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First Report on Karyotypic, Morphometric and Meiotic Analysis of a Predatory Bombardier Beetle *Pherosophus catoirai* (Coleoptera: Carabidae) from Jammu region of Outer Himalayas, India

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Chromosomal studies and manual karyotyping are the aged techniques for determining the identity of a species on evolutionary scale; however, these techniques are simple, reliable and inexpensive to authenticate the existence of a particular species. In the present work, the chromosome complement and meiotic processes of a predatory bombardier beetle *Pherosophus catoirai* were investigated. This species presented 2n=35 as diploid chromosome number and the chromosomal formula was found to be 12m+8sm+12st+X0. Sex mechanism was X0 type with metacentric X chromosome. Y chromosome was absent in this species. Karyotype revealed small chromosomes except X chromosome which is found to be largest in the spermatogonial metaphase stage. Meiotic stages were pachytene, diplotene, diakinesis and metaphase-I. Present study may find importance to analyse evolution of chromosomes in order Coleoptera particularly in family Carabidae.

Keywords: *Pherosophus,* chromosomes, pachytene, predatory.

Carabidae (ground beetles) represented by 40,000 species worldwide ranging in size from 2 mm to 35 mm having spiny powerful legs, massive jaws and large eyes, these are forbidding predators in the insect world. Carabid beetles are proved to be the dominant predators due to their performance in food chain dynamics and pest control[1]. Conservation of ground beetles through their culture and habitat manipulations leads to the enhancement of natural suppression of arthropod pest and weed populations, thus minimize the use of chemicals to control them. Bombardier beetles (sub-family: brachininae) are known for their peculiar explosive self-defence mechanism by which they propel out very hot spray like secretion from defensive gland that keep the predators away[2]. Chromosomal description of nearly 850 species has been given for the family Carabidae[1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16]. Carabids showed a remarkable diversity in chromosome numbers from 2n=8 to 2n=44 and different types of sex determination[17]. The available karyological studies of the family Carabidae are limited to chromosome numbers without their morphometry and meiotic details. Present work analyses karyotypic, morphometric and meiotic details of a ground beetle *Pherosophus catoirai* Dejean (bombardier beetle) from Jammu district of Outer Himalayas.

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MATERIALS AND METHODS

Beetles were collected from agricultural fields of Jammu region using pitfall traps. Traps were made using biodegradable cups of diameter 7cm. Testis tissue of adult male (fig.2a,b) beetles was used for cytological studies. Male beetles can be identified by the presence of pad like structures on the protarsi while female beetles lack these pads. Preparation of slides was done by staining technique described by Rozek[18] with little modifications.

Adult male beetles were first anaesthetised with vapours of ethyl acetate because they are very active runners. The anaesthetised beetle was dissected on a clean glass slide. The abdomen was dissected very carefully in order to prevent explosion due to accidental rupturing of the explosion apparatus of this beetle. Testes are present in posterior half nearby the explosion apparatus. Then the abdomen was squeezed gently so as to take out the gonads which were then placed in hypotonic solution (0.7% KCl) for 20 minutes. Hypotonic treatment was followed by fixation, the tissue was kept in freshly prepared Cornoys fixative solution (3:1 methanol and glacial acetic acid) for 10-12 minutes and this was repeated for three times. Slides were primed by dabbing technique and were dried in air. The dried slides were stained with 2% working giemsa stain solution in phosphate buffer (pH 6.8). Stained slides were scanned and results were recorded using Olympus CH20i BIMF microscope attached with Sony SSC-DC378P camera under 1000X magnification.

RESULTS

Spermatogonial metaphase complement (fig.1a) revealed 2n=35 as diploid chromosome number. Karyotype (fig.1b) consisted of 17 autosomal pairs including 6 pairs of metacentric chromosomes, 4 pairs of sub-metacentric chromosomes, 6 pairs of sub-telocentric chromosomes, one pair of telocentric chromosomes and a single metacentric sex chromosome. The y chromosome was absent in

Table 1. Karyomorphometry of male *Pherosophus catoirai* Dejean showing 2n=35 (12m+8sm+12st+2+X0).

