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New cytological data of dung beetle species from the genus *Onthophagus* Latreille (Coleoptera: Scarabaeidae, Scarabaeinae) from Haryana

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ABSTRACT
The aim of present study was to describe the karyotype of species belonging to the genus *Onthophagus* and to compile the conventional cytogenetic data available in the literature for this group. Chromosomal investigations on three *Onthophagus* spp. viz. *O. fasciatus* (Boucomont, 1914), *O. unifasciatus* (Schaller, 1783) and *O. bifasiatus* (Fabricius, 1781) were analysed using conventional staining and C-banding. All the three species possessed 20 chromosomes and a modal polyphagan karyotype with Xyp as the male sex chromosome mechanism. Most of the autosomal chromosomes were metacentric or submetacentric, while X and y showed acrocentric nature. Modal chromosome number of these species showed the conservative nature of the karyotype. C-bands were located predominantly at peri-centric position. Along with this chiasma frequency, terminalisation coefficient and sperm number count were also calculated which again showed a close relationship among these species.

Keywords: *Onthophagus*, karyotype, Chromosomal investigations, C-banding, chiasma frequency

1. Introduction
Genus *Onthophagus* belongs to family Scarabaeidae in the superfamily Scarabaeoidea. This genus includes around 2000 species, distributed globally [1]. Despite of the large number of species, only 41 species have been analysed (Table 2), predominantly using conventional staining [2, 3, 4, 5, 6, 7]. There is at present no published information on the chromosomes of two of the species of *Onthophagus* presented here. Therefore material in this report provides the first opportunity for comparing the C-banded karyotypes of a suite of small scarabaeoid dung beetles of genus *Onthophagus*. Although all the species of *Onthophagus* exhibited a modal number of chromosomes, they vary in their chromosomal morphology, percentage relative length and location of C-bands.

2. Materials and methods
Adult male specimens of *O. fasciatus* (Bouc.), *O. unifasciatus* (Schall.) and *O. bifasiatus* F. were collected from Seonti forest, Kurukshetra (Haryana, India) in the month of August 2011. The male beetles were sacrificed in 0.56% KCL solution. The testicular material on removal was treated with 0.001% colchicine for 20 minutes. Then it was kept in 1% sodium citrate solution for 20 minutes at room temperature. After the hypotonic treatment the material was fixed in cold 1:3 acetic-methanol for 20 minutes giving 2 or 3 changes. Fixed material was used for the preparation of slides by air drying method [8]. The method was as follows:

The testicular material was taken in a small amount of 50% glacial acetic acid on a clean grease free slide, which was immersed in dehydrated ethanol and cleaned by a piece of muslin cloth. The testes were macerated by means of dissecting needles. The slides were then allowed to dry in air and stained in 2% Giemsa stain.

C-bands were determined using the methods of Sumner [9]. Evaluation of chromosomal morphology was based on ten spermatogonial metaphases. Selected stages were micro-photographed using oil immersion objective (100X) and digital compact camera (Olympus, C-7070). Chiasma frequency per bivalent was calculated from randomly scored diakinetic/ metaphase I stages in each species by applying the formula as

Chiasma frequency = Total number of chiasmata per cell/ no. of bivalents per cell.

Percentage relative Length of each chromosome was calculated, which represented the length of each chromosome expressed as a percentage of the total haploid autosome length in the nucleus.

