



Original Article

Meiotic Chromosomes and Karyotype of *Bufo viridis* (Laurenti, 1768) from Jammu and Kashmir

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ABSTRACT

Somatic karyotype of an Anuran amphibian- *Bufo viridis* (Family- bufonidae) consists of twenty two chromosomes ($2n=22$). The meiotic stages (spermatogonial metaphase, diakinesis, metaphase I and metaphase II) have shown the haploid number to be exactly half of the diploid chromosome number, that is $n=11$. The karyotype comprises of six pairs of larger metacentric or sub-metacentric chromosomes and five pairs of smaller metacentric chromosomes. Only the fourth chromosome pair has been found to be sub-metacentric while all the others are metacentric type of chromosomes.

Keywords: Amphibia, Anura, Bufonidae, Meiotic chromosomes, Karyotype.

INTRODUCTION

The green toad- *Bufo viridis* (Laurenti, 1768) viz *Pseudepidalea viridis* [11]) is broadly distributed throughout the Palearctic region including the Central and Middle Asiatic countries. In India it has been reported from the temperate regions including the higher reaches of North-West Himalayas also covering temperate parts of Jammu and Kashmir. Anuran amphibians have been studied on the basis of morphological, taxonomic and phylogenetic relationships as well as ecological perspectives from Indian sub-continent. Cytogenetic works related with the anurans began the most diverse family bufonidae. Preliminary studies of the genus *Bufo* were purely based on the study of chromosome morphology [4, 13, 21]. Later many studies have been carried out regarding the conventional karyotypic studies, banding patterns as well as molecular genetics [10, 19]. Studies have revealed that the bufonids have characteristically retained the typical diploid chromosome number as twenty two [12, 15, 16, 22] with only slight deviations where some workers have reported the basic chromosome number to be twenty [1, 5, 8, 9].

One of the peculiar characters of green toads is their possession of the bisexual diploid, triploid and tetraploid populations which makes them unique genus as well as a unique vertebrate [18, 20]. The genus *Bufo* is again of particular interest for studies regarding evolution and reproductive isolation mechanisms due to its cosmopolitan distribution and easy interbreeding in nature [2].

In present study we are going to describe for the first time the diploid number of chromosomes and meiotic behavior of the chromosomes from testicular tissue for the green toad *Bufo viridis* from Jammu and Kashmir, India, done by using in vivo colchicine treatment of bone marrow cells and testis.

METHODS

Samples of males and females of *Bufo viridis* were collected from Bhaderwah town, Jammu province of India during 2009. Each sample was given intramuscular or intraperitoneal injection of 0.5% of colchicine at the rate of 1ml per 100gram body weight for three hours before sacrificing. The bone marrow was flushed out with 0.48% sodium chloride solution into the cavity blocks. The minced tissue of bone marrow and testes was treated with hypotonic solution for 35-40minutes. A fresh fixative of 1:3 glacial acetic acid and absolute alcohol was added to the tissue for 30minutes changing the fixative after every 10 minutes. The slides were prepared by using air drying dabbing method. The chromosomes were stained with 2% Giemsa stain for 20-25 minutes. More than 50 metaphase complements from five males and six females were examined under bright field illumination, using 100X oil immersion objective and 10X eyepiece, by using NIKON YS100 binocular research microscope. Also the testicular tissue was used to study the meiotic chromosomes by the same method.



Figure 1: Adult male and female
Bufo viridis



Figure 2: Adult female



Figure 3: Four legged-tadpole stage

RESULTS AND DISCUSSION

The present study deals with the analysis of meiotic chromosomes studied from testicular cells and the somatic karyotype of the green toad *Bufo viridis*, which has been collected from a remote temperate area of Bhaderwah tehsil in Jammu and Kashmir of biodiversity rich Indian sub-continent. The species is well distributed in temperate regions of both provinces, that is, Kashmir and Jammu, and many records are available regarding the morphology, taxonomy and phylogeny. But none of the authors have yet described these aspects taking the cytogenetic perspective in consideration. We have begun the work for which a preliminary karyotypic constitution and meiotic behaviour of chromosomes has been described. The diploid chromosome number was found to be twenty two. The karyotype was typical bufonid one with chromosomes classified into two major groups (Fig. 1) according to decreasing size [14]. The eleven pairs of chromosomes karyotyped were placed into two groups comprising Group A of six pairs of larger metacentric or sub-metacentric chromosomes and Group B of smaller metacentric chromosomes. Analysis of the values of arm ratios and centromeric indices revealed that out of the eleven pairs only the fourth pair was sub-metacentric while the rest were metacentric biarmed chromosomes. Thus haploid chromosome formula for *Bufo viridis* was determined as $n=10M+1SM$ (Fig. 1). All the chromosomes were biarmed and no acrocentric or sub-telocentric chromosomes were observed. The fundamental arm number (FN) was thus calculated as $FN=44$.