Chromosome pair number	Mean length of the short arm (p) in µm	Mean length of the long arm (q) in µm	Absolute length of the chromosome (p+q) in µm	Arm ratio (q/p)	Centromeric index	Nomenclature
1	0.50	0.52	1.02	1.04	49	Metacentric
2	0.22	0.50	0.72	2.27	30	Sub-metacentric
3	0.11	0.52	0.63	4.72	17	Sub-telocentric
4	0.11	0.49	0.60	4.45	18	Sub-telocentric
5	0.11	0.48	0.59	4.36	19	Sub-telocentric
6	0.21	0.31	0.52	1.47	40	Metacentric
7	0.10	0.40	0.50	4	20	Sub-telocentric
8	0.10	0.38	0.48	3.8	21	Sub-telocentric
9	0.10	0.37	0.47	3.7	21	Sub-telocentric
10	0.16	0.29	0.45	1.81	35	Sub-metacentric
11	0.15	0.25	0.40	1.66	37	Metacentric
12	0.15	0.24	0.39	1.60	38	Metacentric
13	0.18	0.19	0.37	1.05	49	Metacentric
14	0.10	0.25	0.35	2.5	29	Sub-metacentric
15	0.16	0.17	0.33	1.06	48	Metacentric
16	0.09	0.20	0.29	2.22	31	Sub-metacentric
17	0	0.15	0.15	-	-	Telocentric
X	0.52	0.53	1.05	1.01	49	Metacentric
Y (absent)	-	-	-	-	-	-
Total	3.07	6.34	9.41			

this species. The diploid chromosomal formula is calculated to be 12m+8sm+12st+X0. Idiogram of chromosomes is constructed (fig.1c) to represent the diagrammatic summary of the karyotype that makes individual chromosomes comprehensible. All the chromosomes were smaller in size as compare to the chromosomes of other families with diploid number close to 20. Karyomorphometrical analysis (table 1) showed the absolute length of autosomes to vary from 1.02 µm to 0.29 µm.

