

Research Paper

Karyological analysis of Indian toad, *Bufo stomaticus* Lutken, 1862 from Haryana (India)

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Abstract

The purpose of the present study was to investigate the karyotype of Indian toad, *Bufo stomaticus* as accurate as possible using air drying technique and conventional Giemsa staining. Karyotype of *Bufo stomaticus* Lutken, 1862 (Bufonidae) was investigated by examining metaphase chromosomes in both sexes. Spleen and testis tissue were used for karyotype analysis from 6 males and 4 female specimens. After taking the tissue in the presence of 0.1% colchicine for 12 hours in normal saline, the metaphase spreads were performed on microscopic glass slides and then air dried. Conventional Giemsa staining was applied to stain the chromosomes. The results indicated that *B. stomaticus* has $2n = 22$ chromosomes, consisting of all metacentric chromosomes. These chromosomes were arranged in two groups: A and B. Group A consisted of 6 pairs of large chromosomes and group B 5 pairs of small chromosomes. Aneuploidy was also reported in a female individual. Secondary constriction was observed on 7th chromosome pair. The female somatic karyotype has been found to resemble in all respects to the one observed in male except for the 4th and 11th pair of chromosomes. The total haploid mean length was calculated to be 105.671 μm in male and 118.372 μm in case of female. Centromeric index, arm ratio and fundamental number (NF) was also determined.

Keywords: Aneuploidy, Chromosomes number, Fundamental number, Karyotype, Morphometric.

Introduction

The family Bufonidae contains about 500 species belonging to over 35 genera. More than half of the species of this family are contained within the genus *Bufo*. In India only 25 species of Bufonidae are found^[1]. Toads of genus *Bufo* share the presence of parotid glands^[2]. Typically they are relatively poor jumpers and are ground dwellers that produce large clusters of small eggs^[3]. Because of their cosmopolitan distribution and variable life histories, these toads are of interest to investigators researching evolutionary history and biogeography. The *Bufo* species have great karyotypic uniformity and, with few exception, they have shown $2n = 22$ chromosomes. The chromosomal study of genus *Bufo* started in 1932 when Makino^[4] reported the diploid number of 22 chromosomes in *Bufo americanus*. There are some limited reports on *Bufo* populations from all over the world^[5].

Indian *Bufo* has not received much attention from cytogenetic point of view from Haryana. Therefore, the present study was carried out to analyse the karyotype of both sexes of *Bufo stomaticus* from Haryana.

Materials and Methods

Roadside injured / dead individuals of *Bufo stomaticus* Lutken, 1862 were collected from Kurukshetra University, Kurukshetra (Haryana). Samples were taken from 6 males and 4 females of *Bufo stomaticus* for present study. Samples were given treatment of 0.1% colchicine for 12 hours in normal saline. Chromosome

preparations were made following Tjio and Whang [6]. Testes from males and spleen from both male and female toads were placed in 0.56% KCl solution for 15 minutes at 37°C. Cell suspension was centrifuged at 1000 rpm for 8-10 minutes. Cell pellet was fixed in 1:3 glacial acetic acid and methanol for 30 minutes. The cells were again pelleted by centrifugation and the supernatant was discarded. A fresh fixative was added to the cells and left for 30 minutes. The slides were prepared by placing 2-3 drops of cell suspension on a clean grease free slide from a height of about 2 feet. The slides were then allowed to dry at room temperature. The air dried slides were stained in 4% Giemsa solution (pH=7).

All the stained slides were scanned under Olympus CX41 Trinocular microscope for well spread metaphase plates. The selected stages were photographed by using Olympus C-7070 wide zoom camera, at magnification of 100X. For each chromosome, centromeric index, arm ratio, relative percentage length were calculated [7].

Results and Discussion

The diploid chromosome number of *Bufo stomaticus* was reported 22 and NF=44. The spermatogonial metaphase showed presence of 22 chromosomes (Figure 1). Chromosomes can be readily arranged into 2 groups in descending order. Group A comprised of 6 pairs of relatively long chromosomes. Group B comprised of 5 pairs of relatively short chromosomes. The size of the chromosomes ranged from as low as 4.155µm of 11th pair of chromosomes to as high as 15.505 µm of 1st pair of chromosomes (Table 1). The total haploid mean length was calculated to be 105.671 µm.

