CHROMOSOMAL STUDIES OF TWO FRESHWATER SNAILS, *BELLAMYA BENGALENSIS* AND *MELANOIDES TUBERCULATA* (MOLLUSCA: GASTROPODA: PROSOBRANCHIA) FROM JAMMU & KASHMIR, INDIA

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ABSTRACT

During the present study, mitotic chromosomes of two freshwater snails *Bellamya bengalensis* and *Melanoides tuberculata* were analyzed using gill tissues of specimens collected from the type locality (Ghomanhasan stream, Jammu, J&K, India), both belonging to class Gastropoda and subclass Prosobanchia but to two different families, namely Viviparidae and Thiaridae. The observed diploid chromosome number was 2n=22 with ZW (female) /ZZ (male) sex determining mechanism in both the species. The karyotype of *B. bengalensis* consisted of twelve metacentric pairs, eight submetacentric pairs and ZW/ZZ pair of sex chromosomes and *M. tuberculata*, fourteen metacentric pairs, six submetacentric pairs and ZW pair of sex chromosomes. Results of the present study have scientific and practical significance complementary to biochemical and molecular studies in animal taxonomy.

KEYWORDS: Chromosomes, Karyotype, *Bellamya, Melanoides*

INTRODUCTION

Phylum Mollusca constitutes one of the major divisions of the animal kingdom and are of unusual interest both in regard to the diversity of organization and in the multitude of living species. The molluscs greatly vary in form, structure, habit and habitats. They are highly adaptive and occupy all possible aquatic and terrestrial habitats. In Molluscs, approximately 60,000 species of Gastropods are known, out of which only 332 species have been worked out cytogenetically till date (305 were karyotyped and 27 included banding techniques), showing the increasing knowledge of chromosome morphology over the last three decades. It includes three subclasses: the Prosobranchia, which contained the majority of gastropods; the Pulmonata, which included the land snails; and the Opisthobranchia, which included the sea hares and sea slugs. The latter subclass consisted of animals with reduced shells or none at all. Most gastropods are motile but some, e.g. the slipper shell (*Crepidula*) are sedentary and some such as the sea butterflies swim and others including the terrestrial snails move by means of a well-developed foot.

There are relatively few studies on the karyotype and sex determination in prosobranchs. Karyological studies of some species of subclass Prosobranchia were described by several authors (Jacob, 1959; Patterson, 1973; Vitturi and Catalano, 1984; Yaseen, 1994; Choudhary and Pandit, 1997). It has been observed that majority of species of the subclass Prosobranchia exhibit a wide range of haploid chromosome numbers ranging from n=7 to n=36. Vitturi et al. (1982 and 1988), Nakamura (1986) and Yaseen (1994) examined the chromosome numbers of a diversity of prosobranchs. They found that the Patellogastropoda has 9, the Pleurotomarioidea, Fissurelloidea and Neritoidea has 11-21 and the Trochoidea has 18 chromosomes. Mesogastropoda has 7-20 and Neogastropoda 28-36 chromosomes. So, Prosobranchia mainly presents a variation in chromosome number from n=7 in *Viviparous contectus* to n=18 in a member of the family Pitonidae and to n=20 in several Pleuroceridae (Patterson, 1969; Patterson and Burch, 1978). 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 karyological studies of two genera of gastropod snails, namely *Bellamya bengalensis* and *Melanoides tuberculata* which belong to the same subclass Prosobranchia and to two different families, Viviparidae and Thiaridae in a trial to clarify their taxonomic status.

**MATERIAL AND METHODS**

The snails were collected from Ghomanhasan stream, Jammu all year round from Feb, 2011 to March, 2012, from slow running fresh water streams. Snails were taken alive to the laboratory, then maintained in tanks of aerated water and fed continuously to promote growth. Chromosome preparations were obtained from the gills. After taxonomic verification of each snail, pooled snails (about 10 snails for each species) 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 as hypotonic solution, at room temperature for 30 min. The tissues after hypotonic treatment were fixed in freshly prepared fixative i.e. 3:1 Methanol and Glacial acetic acid and kept for 20 min giving 3 changes in it. The slides were stained with 4% Giemsa buffer solution for 30 min and later rinsed with distilled water and air dried, 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 using Nikon YS100 binocular research microscope and Samsung SDC-313 camera respectively. Well spread suitable mitotic stages were photomicrographed at a magnification of 1000x. The morphological classification of chromosomes proposed by Levan *et al.* (1964) has been followed to categorize the chromosomes as metacentrics, submetacentrics, subtelocentrics or telocentric. The chromosomes were classified into uniarmed and biarmed following Chen and Ebelling (1971) to calculate the fundamental arm number (FN). 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**

