitive for both the family and the entire Coleoptera order.

The subfamily Scarabaeinae, consisting of true dung beetles that feed exclusively on dung, plays a vital role in ecosystem services such as soil aeration, fertilization, nutrient cycling, pasture improvement and the biological control of pests and parasites. These contributions significantly enhance environmental health and provide agricultural benefits. This subfamily is highly diverse, comprising approximately 5,000 species across 234 genera worldwide, as documented by Hanski & Cambefort in 1991 [5]. It exhibits a wide range of chromosomal variations in number, shape, and size, ranging from 2n=8 in *Eurysternus caribaeus* to 2n=24 in *Oniticellus spinipes* and up to 2n=36 in *Gymnopleurus miliaris*, with the Xyp sex chromosome mechanism being the most commonly observed (Smith & Virkki 1978, Kaur & Yadav 2014) [15]. *Oniticellus cinctus*, in particular, is widely distributed across tropical Asia and is valued in traditional Chinese medicine for its detoxifying and anti-inflammatory properties. Additionally, dung beetles, including *Oniticellus cinctus*, play a crucial role in limiting the spread of helminth parasites among animals. This study examines the chromosomal structure and sperm count per bundle in *Oniticellus cinctus*.

Materials and Methods

Collection: Adult male specimens of *Oniticellus cinctus* were collected from the village Kirmich of Kurukshetra, Haryana, India. The latitude 29^0 54' 7.0332" and longitude 76^0 47' 10.684" are the geo coordinates of the Kirmach.

Slide preparation: Beetles were sacrificed in 0.56% potassium chloride (KCl) solution for the removal of testicular material. Testes were then treated with 0.001% colchicine for 20 minutes to arrest cell division, followed by a 20-minute incubation in 1% sodium citrate solution for hypotonic treatment. Subsequently, the tissue was fixed in a fixative composed of acetic acid and methanol (1:3) for 30 minutes. Fixed testicular tissues were then processed for slide preparation using the air-drying technique.

Staining Procedure: Chromosomal structures and behavior were examined following the standard staining procedure described by Yadav & Lyapunova 1983 [16]. Testicular material was placed in 2–3 drops of 50% glacial acetic acid on grease-free, ethanol-cleaned slides. The tissue was macerated with a fine needle, air-dried and stained using 2% Giemsa solution.

Photography: Photomicrographs of a minimum of 10 well-defined metaphase spreads were captured using an Olympus C-7070 digital compact camera. These images were analyzed for chromosome number, morphology, and karyomorphometric characteristics. Chromosomes were

classified based on centromere position into metacentric (m), submetacentric (sm), subtelocentric (st) and telocentric (t) categories, according to the criteria established by Levan *et al* 1964 *vivo*[14].

Analysis: Karyomorphometric analysis was performed on 10 metaphase plates exhibiting clearly visible centromeres and intact, non-overlapping chromosomes, as per the methodology of Yadav & Lyapunova 1983. Parameters measured included percentage relative length of chromosomes

$$Percentage\ Relative\ length\ of\ chromosome = \frac{\text{Length of individual chromosome}}{\text{Total length of chromosomes in one set}} X\ 100$$

Additionally, the silver staining method described by Bloom & Goodpasture 1976 [3], is used to visualize the nucleolar organizer regions (NORs).

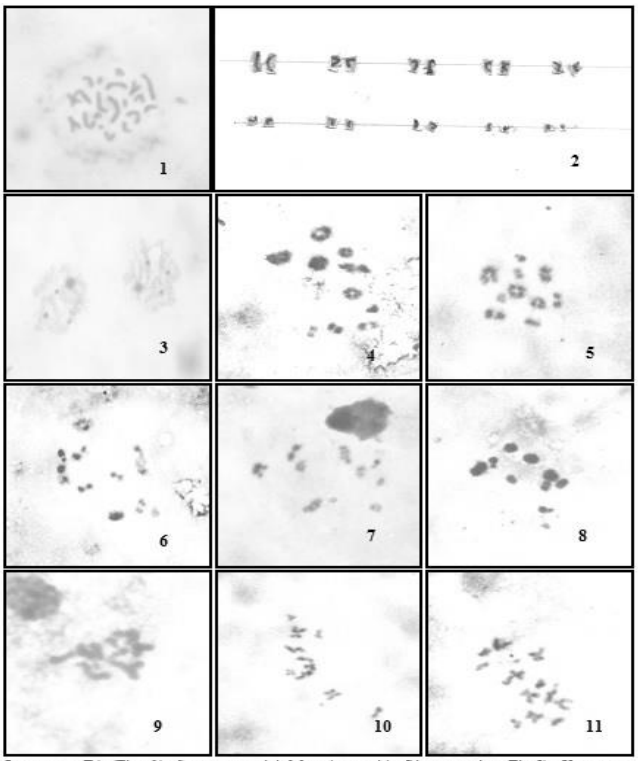
Results and Discussion

Spermatogonial metaphase analysis revealed a diploid chromosome number of 2n = 20 (Fig. 1). The karyotype comprises five pairs of metacentric autosomes (pairs 1, 2, 5, 6, and 8), three pairs of submetacentric autosomes (pairs 3, 4, and 7), and one pair of acrocentric autosomes (pair 9). The sex chromosomes include an acrocentric X and a small y chromosome (Fig. 2). The percentage relative length of autosomes ranged from 6.97% to 20.0%, while the X and y chromosomes measured 5.32% and 4.46%, respectively (Fig. 12). During diakinesis, three ring-shaped and six rod-shaped autosomal bivalents were observed, along with a sex "parachute" structure (Figs. 4-6). At prometaphase I, chromosomal condensation and complete terminalization of chiasmata were evident (Fig. 7). The metaphase I plate consisted of nine autosomal bivalents—arranged as rings and dumbbells—and a sex pseudobivalent in the form of a parachute (Fig. 8). The mean chiasma frequency per bivalent was 1.3, with a terminalization coefficient of 1.0. The meioformula for this species is represented as 9AA + Xyp.