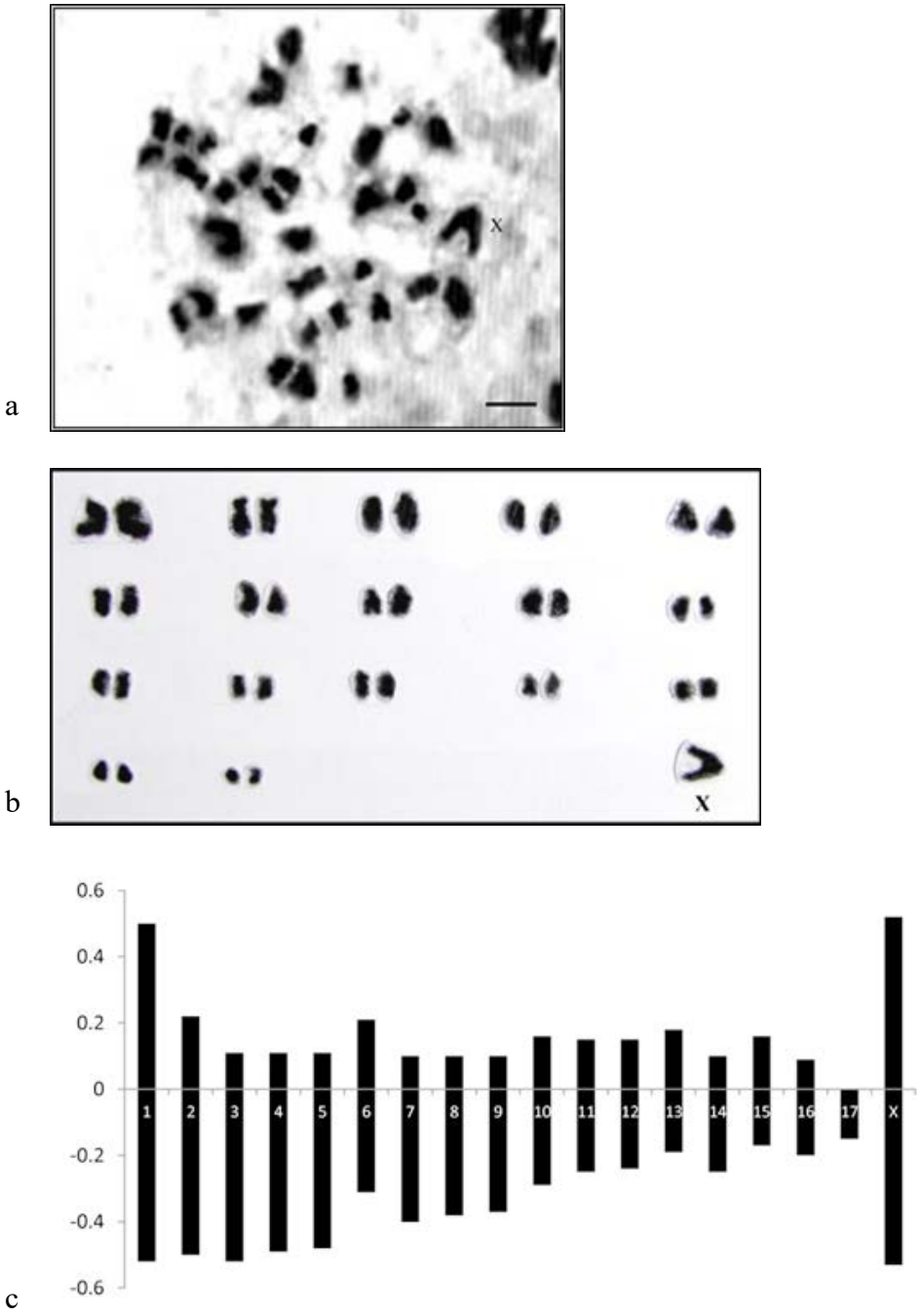

Fig. 1. *Pherosophus catoirai* (Dejean). a. Spermatogonial metaphase, 2n=35 (bar=5 µm), b. Karyotype (12m+8sm+12st+2+X0), c. Idiogram