In the present study, mitotic chromosomes of two freshwater snails *Bellamya bengalensis* and *Melanoides tuberculata* were analyzed using gill tissues of specimens collected from the type locality (Ghomanhasan stream, Jammu, J&K, India), both belonging to class Gastropoda and subclass Prosobranchia but to two different families, namely Viviparidae and Thiaridae.

*Bellamya bengalensis* (Mud Snail)

*B. bengalensis* is a freshwater gastropod snail, which is present in almost all types of lowland water bodies, mainly stagnant water and in water resources like ponds, marshes, ditches and paddy fields etc. (Figure 1 & 2) and the taxonomic characters are; Medium sized to large dextral and conical shell with ovate, corneous and nucleated operculum, Adult female animal with brood pouch containing offspring snails, males with one tentacle longer than other.
Chromosomal Studies of Two Freshwater Snails, *Bellamya bengalensis* and *Melanoides tuberculata* (Mollusca: Gastropoda: Prosobranchia) from Jammu & Kashmir, India

**Chromosomal Observations from Female *B. bengalensis***

- Somatic metaphase obtained from gills (Figure 3)
  - Diploid chromosome number recorded, $2n=22$

- Somatic karyotype prepared from metaphases of gill cells (Figure 4)
  - Number of metacentrics = six pairs
  - Number of submetacentrics = four pairs
  - Sex chromosomes = Heteromorphic pair, $ZW$ (where $Z$ and $W$ are metacentric and submetacentric chromosomes respectively)
  - Chromosomal formula = $12m+8sm+ZW$

![Figure 3: Metaphase Complement from Gill Tissue of Female *B. bengalensis* (2n=22)](image1)

![Figure 4: Karyotype of Female *B. bengalensis* Prepared from Metaphase Complement of Gill Tissue Showing 2n=22 (12m+8sm+ZW)](image2)

- Morphometric data of somatic karyotype (Table 1)
  - Actual mean length of the largest chromosome = 1.00 µm
  - Actual mean length of the smallest chromosome = 0.36 µm
  - Relative length percentage of the largest chromosome = 100
  - Relative length percentage of the smallest chromosome = 36
  - Ratio of the largest to the smallest chromosome = 2.77
  - Mean total haploid length = 6.28 µm
  - Total diploid number = 12.56 µm

- Histogram of the chromosomes (Figure 5)
- Idiogram of the chromosomes (Figure 6)
- Fundamental arm number = 44
Chromosomal Observations from Male *B. bengalensis*

- Somatic metaphase obtained from gills (Figure 7)
  - Diploid chromosome number recorded, 2n=22
- Somatic karyotype prepared from metaphases of gill cells (Figure 8)
  - Number of metacentrics = six pairs
  - Number of submetacentrics = four pairs
  - Sex chromosomes = Homomorphic pair, ZZ (where both Z are metacentric chromosomes)
  - Chromosomal formula = 12m+8sm+ZZ
- Morphometric data of somatic karyotype (Table 2)
  - Actual mean length of the largest chromosome = 0.75 µm
  - Actual mean length of the smallest chromosome = 0.34 µm
  - Relative length percentage of the largest chromosome = 100
  - Relative length percentage of the smallest chromosome = 45.33
  - Ratio of the largest to the smallest chromosome = 2.30
  - Mean total haploid length = 6.48 µm
  - Total diploid number = 12.96 µm
- Histogram of the chromosomes (Figure 9)
Chromosomal Studies of Two Freshwater Snails, *Bellamya bengalensis* and *Melanoides tuberculata* (Mollusca: Gastropoda: Prosobranchia) from Jammu & Kashmir, India

- Idiogram of the chromosomes (Figure 10)
- Fundamental arm number = 44

**Observations of Differential Banding in *B. bengalensis***

In C- Banding (Figure 11 & 12) :- Most of the chromosomes exhibited centromeric C-bands, whereas chromosome pair number 3, 5, 8 and 9 showed clear telomeric C-bands and in NOR- Banding (Figure 13 & 14) :- Fairly dark and conspicuous one pair of NOR was obtained by silver staining on the long arm of a submetacentric pair (7th pair).