Karyomorphometric analysis (table 1) was done using the length of short arm and long arm, centromeric index and other such parameters. The length of the first/largest chromosome was found to be $3.40 \mu\text{m}$ and that for the last/smallest chromosome it was found to be $0.84 \mu\text{m}$. Only one submetacentric chromosome pair, that is, number four with a length of $2.80 \mu\text{m}$ was present. Total complement length was found to be $43.26 \mu\text{m}$ and relative length percentage ranged between $3.88 \mu\text{m}$ (last pair) and $15.72 \mu\text{m}$ (first pair) while values between 0.36 (pair number 4) and 0.50 (pair number 6) were observed for centromeric indices. No heteromorphic chromosomes were observed which could be considered as sex chromosomes. The Meiotic stages were studied from the testicular tissue as:

- i. **Spermatogonial metaphase:** Twenty two chromosomes were observed which confirmed with the diploid number from somatic cells (Fig. 4).
- ii. **Diakinesis:** Eleven well condensed ring shaped thick bivalents were observed. Chiasmata terminalisation was over and the bivalents appeared ring shaped (Fig. 5).
- iii. **Metaphase I:** Eleven bivalents were further condensed. The larger five pairs were late terminalising and still appeared as ring shaped structures whereas the other six pairs had completed terminalisation and thus appeared rod shaped (Fig. 6) .
- iv. **Metaphase II:** Eleven distinct well-condensed, biarmed chromosomes were observed with distinct centromere, long arm and short arm (Fig. 7).

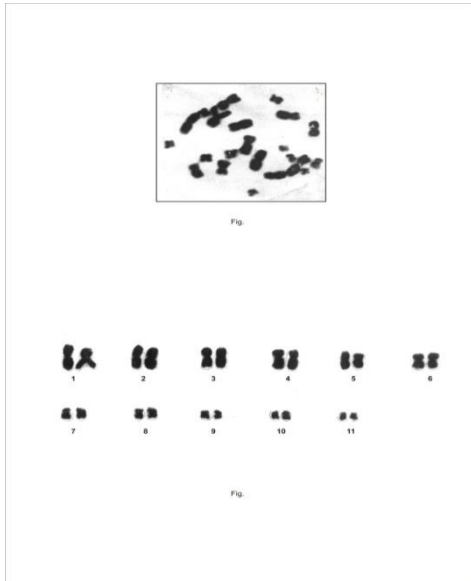


Figure 1 : Somatic karyotype of male *Bufo viridis*

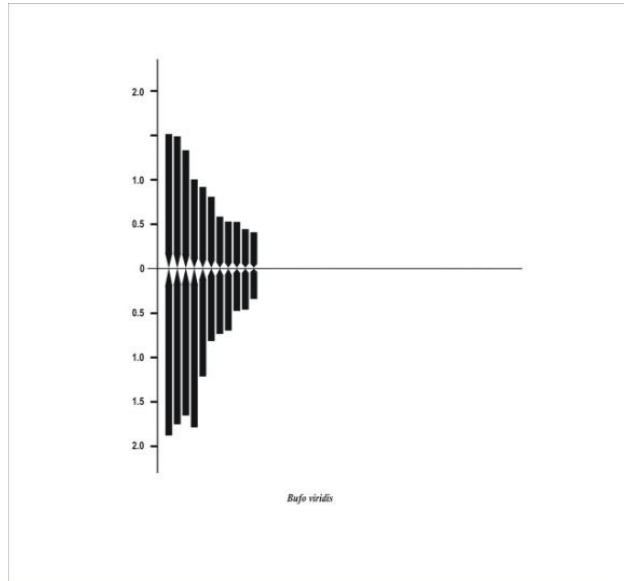


Figure 2: Idiogram of *Bufo viridis*

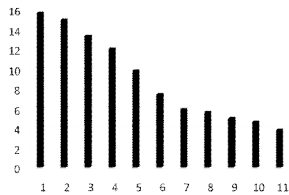


Figure 3: Histogram of chromosomes constructed On the basis of relative length percentage



