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Karyotypic characterisation and C-banding of representatives from Geotrupinae and Dynamopinae (Coleoptera: Scarabaeidae)

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Abstract: *Meiotic chromosomes obtained from members of the coleopteran subfamilies Geotrupinae and Dynamopinae were studied using standard and C-banding techniques. The study presents detailed karyotypic description of two Geotrupinae species viz. Bolboceras indicum Westw. and Bolboceras quadridens F. and one Dynamopinae species, Dynamopus athleta Sem. with special emphasis on the distribution and variability of constitutive heterochromatin. The karyotypes in B. indicum and B.quadridens consisted of 2n=20 (9II + Xyp), with seven metacentric and two acrocentric pairs of autosomes which gradually decreased in size. For D. athleta the karyotype was 2n= 22 (10II + Xyp) with meta- and submetacentric autosome showing gradations in size. In all the three species the constitutive heterochromatin (CH) was predominantly pericentromeric.*
Keywords*: Geotrupinae, Scarab beetles, Dynamopinae, Chromosomes, C-banding, Karyotype*

I. Introduction

The cosmopolitan beetle family Scarabaeidae comprises approximately 2000 genera and 25,000 species [1]. Some of their representatives show important functions as pollinators of plants, organic matter recyclers and biological controllers of agricultural pests and acting moreover as indicators for the analysis of biodiversity in tropical forest [2]. Similarly, representatives of the subfamilies Geotrupinae and Dynamopinae are ecologically important as nutrient recyclers because of their feeding and burrowing habits. The cytological information of these beetles is useful in many ways. Theoretically, it is important to understand the mechanism governing the transmission of genetic information, hence speciation and for classification at taxonomical levels. It is also useful in the field of applied economic entomology, where it may be utilized on the one hand for genetic improvement of economically useful insects and on the other hand for controlling the damage by noxious beetles like pests.

Despite the large number of species, there are few studies about the chromosomal diversity of Scarabaeidae representatives and approximately, only 390 species (1.57%) have been analysed, predominantly using conventional staining [1]. This family shows conserved karyotypes with more than 50% of the species presenting the diploid number 2*n* = 20, Xyp sex mechanism and biarmed chromosomes. This condition has been considered primitive to this group and also to the whole order Coleoptera [3, 4, 5, 6, 7, 8]. Out of 1,000 species of Geoptrupinae, only 13 species have been cytologically analysed. Whereas only one species of the subfamily Dynamopinae has been recorded from India [9] and had been cytologically analysed [10].

About 70 Scarabaeidae species have so far been subjected to differential or molecular cytogenetic techniques, such as C banding, base-specific fluorochromes, silver nitrate staining or fluorescence *in situ* hybridization [5, 6, 7, 11, 12, 13, 14]. The constitutive heterochromatin in this family is predominantly located in the pericentric region of the chromosomes. Moreover, species with telomeric, interstitial CH and diphasic chromosomes have been described in this family [5, 6, 13, 15, 16, 17, 18].

Previously, only conventional staining was used for the cytological investigations of *B. indicum, B. quadridens* and *D. athleta* [10]. In the present study, conventional staining and C-banding were used to characterise the karyology of these species.

II. Materials And Methods

Adult male specimens of *Bolboceras indicum* Westw., *B. quadridens* F. and *Dynamopus athleta* Sem. were collected from Seonti forest, Kurukshetra (Haryana, India) in the month of August 2010. Chromosomal preparations were obtained, using the air drying method [19] from male gonads. C-bands were determined using the procedure described for C-banding in insects [20]. Evaluation of chromosomal morphology was based on ten spermatogonial metaphases. Percentage relative length of chromosomes was also calculated. Selected stages were micro photographed using oil immersion objective (100X) and digital compact camera (Olympus, C-7070). Chiasma frequency per cell was calculated.