The second meiotic division followed a typical pattern and showed no notable deviations. During early prophase II, autosomes appeared as condensed, thick chromatids (Fig. 9). Two types of metaphase II plates were recorded: one containing the X chromosome (Fig. 10), and the other containing the y chromosome (Fig. 11), each accompanied by nine autosomes. The average number of spermatozoa per bundle in this species was 130. At the early pachytene stage, dot-like silver-stained regions were observed on thick thread-like chromatids, along with a large amorphous sex vesicle (Fig. 3).

The Scarabaeidae family maintains a relatively conserved genetic composition, characterized by a chromosome number of 2n=20, the Xyp sex determination mechanism, and predominantly metacentric chromosomes, as reported by multiple researchers (Yadav & Pillai 1977, Smith & Virkki 1978, Kaur & Yadav 2011, 2014, 2018, 2021, 2024, Kaur 2019, 2022, Kaur & Kakkar 2022) [6, 7, 8, 9, 10, 11, 12, 13]. However, the subfamily Scarabaeinae exhibits notable chromosomal diversity in both structure and size compared to other scarabs. Cytogenetic studies have identified more than 170 species within Scarabaeinae, with chromosome numbers ranging from 2n=8 in *Eurysternus caribaeus* to 2n=24 in *Oniticellus spinipes* and up to 2n=36 in *Gymnopleurus miliaris*, while the Xyp system remains the predominant sex chromosome configuration (Smith & Virkki 1978, Yadav *et al* 1990, Kaur & Yadav 2014) [19]. This variation positions Scarabaeinae as a less conservative subgroup within Coleoptera. The presence of diverse chromosome sizes and structures within a single karyotype in Scarabaeinae suggests the occurrence of structural chromosomal rearrangements.

This report confirms the chromosome number of *Oniticellus cinctus* and the first detailed morphometric chromosome analysis of this species. Within the Scarabaeidae family, chiasma formation is generally low, with most species displaying a single chiasma positioned near the terminal region of each bivalent, indicating a degree of genetic stability. This pattern has also been observed in *Oniticellus cinctus*, as noted by Yadav and Pillai (1977) [17].



O. cinctus (F.) (Fig. 1): Spermatogonial Metaphase with Giemsa stain; (Fig.2): Karyotype; (Fig.3): Pachytene showing silver staining; (Fig. 4): Diakinesis; (Fig. 5&6): Diakinesis with Xyp; (Fig. 7): Prometaphase I; (Fig. 8) : Metaphase I; (Fig. 9): Prophase II; (Fig.10): Metaphase II with X; (Fig. 11): Metaphase II with y

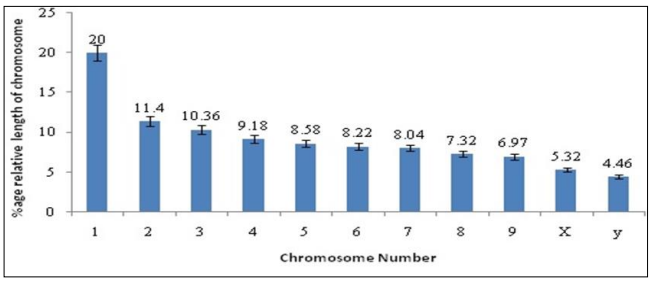


Fig 12: Percentage relative of chromosomes in *Oniticellus cintus*

Research suggests that variations in chromosome numbers within this group stem from evolutionary modifications, including: (i) chromosome loss, (ii) enlargement of the Y chromosome, (iii) a reduction in autosome numbers due to fusion events, (iv) a decrease in chromosome count resulting from the fusion of the X chromosome with an autosome. This data revealed the Scarabaeidae family as conservative for the chromosome number but other morphometric parameters showed the variation in subfamily Scarabaeinae

between genera. Arcanjo *et al* 2013 [2] reported the detailed chromosomal analysis of *Phanaeus* (*N.*) *splendidulus* and described the six pairs of acrocentric chromosomes, one pair of metacentric and two pairs of submetacentric in the karyotype. Whereas in present report species from the genus *Oniticellus* in the same subfamily Scarabaeinae showed the five pairs of metacentric, three pairs of submetacentric and only one pair of acrocentric chromosomes were reported.

Variations in the centromeric positions of chromosomes among different genera within the same subfamily indicate a degree of karyotypic diversity. A conventional chromosomal analysis by Carbell-De-Mello *et al* (2008) revealed significant karyotype variation among members of the subfamily Scarabaeinae. This variability was attributed to various chromosomal rearrangements, including autosome–autosome and X–autosome fusions, pericentric inversions, Y chromosome loss, and chromosomal fissions. Such rearrangements are also key contributors to the overall karyotypic diversity observed within the Scarabaeidae family. Applying differential and molecular cytogenetic methods to members of this group could help identify distinct chromosomal markers, thereby enhancing our understanding of chromosomal differentiation across various tribes of Scarabaeinae.

Conflict of Interest

There aren't any disclosed conflicts of interest.

Acknowledgments

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