3. Results

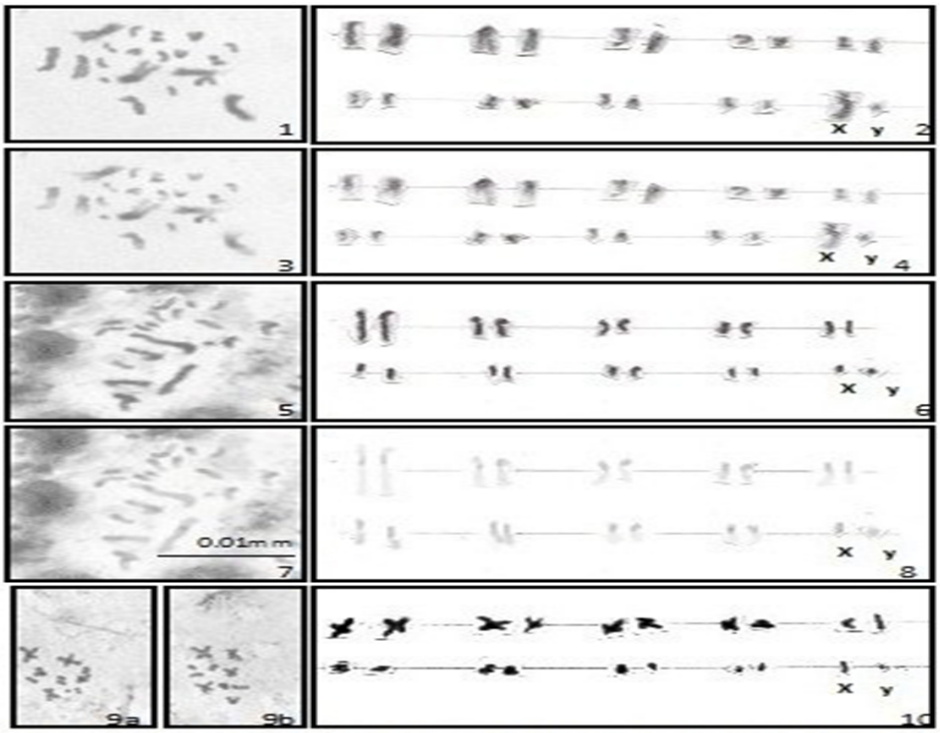
O. fasciatus (Boucomont, 1914)

The diploid number of 20 chromosomes was depicted by spermatogonial metaphase (Fig. 1). The karyotype was comprised of one pair of metacentric (pair 1), five pairs of submetacentric (pairs 2-6) and three pairs of acrocentric (pairs 7-9) autosomes along with sub telocentric X and acrocentric y chromosomes (Fig. 2). Percentage relative length of autosomes varied from 4.94 to 15.90, whereas that of X and y was 16.60 and 4.42, respectively (Table 1). The X chromosome was the largest element of the karyotype and y the smallest.

Metaphase-I possessed nine rod shaped autosomal bivalents and the sex pseudo bivalent resembling a parachute (Fig. 11). Mean chiasma frequency and terminalization coefficient per bivalent at metaphase-I was 1.0 and 1, respectively. The meio-formula of this species is 9AA+Xyp.

First maturation division resulted in the formation of two types of secondary spermatocytes and hence two types of metaphase-II plates 9A+X (Fig. 13) and 9A+y (Fig. 14). The average number of spermatozoa per bundle counted for this species was 120.

C-banding: C-banding was applied to the fixed stages of spermatogonial metaphase (Fig. 3) and metaphase-I (Fig. 12). Karyotype revealed the peri centromeric localisation of constitutive heterochromatin in pairs 1,2,3,4,7 and 8 of autosomes while very small and light bands were observed in X and y chromosomes (Fig. 4). Prominent bands were obtained in autosomal bivalents and sex parachute in metaphase-I (Fig. 12).



Figures 1-4: *O. fasciatus* 1. Spermatogonial metaphase. 2. Karyotype; C-banded 3. Spermatogonial metaphase. 4. Karyotype; Figures 5-8: *O.unifasciatus* 5. Spermatogon metaphase. 6. Karyotype; C-banded 7. Spermatogonial metaphase. 8. Karyotype; Figures 9-10: *O. bifasciatus* 9a Metaphase II with y. 9b Metaphase II with X. 10. Karyotype from Metaphase II

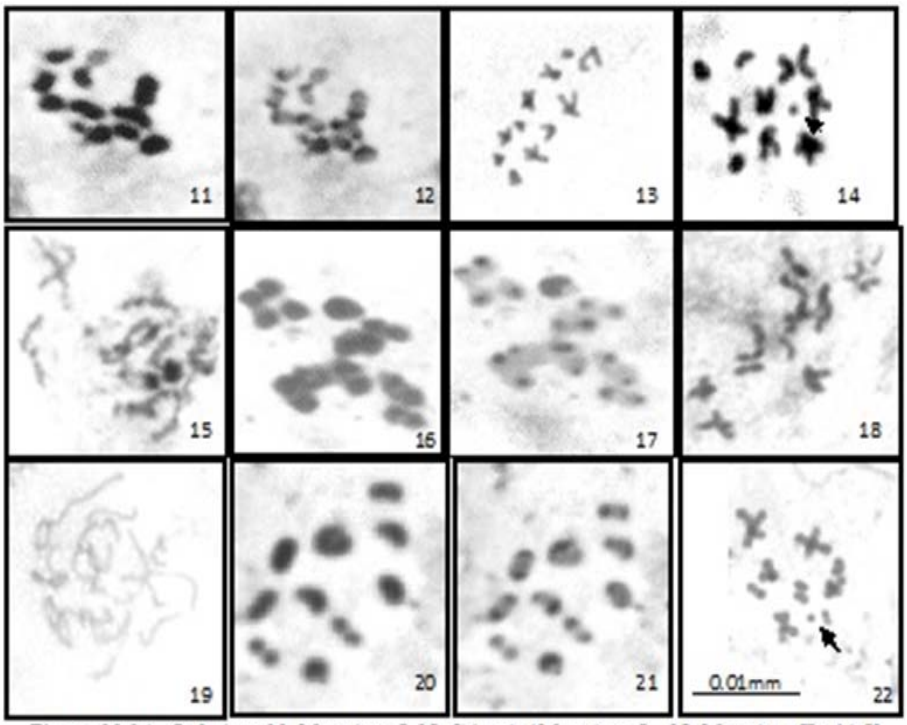
O. unifasciatus (Schaller, 1783)