**Melanoides tuberculata** (Red Rimmed Melania)

*M. tuberculata* is a freshwater snail with an operculum, parthenogenetic, burrowing species that tends to be most active at night and feeds on algae (Figure 15). **Taxonomic characters:** Shell dextral with ovate, horny and paucispiral operculum. Shell light brown and frequently mottled with rust-colored spots that may form a spiral row below suture.
Figure 15: Shell of *Melanoides tuberculata*

Chromosomal Observations from Female Specimen of *M. tuberculata*

- Somatic metaphase obtained from gills (Figure 16)
  - Diploid chromosome number recorded, $2n=22$
- Somatic karyotype prepared from metaphases of gill cells (Figure 17)
  - Number of metacentrics = seven pairs
  - Number of submetacentrics = three pairs
  - Sex chromosomes = Heteromorphic pair, $ZW$ (where $Z$ and $W$ are metacentric and submetacentric chromosomes respectively)
  - Chromosomal formula = $14m+6sm+ZW$

Figure 16: Metaphase Complement from Gill Tissue of Metaphase Complement of Female *M. tuberculata* ($2n=22$)

Figure 17: Karyotype of Female *M. tuberculata* Prepared from Gill Tissue Showing $2n=22$ ($14m+6sm+ZW$)

- Morphometric data of somatic karyotype (Table 3)
  - Actual mean length of the largest chromosome = 0.67 µm
  - Actual mean length of the smallest chromosome = 0.30 µm
  - Relative length percentage of the largest chromosomes = 100
  - Relative length percentage of the smallest chromosomes = 44.77
  - Ratio of the largest to the smallest chromosome = 2.23
  - Mean total haploid length = 5.17 µm
  - Total diploid length = 10.34 µm
- Histogram of the chromosomes (Figure 18)
- Idiogram of the chromosomes (Figure 19)
Chromosomal Studies of Two Freshwater Snails, *Bellamya bengalensis* and *Melanoides tuberculata* (Mollusca: Gastropoda: Prosobranchia) from Jammu & Kashmir, India

- Fundamental arm number = 44

![Figure 18: Histogram of Female *M. tuberculata*](image1)

![Figure 19: Idiogram of Female *M. tuberculata*](image2)

Observations of Differential Banding in *M. tuberculata*

In C-Banding (Figure 20 & 21): Chromosome pair number 1, 3, 4, 6, 7, 8, 9 and 11 showed telomeric C-bands while rest showed centromeric C-bands and in NOR-Banding (Figure 22 & 23): Fairly distinct and darkly stained NORs of equal sizes were observed on the short arm of a submetacentric pair (3rd pair).

![Figure 20: C-Banded Metaphase Complement of Female *M. tuberculata*](image3)

![Figure 21: Karyotype of C-Banded Metaphase Complement Female *M. tuberculata*](image4)

![Figure 22: NOR-Banded Metaphase Complement of Female *M. tuberculata*](image5)

![Figure 23: Karyotype of NOR-Banded Metaphase Complement of Female *M. tuberculata* (3rd Pair: Short Arm of Submetacentric Pair)](image6)

DISCUSSIONS

The karyotypes of the freshwater *Bellamya bengalensis* and *Melanoides tuberculata* snails indicated that the diploid chromosome number of both was 2n = 22 with ZW (female)/ ZZ (male) sex determining mechanism. In family Viviparidae, fifteen species have been studied cytologically so far by different workers viz. Patterson (1965); Stern (1975); Zhou et al. (1988); Yaseen et al. (1991); Choudhary and Pandit (1997); Park et al. (1997) and Barsiene et al. (2000). The diploid chromosome number in two geographically isolated populations of *Bellamya bengalensis* viz. Orissa population (Choudhary and Pandit, 1997) and Jammu population (present study) has been recorded to be the same (2n=22: FN=44),
while they differed in karyotypic details. Choudhary and Pandit (1997) reported 14m+8sm chromosomes, while in the present study; chromosomal formula of 12m+8sm+ZW/ZZ was reported. The variation in chromosome morphology may be due to old techniques used by earlier workers. The ZW/ZZ sex determining mechanism was reported for the first time in B. bengalensis, while it has been already reported in four species of family Viviparidae viz. Viviparus ater, V. acerosus and V. mamillatus having diploid chromosome number of 2n=18 and chromosome formula of 14m+2sm+ZW/ZZ (FN=36), V. viviparus having 2n=18 with 16m+2sm (FN=36) (Barsiene et al., 2000), whereas XX/XY sex determining mechanism was reported in Tulotoma angulata and V. subpurpureus having 2n=26 with 6m+14sm+4t+XX/XY (Patterson, 1965) and 14m+6sm+4st+XX/XY (Stern, 1975) respectively. Barsiene et al. (2000) reported 2n=14 in V. contectus with 10m+4sm (FN=28) and no sex determining mechanism.