The somatic metaphase in the spleen cells of the female showed 21 chromosomes instead of 22 (Figure3). All the chromosomes were metacentric in nature. It appeared that tandem fusion has occurred between the one homologous of 11th pair and one homologous of 4th pair of chromosomes. This tandem fusion result the aneuploid situation (2n-1). The pair no. 7th showed knob like structure on one chromosome. This knob like structure was assumed to be secondary constriction.

The female somatic karyotype (Figure 4) has been found to resemble in all respects to the one observed in male except for the 4th and 11th pair of chromosomes. The size of chromosomes in this case varied from 4.024 µm of 11th pair to 16.598 µm of the 1st pair. The total haploid mean length was calculated to be 118.372 µm. The sex chromosomes could not be distinguished in either male or female individuals.

This is the first report on karyological analysis of toad, *Bufo stomaticus* from Haryana (India). Earlier workers have also established diploid number of chromosome as 22 for the genus *Bufo* [4, 8-16]. Chromosome morphology showed a slight modification. In male and female *Bufo stomaticus*, all the chromosomes were metacentric. In present report, secondary constriction was reported on pair no. 7, unlike any other species of *Bufo* where secondary constriction were reported in 1st, 3rd, 4th and 6th pair [12, 15]. Aneuploidy is the condition of having less than or more than the normal diploid number of chromosomes, and is the most frequently observed type of cytogenetic abnormality. It is the second major category of chromosome mutations in which chromosome number is abnormal. Aneuploid situation is not observed by other workers in *Bufo stomaticus* but there are some limited reports in other *Bufo* spp. [17, 18].

Table 1: Morphometric data of chromosomes of male *Bufo stomaticus*

Chrm. Pair no.	Short Arm(p) (µm)	Long Arm(q) (µm)	Total Length (p+q)	%age Relative Length (RL %)	Arm Ratio	Centromeric Index (CI)	Chromosome Morphology
1	7.503	8.002	15.505	14.672	1.006	48.390	Metacentric
2	6.512	8.012	14.524	13.744	1.230	44.836	Metacentric
3	6.027	8.044	14.071	13.248	1.334	42.832	Metacentric
4	6.000	6.003	12.003	11.358	1.000	49.987	Metacentric
5	4.003	6.002	10.005	09.468	1.499	40.010	Metacentric
6	4.000	6.001	10.001	09.464	1.599	38.465	Metacentric

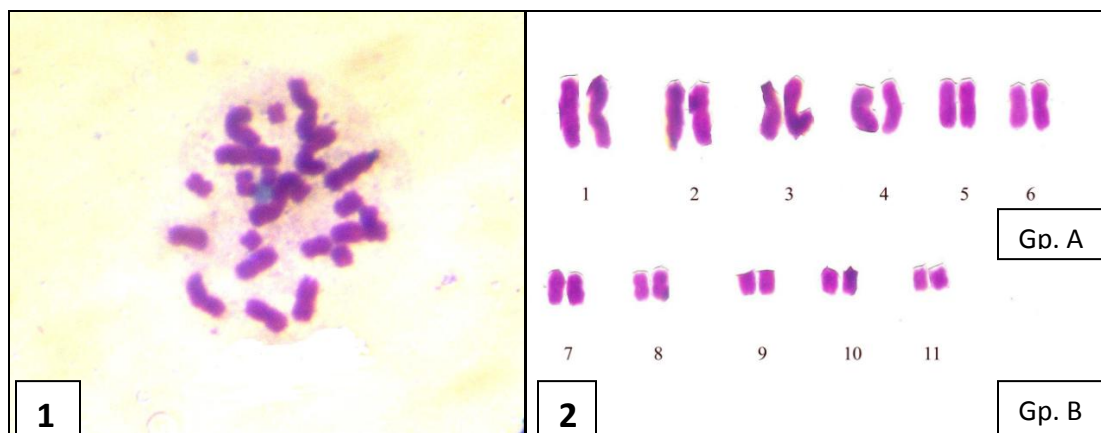
7	3.903	4.012	07.915	07.490	1.027	49.311	Metacentric
8	3.012	3.012	06.024	05.700	1.000	50.000	Metacentric
9	3.002	3.002	06.004	05.681	1.000	50.000	Metacentric
10	3.000	3.001	06.001	05.678	1.000	49.991	Metacentric
11	2.075	2.080	04.155	03.932	1.002	49.939	Metacentric