This species revealed a diploid set of 20 chromosomes in spermatogonial metaphase (Fig. 5). Chromosomes were categorised in three pairs of metacentric (pairs 1, 3, 4), five pairs of sub metacentric (pairs 2, 5-8) and one acrocentric (pair 9) autosomes, while X and y were acrocentric sex chromosomes (Fig. 6). Percentage relative length of autosomes varied from 6.76 to 13.24, whereas that of X and y was 6.28 and 2.38, respectively (Table 1). The X chromosome was smaller than the last pair of autosome and the y was the smallest element of the karyotype.

At pachytene stage the thick chromatid threads were found overlapping each other and gave woolly appearance (Fig. 15). Metaphase-I revealed nine rod shaped autosomal bivalents and a sex parachute (Fig. 16). Mean chiasma frequency and terminalisation coefficient per bivalent at metaphase I was 1.0 and 1, respectively. The meio-formula of this species is 9AA+Xyp.

One metaphase-II plate was encountered with X chromosome (Fig. 18), in addition to nine autosomes which were always present. All the chromosomes showed clear chromatid separation at this stage, thus revealing finer details of their morphology. The average number of spermatozoa per bundle counted for this species was 120.

C-banding: C-banded spreads of spermatogonial metaphase (Fig. 7) and metaphase-I (Fig. 17) were obtained. C-banded karyotype of *O. unifasciatus* possessed pericentromeric distribution of constitutive heterochromatin in all the pairs of autosomes and X chromosome, while y being euchromatic with no band (Fig. 8), which is quite unusual.



Figures 11-14: *O. fasciatus* 11. Metaphase-I, 12. C-banded Metaphase-I, 13. Metaphase II with X, 14. Metaphase II with y; Figures 15-18: *O.unifasciatus* 15. Pachytene, 16. Metaphase-I, 17. C-banded Metaphase-I, 18. Metaphase II with X, Figures 19-22: *O. bifasiatus* 19. Spermatogonial prophase, 20. Metaphase-I, 21. C-banded Metaphase-I, 22. Metaphase II with y. Arrow shows y chromosome in fig. 14&22

***O. bifasiatus* (Fabricius, 1781)**

The diploid chromosome number 20 was inferred by 10 bivalents at metaphase-I plates (Figs. 20 and 21). Spermatogonial metaphase was not available for study in the material under investigation. Spermatogonial prophase exhibited large chromosomal threads which acquired a typical morphology and scattered throughout the cytoplasm (Fig. 19). The karyotype was prepared from the metaphase II spreads (Figs 9a & 9b). Chromosomes were categorised in one pair of metacentric (pair 1), five pairs of sub metacentric (pairs 2-6) and three pairs of acrocentric (pairs 7-9) autosomes, while X and y were acrocentrics (Fig. 10). Percentage relative length of autosomes varied from 3.18 to 11.44, whereas that of X and y was 5.69 and 2.14, respectively (Table 1). The X chromosome was smaller than the seventh pair of autosome and the y was the smallest element of the karyotype.

Metaphase-I revealed nine highly condensed rod shaped autosomal bivalents and heteromorphic sex bivalent in the form of a parachute (Fig. 20). Mean chiasma frequency and terminalisation coefficient per bivalent at metaphase-I was 1.0 and 1, respectively. The meio-formula of this species is 9AA+Xyp.