III. Results And Discussion

Bolboceras indicum Westw. 2n=20 (9II+Xyp):

The chromosome number found in spermatogonial metaphase was 2n= 20 with meioformula 9II+ Xyp (Fig. 1). This is in accordance to that observed earlier by Yadav [10]. The karyotype of this species presented metacentric (pairs 1 to 7) and acrocentric (pair 8 and 9) autosomes, with gradual size reduction. The sex chromosomes comprised of a metacentric X and dot like y chromosome (Fig. 2). Percentage relative length of autosomes varied from 6.11 to 14.93, whereas that of X chromosome was 7.81 and of y was 3.48 (Table 2). During the pachytene stage autosomal bivalents showed characteristic fuzzy appearance (Fig. 8). At metaphase-I nine autosomal bivalents were dumb-bell shaped and one Xyp bivalent (Fig. 9). The chiasma frequency was found to be 9 per nucleus at this stage. The C-bands are localised in the pericentromeric region in most of the analysed species of Scarabaeidae [16, 21] similar results were also obtained in the present study in the spermatogonial metaphase (Fig. 7) and metaphase-I (Fig. 10). Metaphase II was characterised by the presence of seven metacentric and two acrocentric autosomes and dot shaped y chromosome (Fig. 11).

Bolboceras quadridens F. 2n= 20 (9II+Xyp)

Spermatogonial metaphase depicted 20 chromosomes (Fig. 3). The karyotype is composed of seven pairs of metacentric (pairs 1-7) showing size gradation and two pairs of acrocentric (pair 8 and 9) autosomes, metacentric X chromosome of the size of third pair of autosomes and y being the smallest spherical element of the set (Fig. 4). These results agreed with the earlier reports [10]. Percentage relative length of autosomes varied from 6.53 to 13.5, whereas that of X chromosome was 11.04 and of y was 4.47 (Table 2). Diakinesis depicted nine rod shaped autosomal bivalents and a sex heterovalent (Fig. 13). At metaphase-I all the autosomal bivalents were rod shaped, however, sex bivalent formed a parachute (Fig. 14). The chiasma frequency was 9 per nucleus at this stage. Pericentromeric bands of heterochromatin were found at spematogonial metaphase (Fig. 12) and metaphase-I (Fig. 15). Metaphase-II plate with nine autosomes and dot shaped y chromosome confirmed the morphology of the chromosomes (Fig. 16).

Dynamopus athleta Sem. 2n=22 (10II+Xyp)

The diploid set of 22 chromosomes in spermatogonial metaphase (Fig. 5) was obtained. Karyotype contained ten pairs of meta- , submetacentric autosomes with gradual decrease in size; the submetacentric X chromosome was smaller than ninth pair of autosomes while y being the smallest element of the complement (Fig. 6). This was found in agreement with earlier description given on scarab beetles [10]. Percentage relative length of autosomes varied from 5.37 to 13.5, whereas that of X chromosome was 6.81 and of y was 4.18 (Table 2). The general course of meiosis was typical. Diakinesis stage was characterised by eight rods and two ring shaped autosomal bivalents and sex heterovalent (Fig. 18). At metaphase-I the autosomal bivalents were highly condensed with eight rings and two rods, whereas heteromorphic sex pseudobivalent was in the form of Xyp (Fig. 19). The chiasma frequency at this stage was 12 per nucleus. The morphology of individual chromosome was clearly visible during metaphase-II. Two types of metaphase II plates were encountered, one with X-chromosome (Fig. 21) and the other with y chromosome (Fig. 22) in addition to 10 autosomes. Pericentromeric C-bands were obtained at spermatogonial metaphase (Fig. 17) and metaphase-I (Fig. 20).

Existing literature on Geotrupinae elucidates the cytology of 13 species (Table 1), out of which eight species belonging to the genus *Geotrupes* and one species of genus *Thorectes* are having a uniform 2n=22. *Athyreus excavates* [22], *Bolbelasmus arcuatus* [3] , *Bolboceras indicum* and *B. quadridens* [10 and Present Report] , however, possess basic scarab karyotype, 2n=20. Dynamopinae is chromosomally known by *Dynamopus athleta* only having 2n=22 (10+Xyp) and karyological kinship with *Geotrupes*. These results suggest that in geotrupids the evolution has progressed in the line of increasing chromosome number from 2n = 20 in *Bolboceras* to 2n = 22 in *Geotrupes*. Some researchers, assigned this increase to the fragmentation of two metacentrics because of the presence of acrocentrics in *Geotrupes* [23].