Bellamya aeruginosa, B. angularisa and B. quadrata are reported to have 2n=16 with 4m+8sm+4st chromosomes (FN=32) in all the species respectively (Zhou et al., 1988). Yaseen et al. (1991) accounted 2n=18 in B. unicolor with 10m+4sm+4st chromosomes (FN=36). In Cipangopaludina chinensis, diploid chromosome number of 2n=18 was reported with different chromosome formula viz. 10m+6sm+2st; FN=36 (Zhou et al., 1988) and 4m+14sm; FN=36 (Park et al., 1997) and 2n=18 in C. cathayensis and C. japonica with 10m+6sm+2st (FN=36) (Zhou et al., 1988) and 4m+14sm (FN=36) (Park et al., 1997) respectively. In all the species of family Viviparidae, only biarmed chromosomes were present, with diploid chromosome number and fundamental arm number ranging in between 14-26 and 28-52 respectively. The comparative karyological analysis of Viviparids led researchers to suggest that the main mechanism of evolutionary changes in this group of snails is Robertsonian translocation of chromosomes (Rainer, 1963).

Karyological information in the prosobranch family Thiaridae was comparatively scarce and only two species were studied till date. Yaseen (1996) from Egypt, reported a diploid number of 2n=22 in Melanoides tuberculata with 20m+2st chromosomes (FN=44). During the present study, in the Jammu population of M. tuberculata a diploid chromosome number of 22 with 14m+6sm+ZW/ZZ chromosomes (FN=44) with ZW/ZZ sex determining mechanism was recorded. Diploid chromosome number of 2n=22 reported in both populations i.e. Egypt and Jammu population was similar with prevalence of only biarmed chromosomes and the sex determining mechanism was reported for the first time in family Thiaridae. The variation in chromosome morphology may be due to pericentric inversions. Barsiene et al. (1998) observed 2n=36 in Melanopsis dufouri with 4sm+32st chromosomes (FN=72).

Moreover, the presence of ZW/ZZ sex determining mechanism was determined in both families of Viviparidae and Thiaridae by different workers (Barsiene and Ribi, 1998; Barsiene et al., 2000; Kongim et al., 2006, 2010; Thiriot-Quievreux, 2002). In conclusion, cytogenetic studies contribute useful information supplementary to the morphological, biochemical and other characters used for systematic analysis of freshwater snails.

ACKNOWLEDGEMENTS

Authors are thankful to Head, Department of Zoology, Prof. K. K. Sharma for providing necessary lab facilities and equipments for the present work.

REFERENCES


APPENDICES

Table 1: Morphometric Data of Karyotype of female B. bengalensis Showing 2n=22 (12m+8sm+ZW)

<table>
<thead>
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<th>Chromosome Pair No.</th>
<th>Mean Length of the Short Arm (p) in µm</th>
<th>Mean Length of the Long Arm (q) in µm</th>
<th>Absolute Length (p+q) of the Chromosome in µm</th>
<th>Arm Ratio (q/p)</th>
<th>Relative Length Percentage</th>
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Table 2: Morphometric Data of Karyotype of Male B. bengalensis Showing 2n=22 (12m+8sm+ZZ)

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<th>Chromosome Pair No.</th>
<th>Mean Length of the Short Arm (p) in µm</th>
<th>Mean Length of the Long Arm (q) in µm</th>
<th>Absolute Length (p+q) of the Chromosome in µm</th>
<th>Arm Ratio (q/p)</th>
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Chromosomal Studies of Two Freshwater Snails, *Bellamya bengalensis* and *Melanoides tuberculata* (Mollusca: Gastropoda: Prosobranchia) from Jammu & Kashmir, India

Table 3: Morphometric Data of Karyotype of Female *M. tuberculata* Showing $2n=22$ (14m+6sm+ZW)

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