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Full Length Research Article

CYTOGENETICS OF PILL CLAM, *PISIDIUM MITCHELLI* (BIVALVIA: PISIDIIDAE)

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ABSTRACT

A cytogenetic study was carried out on the chromosomes of pill clam, *Pisidium mitchelli* (Bivalvia: Veneroida: Pisidiidae) collected from River Chenab fed stream Gho-Manhasan (Jammu, J&K, India). Chromosomes were studied using Giemsa staining and two banding techniques viz. C-banding & Ag-NOR banding. *P. mitchelli* shows a diploid chromosome number of 2n=62 consisted of seven metacentric, three submetacentric, eighteen subtelocentric and three telocentric chromosomal pairs. The fundamental arm number (FN) was 118. The mean haploid length and total diploid length was 21.58µm and 43.16µm respectively. Preliminary C-band studies revealed the presence of terminal blocks besides the presence of centromeric heterochromatin. The nucleolar organizer region (NOR) was found on one chromosome pair i.e. on the metacentric chromosome pair no. 6. The cytogenetic relationship between *P. mitchelli* and other species of Veneroida was also discussed.

INTRODUCTION

Family Pisidiidae includes the smallest freshwater bivalves and is distributed worldwide. According to Kuiper (1983), the origin of *Pisidium* is certainly Mesozoic and their centre of evolution lies without doubt in the Holarctic Region. *P. mitchelli* are very small or minute freshwater clam, commonly known as Pill Clam. They are very abundant in slow running rivers and streams and are occasionally found in the littoral zone along the shores of lakes and large ponds, where they prefers lentic areas with a soft muddy bottom. They mainly feed on bacteria and organic residues suspended in the water. Shell was very small, obliquely ovate, greatly inflated with external ligament. In view of the fact that it can utilise habitats such as springs, small creeks and peat bogs where no other bivalves can survive, Korniushin (2000) is of the opinion that investigation of *Pisidium* species is important not only for understanding the structure and history of the fauna, but also because they could be used for monitoring environmental conditions. Their investigation is also important in view of reports from elsewhere in the world that representatives of this genus can serve as intermediate hosts for trematode parasites (Cannon, 1972; Rantanen *et al*., 1998). Little is also known about the conservation status of the South African Sphaeriidae but Herbert (1998) was of the opinion that *P. harrisoni* meet the criteria for Red listing. From elsewhere in the world it is reported that native burrowing bivalves are declining at a catastrophic rate (Vaughn and Hakenkamp, 2001). A significant decrease of species richness and density of gastropods and bivalves due to climatic warming also be observed in a river system in France (Mouthon and Daufresne, 2006). The relatively widespread distribution and ability of *Pisidium* to exploit a wide variety of water-bodies also make it an ideal candidate for monitoring heavy metal pollution. Cytogenetic parameters such as chromosome number and morphology have long been used to characterize species and can give valuable clues to phylogeny evolution and taxonomic relationships. The present investigation is concerned with the cytogenetic studies of *P. mitchelli* and its cytogenetic relationship with other species of the order Veneroida is also discussed. This preliminary chromosomal and banding study will surely pave the way for further cytogenetic and molecular studies in future. Moreover the extended molecular studies after this basic step would be of immense help to establish taxonomic status of the specimen populations.

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MATERIAL AND METHODS

Specimens were collected from River Chenab fed stream Gho-Manhasan. River Chenab is one of the largest rivers of the Indus basin and thus it feeds to maximum parts of the Jammu region of J&K. River Chenab gives rise to many streams and Gho-Manhasan is one of them which is located at 32.56ºN 74.95ºE. The snails were collected from Ghomanhasan stream, Jammu from slow running fresh water streams. Snails were taken alive to the laboratory, then maintained in well aerated tanks of water and fed continuously to promote growth. Chromosome preparations were obtained from the gills. After taxonomic verification of each snail, snails were placed directly in 0.1% Colchicine at room temperature, for 24 hrs. Snails were dissected and their gills separated and treated with 0.07% KCl followed by Carnoy's fixative. The slides were stained with 4% Giemsa buffer solution while some slides were kept for C-banding and NOR banding. Slides were processed for C-banding (Sumner, 1990) and silver staining of NORs (Howell and Black, 1980). Scanning and photomicrography of the slides was done. Well spread suitable mitotic stages were photomicrographed at a magnification of 1000x. Idiogram of chromosomes pairs was constructed to represent the shape and size of the chromosomes. Histogram was prepared by taking chromosome pair number on X-axis and corresponding relative length percentage on Y-axis. Chromosomes pairs were arranged in decreasing order of their length.