The constitutive heterochromatin in the present study was located on the peri centromeric region of the chromosomes, similar to that for other Sarabaeidae species [24, 25]. This pattern of distribution has been described for most Coleopteran species studied by C-banding in Alticinae and Carabinae [26, 27, 28] in addition to peri centromeric ones have been observed in the Scarbaeinae [6].

IV. Conclusion

The present study provides the first detailed karyological information by applying the C-banding techniques on these species belonging to subfamilies Geotrupinae and Dynamopinae. This information could join to future cytogenetic data on other taxa belonging to these subfamilies, which will provide a set of potentially informative characters suitable to understand the evolutionary pathways and phylogenetic position of these species within Scarabaeidae, which is still unclear. The use of banding methods is necessary to establish a

standard karyotype in these beetles. Further studies will shed more light on the chromosome evolution in these subfamilies.

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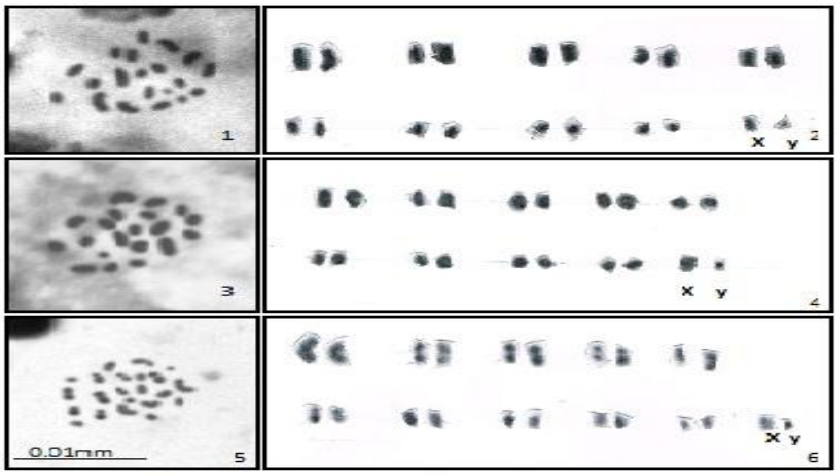
Table 1: The chromosome number and Meioformulae of subfamilies under study

Sr.No.	Species with classification	Diploid number	Mieoformula	References
	Subfamily: Geotrupinae			
1.	*Thorectes intermedius*	22	11II	Vitturi *et al.* 1999;
2.	*Athyreus excavates* Cast.	20	9+Xyp or Xy	Colomba *et al.* 2004
3.	*Bolbelasmus arcuatus* Bates	20	9+Xyp	Virkki 1967
4.	*Bolboceras indicum* Westw.	20	9+Xyp	Smith and Virkki 1978
	B. quadridens F.	20	9+Xyp	Yadav 1983; Yadav *et*
5.	*Geotrupes balvi* Jek.	22	10+Xy	*al.* 1990, PR
6.	*G. hypocrita* Serv.	22	10+Xy	Yadav 1983; Yadav *et*
7.	*G. intermedius* Costa	22	10+Xy	*al.* 1990, PR
8.	*G. mutator* Mars.	22	-	Smith 1953
9.	*G. spiniger* Mars.	22	11 II	Virkki 1954
10.	*G. splendidus* F.	22	10 + Xy	
11.	*G. stercorarius* L.	22	11 II	Salamanna 1966; 1972
12.	*G. stercorosus* Scriba	22	11 II	
13.	**Subfamily: Dynamopinae**			Virkki 1951
1.	*Dynamopus athleta* Sem.	22	10+Xyp	Salamanna 1972
				Virkki 1960
				Virkki 1951
				Virkki 1951
				Yadav 1983; Yadav *et*
				al. 1990, PR