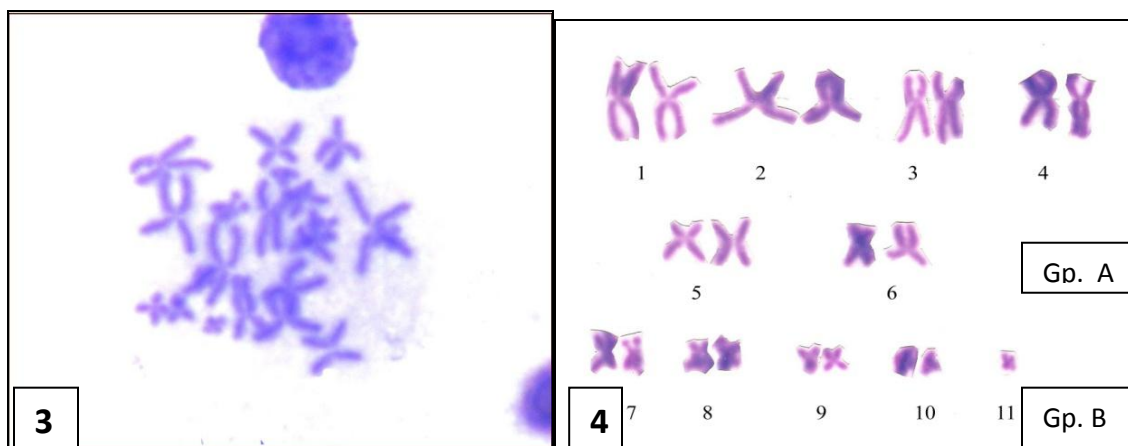
Total haploid mean chromosome length= 105.670 μm

Table 2: Morphometric data of chromosomes of female *Bufo stomaticus*

Chrm. Pair no.	Short Arm(p) (μm)	Long Arm(q) (μm)	Total Length (p+q)	%age Relative Length (RL %)	Arm Ratio	Centromeric Index (CI)	Chromosome Morphology
1	7.078	9.520	16.598	14.021	1.345	42.643	Metacentric
2	7.222	9.273	16.495	13.934	1.287	43.782	Metacentric
3	6.011	9.519	15.530	13.119	1.583	63.147	Metacentric
4	6.019	8.304	14.323	12.099	1.379	42.023	Metacentric
5	5.518	6.042	11.560	09.765	1.094	47.733	Metacentric
6	5.534	5.553	10.887	09.197	1.003	50.831	Metacentric
7	3.527	4.532	08.059	06.808	1.287	43.764	Metacentric
8	3.536	4.515	08.051	06.801	1.276	43.920	Metacentric
9	3.510	3.510	07.020	05.930	1.000	50.000	Metacentric
10	2.910	2.915	05.825	04.920	1.001	49.957	Metacentric
11	2.012	2.012	04.024	03.399	1.000	50.049	Metacentric

Total haploid mean chromosome length= 118.372 μm





**Figure 1-4: Bufo stomaticus 1. Spermatogonial metaphase 2. Karyotype of male
3. Somatic metaphase of female 4. Karyotype of female with aneuploidy**

Conclusion

The diploid number, karyomorphology and morphometrical analysis of metaphase chromosomes in *Bufo stomaticus* have been studied from the spleen cells of female and spleen and testis cells of male after colchicine-air drying Giemsa technique. Diploid chromosome number was found to be $2n=22$. All the chromosomes were metacentric.

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