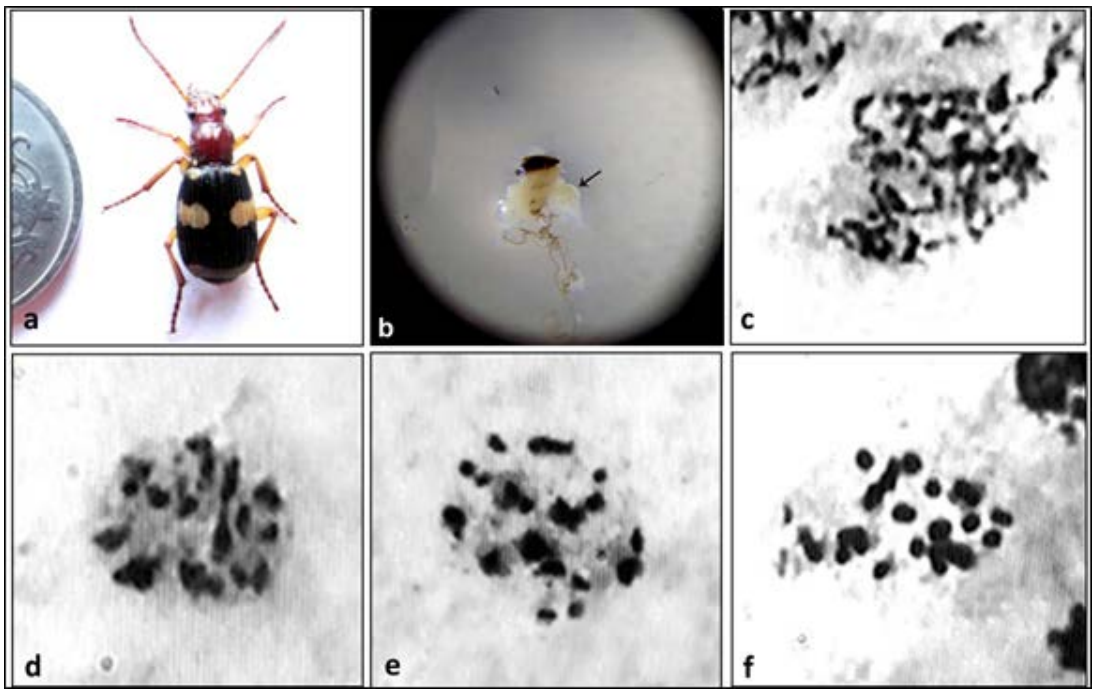

Fig. 2. *P. catoirai*. Meiosis: a. Adult male beetle, b. Testes tissue, c. Pachytene, d. Diplotene, e. Diakinesis, f. Metaphase-I stage (2n=35)

The sex chromosome X was the largest in the complement with an absolute length of 1.05 µm.The y sex chromosome was absent in the complement. The total mean haploid length was calculated as 9.41 µm. The last autosomal telocentric pair is smallest among the autosomes with an absolute length of 0.15 µm.

Meiotic observations included the stages pachytene, diplotene, diakinesis and metaphase-I. Pachytene showed little condensation in chromosomes without visible chromatids (fig.2c). Diplotene represented by 17 condensed bivalents with interstitial or terminal chiasmata. They appeared as small stubby structures and the sex chromosome was univalent (fig.2d). There were present characteristic small ring like chromosomes in diakinesis (fig.2e) X chromosome appeared as single condensed large chromosome in this stage. Metaphase-I characterized by 17 highly condensed autosomal bivalents and a single sex (X) chromosome (fig.2f). This stage also confirmed the diploid chromosome number of present species.

DISCUSSION

Chromosome number of beetles in family Carabidae showed a wide variation from 2n=8 to 2n=44, however 6% of the species exhibited a chromosome number less than $2n=18+Xy_p$, the ancestral karyotype of the order Coleoptera. This separates Carabid beetles from rest of the major groups of Coleoptera (mainly those belonging to Polyphaga) characterised by karyotypes with 2n=20. Smith[19] has proposed that the high diploid chromosome numbers of Caraboidea have arisen from ancestral karyotype of 20 chromosomes through frequent dissociations, so the karyotype with 35 chromosomes should be considered derived one. The presence of only one sex chromosome (X) in this species might be the result of gradual erosion of the y chromosome during the course of evolution as many species of carabidae showed a small sized y chromosome[20]. Studies on the predatory species of this family are scarce. Agarwal[21] reported 35 chromosomes in the species

Pherosophus bimaculatus. She also mentioned sex chromosome mechanism as X0 and X chromosome being metacentric and larger in the complement. Recent cytogenetic work on four species of family Cleridae- *Trichodes favarius*, *Trichodes quadriguttatus*, *Trichodes reichei*, and *Tilloidea transversalis* revealed their chromosome number as 2n=18 with Xy_p sex mechanism[22]. All the chromosomes except y chromosomes were metacentric in morphology. Azambuja et al[23] cytogenetically analysed three mimetic species of genus Alagoasa (Coleoptera: Alticinae) and found diploid chromosome number as 2n=22 with Xy sex chromosomes.

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Conflict of Interest

There is no conflict of interest between any authors in this research article.

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Statement of Informed Consent

All the authors have assured their consent for the publication of manuscript. The first draft of the manuscript was written by Arshad Ayoub Bhatti and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

Not applicable.

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