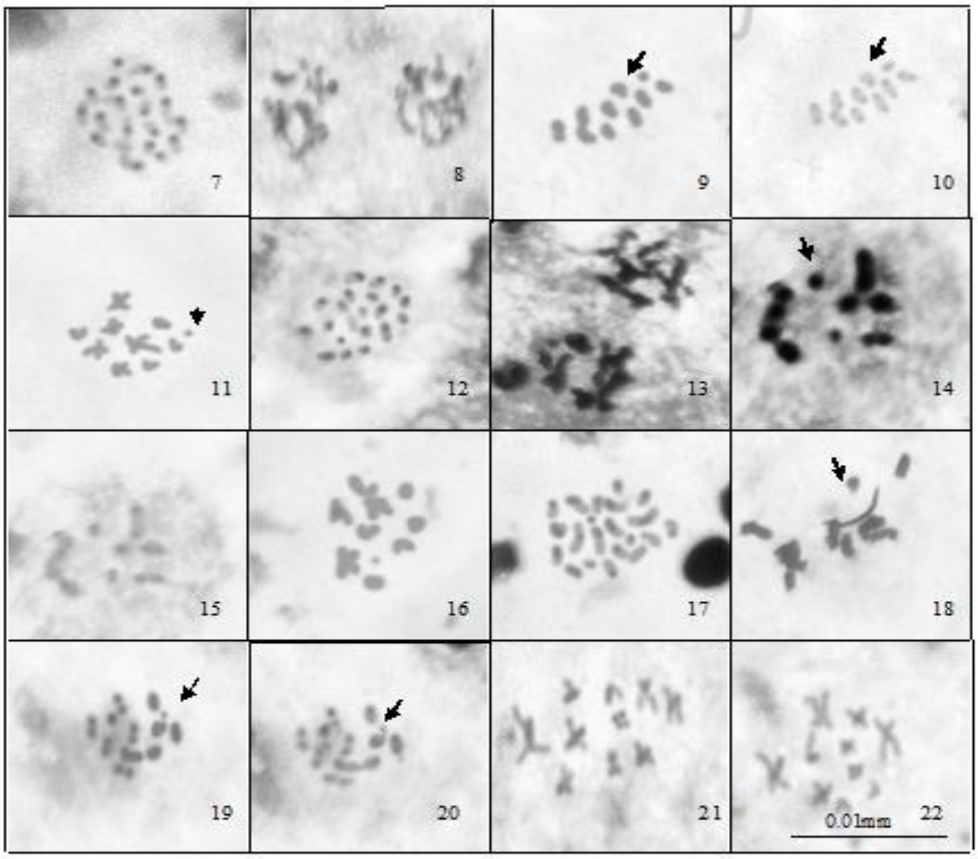
PR= Present Report

Table 2: Percentage relative length of chromosomes of three species

Chromosome pairs	Species names with percentage relative length of chromosomes		
	Bolboceras indicum	*Bolboceras quadridens*	*Dynamopus athlete*
1	14.93	13.5	13.5
2	12.83	11.64	12.07
3	11.44	10.74	10.89
4	11	10.3	9.32
5	9.21	8.36	9.08
6	8.23	8.09	7.84
7	7.95	7.91	7.53
8	6.94	7.46	6.81
9	6.11	6.53	6.57
10	-	-	5.37
X	7.81	11.04	6.81
y	3.48	4.47	4.18



Figures 1-6: *B. indicum* 1. Spermatogonial metaphase, 2. Karyotype; *B. quadridens* 3. Spermatogonial metaphase, 4. Karyotype; *D. athleta* 5. Spermatogonial metaphase, 6. Karyotype;



Figures 7-22: *B.indicum* 7. Spermatogonial metaphase with C-bands, 8. Pachytene, 9. Metaphase I, 10. C-banded Metaphase I, 11. Metaphase II; *B. quadridens* 12. Spermatogonial metaphase with C-bands, 13. Diakinesis, 14. Metaphase I, 15. C-banded Metaphase I, 16. Metaphase II; *D. athleta* 17. Spermatogonial metaphase with C-bands, 18. Diakinesis, 19. Metaphase I, 20. C-banded Metaphase I, 21. Metaphase II with X; 22. Metaphase II with y (Arrows show Xyp except y chromosome in fig. 11)