RESULTS

A total of 100 metaphase stages were selected to establish the diploid chromosome number. Somatic metaphase complements were used for karyological study but no sex chromosome heteromorphism was observed in any of the species. The basic chromosome number was found to be 2n=62. Thirty-one pairs of chromosomes were categorized into metacentric, submetacentric, subtelocentric and telocentric pairs following Levan *et al*. (1964). The chromosomes were classified into uniarmed and biarmed following Chen and Ebelling (1971) to calculate the fundamental arm number (FN). Somatic karyotype prepared from metaphases of gill cells showed 7 metacentric, 3 submetacentric, 18 subtelocentric and 3 telocentric pairs of chromosomes (Fig. 1A & 1B).

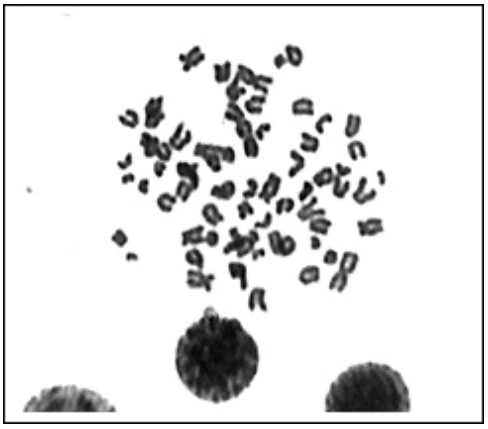

Fig. 1A Metaphase complement from gill tissue of *P. mitchelli* (2n=62)

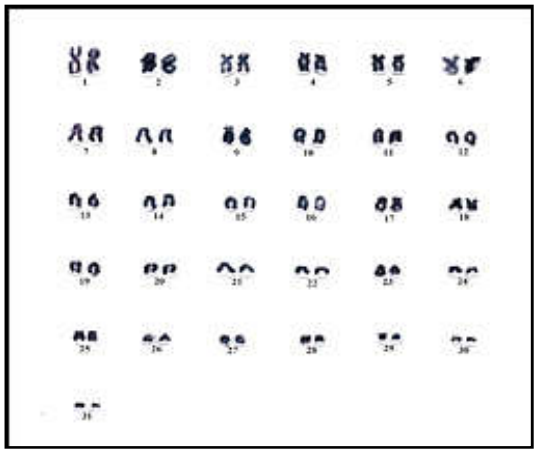

Fig. 1B Karyotype of metaphase complement from gill tissue of *P. mitchelli* showing 2n=62 (2n= 14m+6sm+36st+6t)

Chromosomal formula for the complement was calculated as 2n=14m+6sm+36st+6t and the corresponding fundamental arm number was calculated as FN=118. Mean haploid length was 21.58 µm and Total diploid length was 43.16 µm. In C-banding, the chromosome pair no. 2, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 16, 17, 20, 22, 24, 25, 26, 27, 28, 29, 30 and 31 showed telomeric C-bands where as the centromeric bands were visible in 1, 3, 11, 18, 19, 21 and 23 (Fig. 1C). Ag-NOR banding showed fairly distinct and darkly stained one pair of NORs of equal sizes on the metacentric chromosome pair no. 6 (Fig. 1D). Morphometric data is given in the Table 1. Histogram (Fig. 2A) and Idiogram (Fig. 2B) were prepared using morphometric data.