Metaphase-II plates were of two types, one with X chromosome (Fig. 9a) and the other with y chromosome (Fig. 22), in addition to nine autosomes. The average number of spermatozoa per bundle counted for this species was 128.

C-banding: Application of C-banding revealed large C-bands in the seven autosomal bivalents and sex bivalent in metaphase-I plate, corresponding to the constitutive heterochromatin (Fig. 21).

Table 1: Percentage relative length of chromosomes of species under study

Sr. No.	Species	Percentage Relative length of chromosomes										
		Chromosome Pairs										
		1	2	3	4	5	6	7	8	9	X	y
1.	*O. fasciatus*	15.90	13.57	11.60	9.54	6.47	6.04	5.46	5.36	4.94	16.60	4.42
2.	*O. unifasciatus*	13.24	11.60	11.56	11.33	10.08	8.99	9.03	8.69	6.76	6.28	2.38
3.	*O. bifasiatus*	11.44	11.40	10.38	9.18	9.00	6.78	6.56	4.33	3.18	5.69	2.14

4. Discussion

The Scarabaeinae constitutes a highly diverse subfamily that comprises about 5000 described species belonging to 234 genera spread widely in the world [10]. Although this subfamily is considered to be chromosomally conserved, generally presenting biarmed autosomes and a sex mechanism of the parachute type (Xyp), some chromosome rearrangements have led to changes in chromosome morphology, size and diploid numbers [11].

Table 2: The chromosome number and Meioformula of subfamilies under study PR= Present Report

S. No.	Species with classification Subfamily: Scarabaeinae	Diploid number	Meioformula	References
1.	*O. nthophagus amyntas Ol.*	20	-	Virkki (1951)
2.	*O. andalusicus italicus Goid.*	20	-	Salamanna (1972)
3.	*O. bonasus F.*	20	9+Xyp	Yadav and Dange (1988, 1989)
			9+Xyr	Joneja (1960)
4.	*O. bifasiatus F.*	20	9+Xyp	Yadav and Dange (1988), Yadav et al. (1989) , PR
				PR
5.	*O. fasciatus*	20	9+Xyp	PR
6.	*O. unifasciatus*	20	9+Xyp	Kacker (1970) , Manna and Lahiri (1972), Yadav and Pillai(1977)
7.	*O. catta F.*	20	9+Xyp	Joneja (1960)
				Kacker (1970)
			9+Xyr	Virkki (1951)
8.	*O. dama F.*	20	9+Xyr	Virkki (1954) , Wilson and Angus (2005)
9.	*O. Fractions Prey.*	20	-	Smith (1960), Virkki (1960)
10.	*O. furcatus prey*	-	9+Xy	Vidal (1984)
11.	*O. hecate Panz*	-	9+Xyp	Virkki (1954) , Wilson and Angus (2005)
12.	*O. hirculus Mannerheim*	20	9+Xyp	Virkki (1951), Virkki (1954)
13.	*O. illyricus S. DeG.*	-	9+Xy	Smith and Virkki (1978)
14.	*O. lemur F.*	20	-	Yadav and Pillai(1977)
15.	*O. marginicollis Har.*	-	9+Xyp	Manna and Lahiri (1972)
16.	*O. mopsus (F.)*	20	9+Xyp	Virkki (1951), Virkki (1954)
17.	*O. mopsus gracilicornis Germar*	20	9+Xyp	Virkki (1954)
18.	*O. nuchicornis L.*	20	-	Yadav and Dange (1988, 1989), Yadav et al. (1989)
19.	*O. ovatus F.*	20	-	Yadav and Dange (1988)
20.	*O. pacificus Lansb.*	20	9+Xyp	Virkki (1951)
21	*O. pennsylvanicus Har.*	-	9+Xyp	Yadav and Pillai(1977, 1979)
22.	*O. punctatus Ill.*	-	10II	
23.	*O. quaestus Sharp*	-	9+Xyp	Manna and Lahiri (1972) , Yadav and Pillai(1977,1979)
24.	*O. ramosellus Bates*	20	9+Xyp	Smith and Virkki (1978) Virkki (1951), Wilson and Angus (2005)
25.	*O. rufescens Bates*	-	9+Xyp	Virkki (1951), Wilson and Angus (2005)
26.	*O. ruficapillus Br.*	20	9+Xy	Virkki (1951), Virkki (1954) Wilson and Angus (2005)

