KARYOTYPIC STUDY OF THE COMMON INDIAN TOAD, *DUTTAPHRYNUS MELANOSTICTUS*, FROM JAMMU AND KASHMIR, INDIA

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ABSTRACT

Karyotypic study of two sexes of the toad species, *Duttaphrynus melanostictus*, was carried out using giemsa staining, C- banding and NOR banding methods, from Jammu and Kashmir, India. The basic chromosome number was found to be 2n=22, fundamental arm number (NF) was 44 with all the biarmed chromosomes and no sex chromosome heteromorphism was found. C-banding and NOR banding was also performed. Paracentric C-band was on the long arm of first homologous pair. Centromeric heterochromatin appeared as darkly stained C-bands on all the chromosomes of diploid complement, whereas Ag-NOR staining showed a pair of nucleolar organizer regions present on pair no. 7 on short arm i.e. 7p in both male and female karyotypes.

Keywords: Karyotype, Chromosome, *Duttaphrynus*, C-banding, NOR, fundamental arm number.

INTRODUCTION:

Amphibians are first land vertebrates which have evolved from the aquatic vertebrates and all the higher vertebrates have evolved from them. They are thus the link between aquatic and terrestrial life. There are 7211 amphibians in the world out of which 6354 are anurans (www.amphibiaweb.org accessed on Dec. 17, 2013). Anurans include the globally distributed frogs and toads. The Common Indian toad - *Duttaphrynus melanostictus* belongs to the family Bufonidae (Anura, Amphibia). Presently genus *Duttaphrynus* is represented by thirty species worldwide. Taxonomic revision of anurans is done simultaneously with the discovery of newer and newer species daily which creates a chaotic condition in the amphibian taxonomy. The common Indian toad is widely distributed throughout India as well as parts of Pakistan and Afghanistan. We performed the cytogenetic study of this toad species from Jammu division (327m altitude and 32º44´00´´N: 74º52´00´´E) of Jammu and Kashmir, India. This karyotypic study is first report of the species from Jammu and Kashmir. The study was carried out using both conventional staining and banding methods in order to establish the diploid number as well as distribution of heterochromatin and nucleolar organizer regions in the species.

MATERIALS AND METHODS:

Two male and two female specimens of *Duttaphrynus melanostictus* were collected from District Jammu (altitude 327m) of Jammu and Kashmir during the monsoon breeding season. Before sacrificing, the specimens were injected intramuscularly and intraperitoneally with 0.5% colchicine solution (@ 1ml per 100g body weight) for 3.5 hours. Then the animals were anaesthesized and dissected to take out the intestine, spleen and bone marrow. The tissues were hypotonised with 0.75M KCl solution for 1hour at room temperature. Fixation of the tissue was done in 3:1 methanol-acetic acid fixative, changing the solution for every 10 minutes. The material was then dabbed on clean slides, air-dried and stained with 2% giemsa stain (pH=7) for 30-35 minutes. C-banding was done using Sumner (Sumner, 1972) technique with some modifications. Ag-NOR banding was done using Howell and Black (1980) protocol with slight modifications. Slides were scanned under Olympus research microscope and the best metaphase complements and meiotic stages as well as C-banded and NOR-banded complements were photographed at 100X magnification. Morphometry was done using occulometer.

RESULTS:

Spermatogonial metaphase complement of male and mitotic metaphase complement of female was selected for preparing karyotypes (Fig. 1 and 2). General chromosome form and type was found to be the same in both and no heteromorphic sex chromosomes were observed in the karyotypes. Diploid chromosome number was 2n=22. Eleven pairs of chromosomes were placed into two groups (Fig. 1 and 2) comprising of Group A of six pairs of large chromosomes and Group B of five pairs of small chromosomes. All chromosomes in both the karyotypes were biarmed and of metacentric and submetacentric type. Three pairs, i.e., Pair no. 2, 4 and 5 of group A was found to be submetacentric while all other chromosomes in both the groups were found to be metacentric type. Haploid formula for the complement was calculated as n=8M+3SM and the corresponding fundamental arm number was calculated as NF=44. Mean haploid length (MHL) was 16.84µm and total complement length (TCL) was 33.68µm for male karyotype whereas MHL was 11.58µm and TCL was 23.16µm for female karyotype.

