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Cytogenetics of Two Sexes of the Green Toad, *Pseudepidalea viridis*: First Report from Jammu and Kashmir, India

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Abstract: ***Cytogenetics of the green toad, Pseudepidalea viridiswas studied from Jammu and Kashmir (India). Conventional Giemsa staining showed that the toad species has diploid number (2n) as 22 and all the chromosomes were biarmed (NF=44). Karyotype comprised of 11 pairs of chromosomes arranged in two groups. Fourth pair was submetacentric whereas all other chromosomes were metacentric. Male and female karyotypes were studied but no heteromorphic sex chromosomes were found in either of them. C-banding showed centromeric C-bands in all the chromosomes and few interstitial C-bands on some chromosomes. Ag-NOR staining showed a pair of NORs on short arm of 7th pair of chromosomes. The study is first of its kind from Jammu and Kashmir, India.***

Keywords: Karyotype, Chromosomes, Biarmed, Metacentric, Submetacentric, C-band, NOR

1. Introduction

The family Bufonidae (order Anura) is largest anuran family currently which comprises of 585 species in 50 genera [13]. Bufonids are the toads distributed widely on the globe. Bufonidae is a worldwide hyloid clade of noncontroversial monophyly. Amphibian taxonomy was relatively stable for decades, as summarized by Duellman and Trueb [12]. The advent of direct DNA sequencing methods in the early 1990's enabled tests of previous hypotheses of phylogenetic relationships. In turn, new understandings of phylogeny led to suggestions for taxonomic revision. The first large-scale taxonomic treatment for all of living amphibians [14] proposed radical changes and additional publications have made further taxonomic revisions.

Cytogenetics of the green toad including conventional karyotyping, C-banding and NOR-banding has been describedfor the first time from Jammu. The green toad thrives well in northern parts of India, parts of Pakistan and Afghanistan [23], [15], [9], [13]. The amphibian species of Jammu and Kashmir have not received much attention of cytogeneticists and molecular biologists till now [11], [22]. Karyotypic details in both the sexes as well as C-banding and Ag-NOR banding resultsare described and the species has been characterised.

2. Materials and Methods

Two male and one female specimen of *Pseudepidalea viridis*were collected from Jammu division (altitude 300-4200m) 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 0.5% NaCl solution for 30 minutes at room temperature. Fixation of the tissue was done in 3:1 methanol-acetic acid fixative for 45 minutes changing the solution every 15 minutes. The material was then dabbed on clean slides, air-dried and stained with 2% giemsa stain (pH=7) for 30-35 minutes. Ag-NOR banding was done using Howell and Black [30] protocol with slight modifications. Slides were scanned under Olympus research microscope and the best metaphase complements were photographed at 100X magnification. Morphometry was done using occulometer.

3. Results

A total of 40-50 metaphase stages were selected to establish the diploid chromosome number and sexual heteromorphism. Both male spermatogonial metaphase and female 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=22 (Fig1 and 2). Eleven pairs of chromosomes were placed into two groups comprising of Group A: six pairs of large chromosomes and Group B: five pairs of small chromosomes. All chromosomes in both the karyotypes were biarmed and of two types, that is, metacentric and submetacentric type (following Levan *et al.*, 1964). In *Pseudepidalea viridis*, pair 4 of group A was found to be submetacentric and all the other chromosomes in both the groups were metacentric type. Haploid formula for the complement was calculated as n=10M+1SM and the corresponding fundamental arm number was calculated as NF=44. Mean haploid length was 21.72µmand16.53µm for male and female karyotypes respectively, and total complement length was 43.44µmand 33.06µm for male and female karyotypes respectively. Idiograms were prepared for both male and female karyotpes (Fig. 3 and 4 respectively). Since there was no difference between male and female karyotypes on C-banding (with only a few

differences of chromosome lengthbut no sex specific C-bands), so only male C-banded karyotype has been described. Centromeric heterochromatin was shown in all the chromosomes as C-bands. Homologous pairing of chromosomes and chromosome form was made clear in C-banded karyotype. Paracentric C-bands were present on the short arm of pair no. 1, 2, 3 and on long arm of pair no. 4. No sex specific heterochromatin was found any of the chromosomes. No C-band heteromorphism was seen in any pair of male and female karyotypes (Fig. 5). Ag-NOR banding showed two NORs on short arm of pair number 7 in group B (Fig 6). Chromosomal morphometric data for male and female karyotypes is given in the Table 1 and 2.