27.	*O. taurus Schreb.*	20	9+Xy	Virkki (1954) Yadav et al. (1993)
28.	*O. vacca L.*	20	-	Wilson and Angus (2005) Wilson and Angus (2005)
29.	*O. verticicornis Laich.*	20	-	Wilson and Angus (2005)
30.	*O. quadridens*	20	9+Xyp	Wilson and Angus (2005)
31.	*O.* stratocirrus *Graells*	20	9+Xy	Wilson and Angus (2005)
32.	*O. similis (Scriba)*	20	9+Xy	Wilson and Angus (2005)
33.	*O. coenobite (Herbst)*	20	9+Xy	Wilson and Angus (2005)
34.	*O. lucidus (Sturm)*	20	9+Xy	Wilson and Angus (2005)
35.	*O. ovatus (L.)*	20	9+Xy	Wilson and Angus (2005)
36.	*O. Joanna Golgan*	20	9+Xy	Wilson and Angus (2005)
37.	*O. gazelle (F)*	20	9+Xyp	Wilson and Angus (2005)
38.	*O. albicornis (Baeuvois)*	20	9+Xy	
39.	*O. opacicollis*	20	9+Xy	
40.	*O. maki (Illeger)*	20	9+Xy	
41.	*O. hirtus (Illeger)*	20	9+Xy	

The chromosomes of genus *Onthophagus* are of particular importance as it is the one case so far known where all the cytogenetically known species of a genus have modal number of chromosomes i.e. 2n= 20 including 9 pairs of autosomes ranging from medium metacentric to small acrocentric plus Xyp/ XY/ Xyr sex chromosomes [12, 13, 14, 15, 16].

All cytogenetically known species of genus *Onthophagus* show the modal number of chromosomes 2n=20 according to the reports given by different scientists [2, 3, 4, 5, 12, 13, 17, 18, 19, 20, 21, 22, 23] thereby exhibiting the conservative nature of this genus (Table 2). However, the karyotype of *O. unifasciatus* is distinguishable from that of *O. fasciatus* by the presence of three metacentric chromosomal pairs instead of one metacentric pair. Whereas, three acrocentric pairs (pairs 7-9) are found in *O. fasciatus* and only one acrocentric pair in *O. unifasciatus* which again confirms the earlier reports of centromeric polymorphism in chromosomes of this genus [17]. Again, the percentage relative length of chromosomes of these species also varied from 4.95 to 15.90 of autosomes in *O. fasciatus* and from 6.76 to 13.24 of autosomes, in *O. unifasciatus* (Table 1). It is, therefore, possible that there may be some structural chromosomal differences between the species, resulting from peri centric inversions or trans version, which gave rise to the centromeric polymorphisms in autosomes of *O. fasciatus* and *O. unifasciatus* [17].

Karyotype *of O. fasciatus a*nd *O. bifasiatus* are very similar in the localisation of centromere and the number of chromosomes (Fig. 2 and 10), except the sub telocentric X chromosome in *O. fasciatus* and acrocentric in *O. bifasiatus*. Percentage relative length of the chromosomes in these species also varied (Table 1).

Chiasma frequency per bivalent for all of the three species is 1 which confirms the earlier reports given by [2, 3, 4, 5, 6]. The karyotype data of these species appears to be useful in showing differences between apparently similar species of genus *Onthophagus*. The one instance where the karyotypes fail to show difference between members of a species-pair is that of *O. fasciatus* and *O. unifasciatus*.

Lack of karyotype differences is not in itself evidence of co specificity. There is no feature of the karyotypes which appears to be specific for any of the genera studied, but the absence of entirely heterochromatic chromosome arms in any of the *Onthophagus* species studied is worth noting and agreed the earlier reports [17]. Such arms are present in both *Euoniticellus* and *Euonthophagus*, and are often associated with interspecific variation in *Aphodius* [22]. But in the present studies C-bands were localised at peri centric regions in autosomes whereas Small bands were appear in X and y sex chromosomes in *O. fasciatus* and only X chromosome in *O. unifasciatus* with y being the euchromatic.

5. Conclusions

Chromosomal differences among the species in the present study were due to the peri centric inversion and trans version. Because peri centric inversion leads to the change in the centromeric position if the centromere involved in the inversions as in case of *O. unifasciatus* and *O. fasciatus* , while trans version leads to the change in the chromosomal length without changing the location of centromere as in *O. fasciatus* and *O. bifasiatus*. To confirm the processes involved in the evolutionary changes at the molecular level and to understand the right place of the species belonging to genus *Onthophagus* in taxonomy, further exploration of this genus is needed.

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