Morphometric data of the male and female karyotypes is given in the tables 1 and 2. C-banded karyotype showed centromeric heterochromatin in all the chromosomes as C bands. Paracentric C-band was on the long arm of first homologous pair (Fig. 3). Ag-NOR banded karyotype (Fig. 4) showed a well-defined and conspicuous pair of nucleolar organiser regions (NORs). It was present on pair no. 7 on short arm i.e. 7p in both male and female karyotypes. There was no difference in the size of NORs on the homologous pair.

Fig. 1: Karyotype of the male *D. melanostictus.*

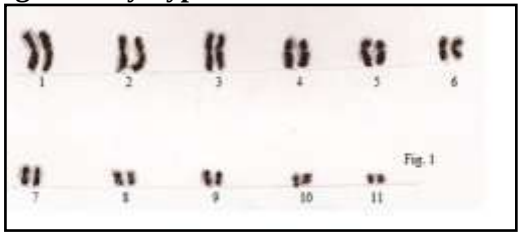

Fig. 2: Karyotype of the female *D. melanostictus*

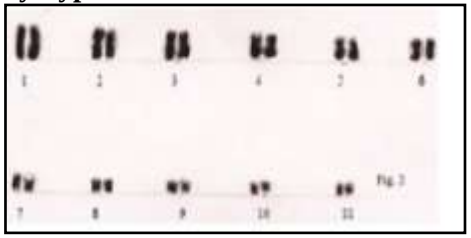

Fig. 3: C-banded complement of *D. melanostictus* showing centromeric C-bands in all the chromosomes of diploid complement and paracentric C-band on the long arm of first homologous pair.

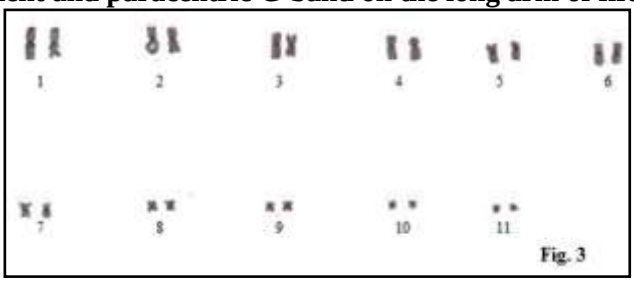

Fig. 4: NOR- banded complement of *D. melanostictus* showing a pair of NORs on short arm of 7th chromosome pair

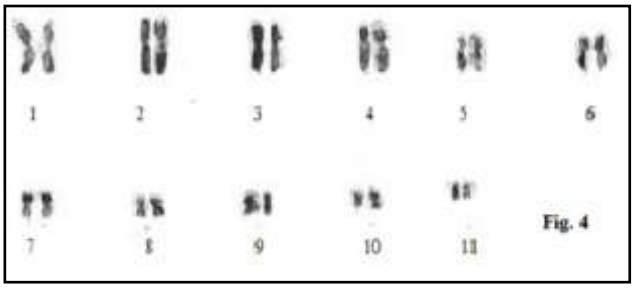

Table 1: Morphometric Data of male *Duttaphrynus melanostictus* (2n=22) from Somatic Metaphase complement

Chromosome Number	Length of Short Arm –p (µm)	Length of Long Arm –q (µm)	Total chromosome length – p+q(µm)	Relative Length Percent	Arm Ratio- q/p	Centromeric Index=p/p+q	Nomenclature
1	1.06	1.09	2.15	18.56	1.03	0.49	Metacentric
2	0.64	1.11	1.75	15.11	1.73	0.36	Submetacentric
3	0.69	0.89	1.58	13.64	1.29	0.43	Metacentric
4	0.51	0.94	1.45	12.52	1.84	0.35	Submetacentric
5	0.31	0.69	1.00	8.63	2.23	0.31	Submetacentric
6	0.45	0.52	0.97	8.37	1.16	0.46	Metacentric
7	0.34	0.46	0.80	6.90	1.35	0.42	Metacentric
8	0.24	0.34	0.68	5.87	1.42	0.35	Metacentric
9	0.23	0.28	0.51	4.40	1.22	0.45	Metacentric
10	0.17	0.21	0.38	3.28	1.24	0.56	Metacentric
11	0.12	0.19	0.31	2.67	1.58	0.38	Metacentric