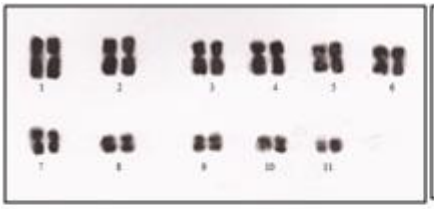

Fig 1. Karyotype of Male *P. viridis*

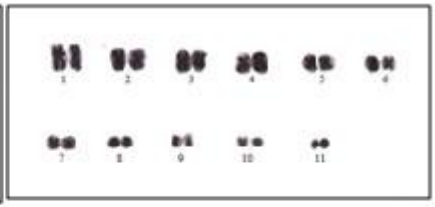

Fig 2. Karyotype of Female *P. viridis*

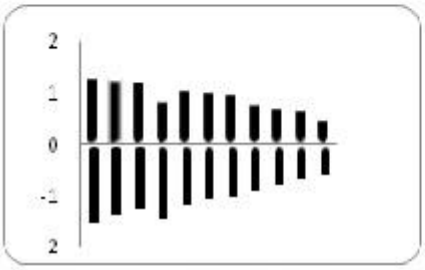

Fig 3. Idiogram of Male *P. viridis*

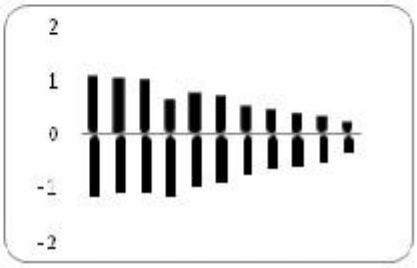

Fig 4. Idiogram of Female *P. viridis*

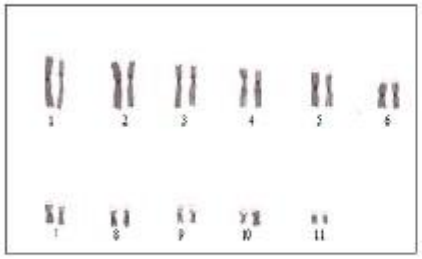

Fig. 5. C-banded karyotype of *P. viridis*

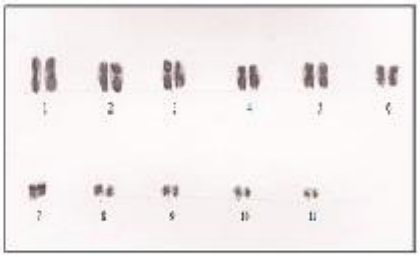

Fig 6. NOR-banded karyotype of *P. viridis* (NOR on 7p).

Table 1: Chromosomal Morphometric Data of male *Pseudepidalea viridis* (2n=22) from spermatogonial 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	1.23	1.53	2.76	12.70	1.24	0.44	Metacentric
2	1.21	1.39	2.60	11.97	1.14	0.46	Metacentric
3	1.17	1.27	2.44	11.23	1.08	0.45	Metacentric
4	0.79	1.47	2.26	10.40	1.86	0.34	Submetacentric
5	1.03	1.20	2.23	10.26	1.11	0.46	Metacentric
6	0.97	1.05	2.02	9.30	1.08	o.48	Metacentric
7	0.93	1.02	1.95	8.97	1.09	0.47	Metacentric
8	0.75	0.90	1.65	7.59	1.20	0.45	Metacentric
9	0.69	0.80	1.49	6.86	1.15	0.46	Metacentric
10	0.63	0.69	1.32	6.07	1.09	0.47	Metacentric
11	0.40	0.60	1.00	4.60	1.50	0.40	Metacentric

Table 2: Chromosomal Morphometric data of female *Pseudepidalea viridis* (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	1.07	1.17	2.24	13.55	1.09	0.47	Metacentric
2	1.05	1.11	2.16	13.06	1.05	0.50	Metacentric
3	1.01	1.11	2.12	12.82	1.09	0.48	Metacentric
4	0.63	1.17	2.80	10.88	1.85	0.35	Submetacentric
5	0.78	0.99	1.77	10.70	1.26	0.44	Metacentric
6	0.69	0.92	1.61	9.73	1.33	0.42	Metacentric
7	0.53	0.77	1.30	7.86	1.45	0.40	Metacentric
8	0.44	0.65	1.09	6.59	1.47	0.40	Metacentric
9	0.39	0.61	1.00	6.04	1.56	0.39	Metacentric
10	0.33	0.53	0.86	5.20	1.60	0.38	Metacentric
11	0.24	0.34	0.58	3.50	1.41	0.41	Metacentric

4. Discussion and Conclusion

The green toad species studied has diploid number, n=22 and NF=44. Bufonid karyotype features are conserved in this species. Almost all the toad species share the conserved karyotypic characters of family Bufonidae. The presence of metacentric and submetacentric type chromosomes strongly depicts karyotypic conservatism as observed in most of bufonid toads. Chromosome numbers of bufonids have been found to be highly conserved over such huge period of evolutionary history. Basic chromosome number is 2n=22. Almost all the bufonids have 2n=22 [4], [17], [6], [18], [27], [28], [21] [26], [20], [2], [3] [29] [22] with only one exception of *Buforegularis* in which 2n=20 [5] [8] [1]. Chromosome form is also highly conserved with most of the karyotypes having symmetrically arranged biarmed chromosomes of metacentric, submetacentric and in few cases sub-telocentric chromosomes [19], [7], [3], [22]. Existence of NORs on 7^{th} pair is yet another proof of karyotypic conservatism in these two species. Most of the toad species do have secondary constriction on 7^{th} pair [24], [25]. This description is first of its kind reporting the cytogenetics of *Pseudepidalea viridis*from Jammu and should be treated as a basic step towards species characterization of the region. This will definitely contribute to the existing cytogenetic information regarding taxonomic re-evaluation of the species as far as the global distribution of species and its taxonomic data is concerned. The present study has revealed that the green toad species possesses the unique bufonid C-banding, NOR-banding and general Giemsa staining cytogenetic profile. Further study is required in future to study population genetics and other cytogenetic trends in the species in order to evaluate its phylogenetic relationships within the species and with other bufonid species. We are still working to trace out other species of the genus *Pseudepidalea* in the region or existence of *P. viridis* subspecies with cytogenetic as well as molecular based studies.

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