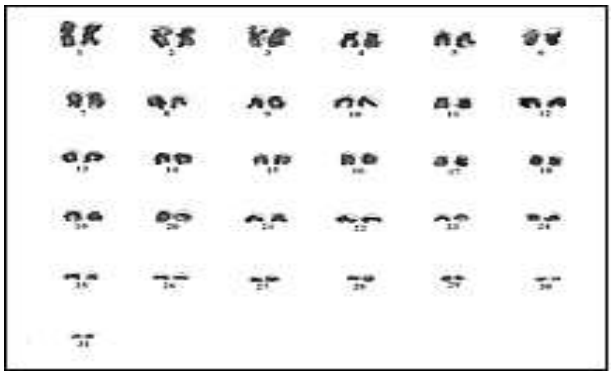

Fig. 1C Karyotype of C-banded metaphase complement of *P. mitchelli*

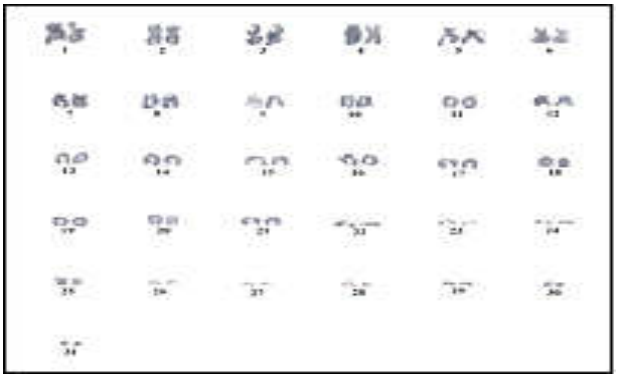

Fig. 1D. Karyotype of NOR-banded metaphase complement of *P. mitchelli* (6th pair: metacentric chromosome)

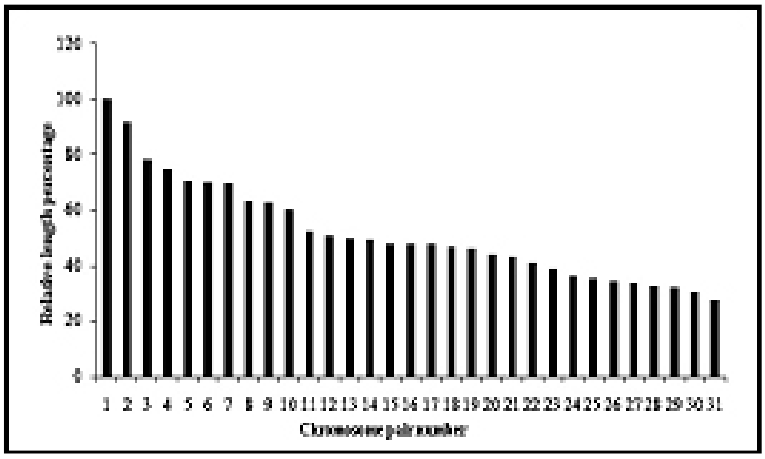

Fig. 2A. A Histogram showing the Relative Length Percentage of chromosomes of *P. Mitchelli*

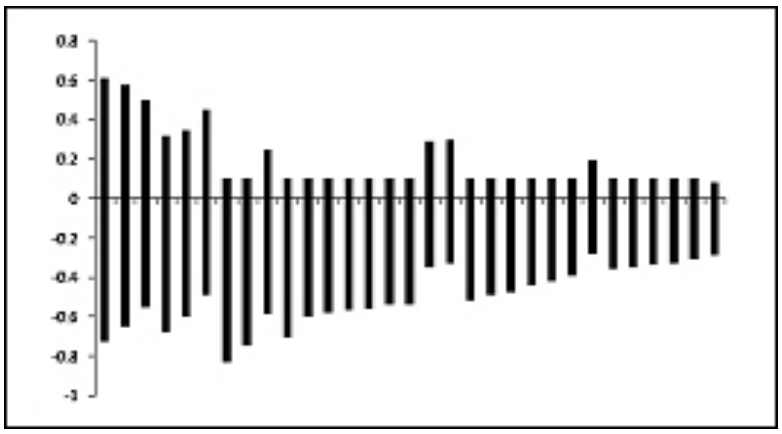

Fig. 2B. An Idiogram of *P. mitchelli* constructed on the basis of chromosome numbers and position of the centromere

Table 1. Morphometric data of karyotype of *P. mitchelli* showing 2n=62 (14m+6sm+36st+6t)