Table 2: Morphometric Data of female *Duttaphrynus melanostictus* (2n=22) from Somatic Metaphase complement

Chromosome Number	Length of Short Arm –p (µm)	Length of Long Arm –q (µm)	Total chromosome length – p+q(µm)	Relative Length Percent	Arm Ratio- q/p	Centromeric Index=p/p+q	Nomenclature
1	1.17	1.23	2.40	14.63	1.05	0.48	Metacentric
2	0.81	1.47	2.30	13.65	1.83	0.35	Submetacentric
3	1.09	1.11	2.20	13.06	1.02	0.49	Metacentric
4	0.67	1.22	1.89	11.22	1.82	0.35	Submetacentric
5	0.61	1.09	1.70	10.09	1.78	0.36	Submetacentric
6	0.72	0.86	1.58	9.63	1.19	0.45	Metacentric
7	0.55	0.68	1.23	7.30	1.23	0.44	Metacentric

8	0.52	0.67	1.19	7.06	1.28	0.43	Metacentric
9	0.42	0.51	0.93	5.52	1.21	0.45	Metacentric
10	0.33	0.48	0.81	4.80	1.45	0.40	Metacentric
11	0.23	0.38	0.61	3.62	1.65	0.37	Metacentric

DISCUSSION:

Family Bufonidae (toads) is represented by 50 genera and 585 species which are successfully distributed throughout the world. Chromosome numbers of bufonids have been found to be highly conserved over a huge period of evolutionary history. Basic chromosome number is 2n=22. Almost all the bufonids have 2n=22 (Beccari, 1926; Makino, 1932; Volpe and Gebhardt, 1968; Odierna *et al*., 2007; Baraquet *et al*., 2011; Al-Shehri and Al-Saleh, 2012; Yadav and Neeru, 2012) with only one exception of *Bufo regularis* in which 2n=20 (Beckert and Doyle, 1968; Bogart, 1968; Al-Shehri and Al-Saleh, 2008). Chromosome form is also highly conserved with most of the karyotypes having symmetrically arranged biarmed chromosomes of metacentric, submetacentric and in few cases subtelocentric chromosomes (Duda and Koul, 1971; Amaro-Ghilardi, 2008; Baraquet *et al*., 2011; Saba and Tripathi, 2012). C-banding analysis and NOR-banding analysis of bufonid karyotypes have shown diverse types of distribution of the heterochromatin which appears as darkly stained regions or bands along the chromosome length. Moreover, location of heterochromatin as well as nucleolar organizer regions (NOR) is highly variable in different members of family bufonidae (Schmid, 1982; Odierna *et al*., 2004). C-banding of bufonid species chromosomes have provided appropriate species specific banding patterns and have been used in species characterization, confirming polyploidy and comparative accounts (Schmid *et al*., 2004; Jing *et al*., 2009).

General karyotype formula of *Duttaphrynus melanostictus* confirms with the other bufonids, i.e., 2n=22, NF=44 and two groups of chromosomes divisible on the basis of size of chromosomes (Fig. 1 and 2). Metacentric and submetacentric chromosomes were found in group A whereas group B was entirely of small metacentric chromosomes. Pair no. 2, 4 and 5 of group A was found to be submetacentric while rest of all the chromosomes in both the groups were found to be metacentric type. These findings confirm the earlier works like Manna and Bhunya (1966), Maxson (1984), Bannerjee (1987), Jun (1998), Supaprom, and Kullayaprasit (2000), Phimphan and Tanomtong (2012). Centromeric heterochromatin was shown in all the chromosomes as C-bands (Fig. 3). Interstitial paracentric band was observed on long arm of the first or the pair. NOR banding (Fig. 4) showed a well-defined and conspicuous pair of nucleolar organizer regions was found on pair no. 7 on short arm (7p) which resembles with that of other bufonids.

CONCLUSION:

This study is first of its kind from Jammu division of Jammu and Kashmir, India. This investigation has charaterised the species karyotypically and will further help in its phylogenetic evaluation. Since this is the first report of karyotype of the species from Jammu and Kashmir, it is to be treated as a beginning towards further taxonomy based evaluation of the species and its relationship with the other bufonids and anurans of the region.

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