Figure 4: Sperrmatogonial Metaphase

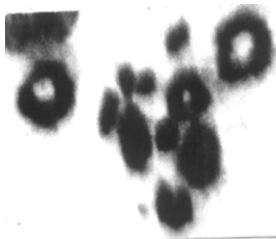


Figure 5: Diakinesis



Figure 6: Metaphase-I

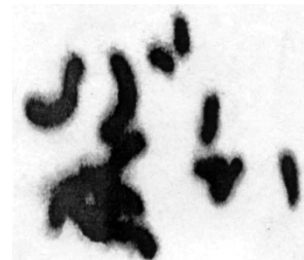


Figure 7: Metaphase-II

The present species *Bufo viridis* shows the presence of twenty two chromosomes in its somatic karyotype and this typical bufonid diploid number has been confirmed from the meiotic stages. Thus diploid number $2n=22$, haploid number $n=11$ and the haploid formula has been concluded to be $1SM+10M$. The retention of the diploid chromosome number 22 is a characteristic of the bufonid species. They have maintained chromosomal stability with unwavering constitution of twenty two chromosomes [2, 3, 6, 7, 10, 17, 18] with a few exceptions like *Bufo regularis* showing the diploid number of twenty chromosomes [1, 5, 8, 9].

Study of meiosis of *Bufo* spp. has been reported in only rare occasions. Duda and Koul (1971) have demonstrated the meiotic stages in *Bufo* spp. from Kashmir. They recorded that the twenty two elements regularly form eleven bivalents during meiosis as determined from diakinesis and metaphase-I stages. Out of the eleven bivalents at metaphase-I, five were large ring shaped elements (last to terminalise) and the remaining six small rod shaped elements (terminalisation complete) were present. In our studies we also arrived at the same results but with slight variations, that is, six large and five small ring shaped bivalents at diakinesis and at metaphase-I, five large thickly

condensed ring shaped bivalents and six small rod shaped bivalents were observed. The stages spermatogonial metaphase and metaphase-II were also observed with twenty two and eleven bivalents chromosomes respectively.

Table 1 Morphometric data of male *Bufo viridis* (2n=22) obtained from somatic metaphase complement.

Chromosome pair No.	Mean Length of Short arm (µm)	Mean Length of Long Arm (µm)	Total Chromosome Length(µm)	Relative Length Percent= $\frac{p+q}{x100}$ Mean total length of haploid set	Arm Ratio (q/p)	Centromeric Index $\frac{p}{p+q}$	Nomenclature
1.	1.53	1.87	3.40	15.72	1.22	0.44	Metacentric
2.	1.50	1.75	3.25	15.02	1.16	0.46	Metacentric
3.	1.34	1.56	2.90	13.41	1.16	0.46	Metacentric
4.	1.01	1.79	2.80	12.94	1.77	0.36	Submetacentric
5.	0.93	1.21	2.14	9.89	1.30	0.43	Metacentric
6.	0.81	0.81	1.62	7.48	1.00	0.50	Metacentric
7.	0.59	0.73	1.32	6.10	1.23	0.45	Metacentric
8.	0.54	0.70	1.24	5.73	1.28	0.43	Metacentric
9.	0.54	0.57	1.11	5.13	1.05	0.49	Metacentric
10.	0.45	0.56	1.01	4.66	1.25	0.44	Metacentric
11.	0.41	0.43	0.84	3.88	1.07	0.48	Metacentric

Morphometric data of Male Somatic Karyotype:

Actual mean length of largest chromosome = 3.4 µm : Actual mean length of smallest chromosome = 0.84 µm

Relative length percent of largest chromosome = 15.72 : Relative length percent of smallest chromosome = 3.88

Ratio of largest to smallest chromosome = 4.04 : Total mean haploid length = 21.63 µm

However, the present study is only preliminary to investigate the basic number, morphology and meiotic behavior of chromosomes for *Bufo viridis*. These conclusions can be later used to contribute for the further cytological perspectives, especially if this is considered to be the first report from Jammu and Kashmir, India. Moreover we are looking forward for further cytogenetic and molecular perspectives of the species which would be utilised for unravelling the phylogeny and intra-specific relationships of the different populations of *Bufo viridis* from Jammu and Kashmir.

ACKNOWLEDGEMENTS

The authors acknowledge the help provided by the Department of Zoology, University of Jammu.

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