Chromosome pair no.	Mean length of the short arm (p) in μm	Mean length of the long arm (q) in μm	Absolute length (p+q) of the chromosome in μm	Arm ratio (q/p)	Relative length percentage	Total complement length percentage	Centromeric index	Nomenclature
1	0.61	0.73	1.34	1.19	100.00	3.10	45.52	Metacentric
2	0.58	0.65	1.23	1.12	91.79	2.85	47.15	Metacentric
3	0.50	0.55	1.05	1.10	78.35	2.43	47.61	Metacentric
4	0.32	0.68	1.00	2.12	74.62	2.32	32.00	Submetacentric
5	0.35	0.60	0.95	1.71	70.89	2.20	36.84	Submetacentric
6	0.45	0.49	0.94	1.08	70.14	2.18	47.87	Metacentric
7	0.10	0.83	0.93	8.30	69.40	2.15	10.75	Telocentric
8	0.10	0.75	0.85	7.50	63.43	1.97	10.52	Telocentric
9	0.25	0.59	0.84	2.36	62.68	1.95	29.76	Submetacentric
10	0.10	0.71	0.81	7.01	60.44	1.88	12.34	Telocentric
11	0.10	0.60	0.70	6.00	52.23	1.62	14.28	Subtelocentric
12	0.10	0.58	0.68	5.80	50.74	1.58	14.70	Subtelocentric
13	0.10	0.57	0.67	5.70	50.00	1.55	14.92	Subtelocentric
14	0.10	0.56	0.66	5.60	49.25	1.53	15.15	Subtelocentric
15	0.10	0.54	0.64	5.40	47.76	1.48	15.62	Subtelocentric
16	0.10	0.54	0.64	5.40	47.76	1.48	15.62	Subtelocentric
17	0.29	0.35	0.64	1.20	47.76	1.48	45.31	Metacentric
18	0.30	0.33	0.63	1.10	47.01	1.46	47.61	Metacentric
19	0.10	0.52	0.62	5.20	46.26	1.44	16.12	Subtelocentric
20	0.10	0.49	0.59	4.90	44.02	1.37	16.94	Subtelocentric
21	0.10	0.48	0.58	4.80	43.28	1.34	17.24	Subtelocentric
22	0.10	0.44	0.55	4.40	41.04	1.27	18.18	Subtelocentric
23	0.10	0.42	0.52	4.20	38.80	1.20	19.23	Subtelocentric
24	0.10	0.39	0.49	3.90	36.56	1.14	20.40	Subtelocentric
25	0.19	0.28	0.47	1.47	35.07	1.09	40.42	Metacentric
26	0.10	0.36	0.46	3.60	34.32	1.07	21.73	Subtelocentric
27	0.10	0.35	0.45	3.50	33.58	1.04	22.22	Subtelocentric
28	0.10	0.34	0.44	3.40	32.83	1.02	22.72	Subtelocentric
29	0.10	0.33	0.43	3.30	32.08	0.99	23.25	Subtelocentric
30	0.10	0.31	0.41	3.10	30.59	0.95	32.25	Subtelocentric
31	0.08	0.29	0.37	3.62	27.61	0.86	21.62	Subtelocentric

DISCUSSION

The chromosome numbers and forms are of particular adaptive evolutionary significance as these are the compound bodies with many different components. Karyotype of a species is the physical basis of its genetic system. Species with high chromosome number are likely to show considerably more genetic recombinations than those with low number of chromosome, and this may be related with their population genetics and reflecting their evolutionary patterns. In family Pisidiidae (Bivalvia: Veneroida), three species were studied cytologically viz. *Sphaerium corneum*, (2n=30 consisted of 26m+4sm chromosomes, FN=60) (Petkeviciute *et al*., 2006; Korinkova and Kral, 2011); *S. nucleus*, (2n=30 consisted of 28m+2sm chromosomes, FN=60) (Korinkova and Kral, 2011) and *S. nitidum* (2n=44 consisted of 24m+8sm+12st chromosomes, FN=88) (Barsiene and Barsyte, 2000).

The diploid chromosome number in *Pisidium mitchelli* has been recorded to be 62 with 14m+6sm+36st+6t, prevalence of 56 biarmed and 6 uniarmed chromosomes whereas in this family earlier workers reported only biarmed chromosomes in the diploid complement of all the species studied so far in this family. This may be due to Robertsonian translocation (centric fission) of chromosomes or polyploidy as there was almost doubling in the chromosome as compared to other species (Rainer, 1963). *Pisidium mitchelli* (2n=62) showed doubling in the diploid chromosome number as compared to other species of order Veneroida, where diploid chromosome number ranges between 30-44; this may be either due be polyploidy or Robertsonian translocation (centric fission) of chromosomes. In conclusion, cytogenetic studies contribute useful information supplementary to the morphological, biochemical and other characters used for systematic analysis of freshwater snails.

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