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Chromosomal Study of *Euphlyctis cyanophlyctis* (Anura, Amphibia) From Jammu Region of Jammu and Kashmir, India

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Abstract | **Original Research Article**

Chromosomal studies of skitter frog- *Euphlyctis cyanophlyctis* was carried out from Jammu and Kashmir, using conventional staining and banding methods. Karyotypes were prepared by conventional staining in both male and female specimens. Diploid chromosomes number for the species was 2n=26 and fundamental arm number was NF=52. Giemsa stained karyotypes of both male and female comprised of thirteen pairs of biarmed chromosomes divided into two groups: first group of five pairs of larger metacentric or submetacentric chromosomes and second group of eight pairs of smaller metacentric or submetacentric chromosomes. Pair number 2, 3, and 4 among the first group and 9, 10 and 11 among the second group chromosomes. All the other chromosomes in both the groups were metacentric. Meiotic stages observed were leptotene, zygotene, diakinesis, metaphase-I and metaphase-II, from testicular cells of males. C-banding analysis of the species showed presence of centromeric C-bands in all the chromosomes of karyotype. NOR-banding showed presence of a pair of nucleolar organizer regions on the long arm of 10th pair.

Keywords: Karyotype, Chromosome, C-Banding, NOR-Banding, Metacentric, Sub-Metacentric.

INTRODUCTION

Amphibians are an amazing group of vertebrates leading dual mode of life i.e. both aquatic and terrestrial. They are the first land vertebrates and all the higher vertebrates have evolved from them. They are thus the link between aquatic and terrestrial life. There are 7145 amphibians in the world out of which 6300 are anurans. (www.amphibiaweb.org accessed on July 1, 2013). Anurans include frogs and toads which are most widely distributed on globe. The skitter frog, *Euphlyctis cyanophlyctis* belongs to the family Dicroglossidae (Anura, Amphibia). Presently genus *Euphlyctis* is represented by six species worldwide. Dicroglossidae comprises of 186 species. Taxonomic revision of ranoid frogs renamed the species *Rana cyanophlyctis* as *Euphlyctis cyanophlyctis*. Cytogenetic records of the species from Jammu and Kashmir, North India, are very scanty (Duda and Koul, 1973). The skitter frog is one of the most widely distributed species in Jammu and Kashmir. Our study was confined to Jammu division (327m altitude and 32º44′00′′N: 74º52′00′′E) of the state and preliminary cytogenetic study was carried out in order to characterize the species using both conventional staining and banding methods. This is an attempt to characterize the species from Jammu in order to contribute for the taxonomic evaluation of the anurans of the state and we are further looking for molecular investigation of the species for taxonomic based assessment and population genetics.

MATERIALS AND METHODS

Three male and two female specimens of *Euphlyctis cyanophlyctis* (Figure 1 and 3 respectively) 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 1% colchicine solution (@ 1ml per 100g body weight) for 4 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 1 hour at room temperature. Fixation of the tissue was done in 3:1 methanol-acetic acid fixative for 50 minutes changing the solution 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 (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

Mitotic metaphase plate of female and spermatogonial metaphase plate of male was selected for preparing karyotype. General chromosome form and type was found to be the same in both and no heteromorphic sex chromosome were observed in the karyotype. Diploid chromosome number was in agreement with other bufonids that is 2n=26. Thirteen pairs of chromosomes were placed into two groups comprising: Group A of five pairs of larger metacentric or submetacentric chromosomes and Group B of eight pairs of smaller metacentric or submetacentric chromosomes. According to chromosome classification of Levan *et al*., (1964), all chromosomes in this karyotype were biarmed and of two types, that is, metacentric and submetacentric type (Figure 2 and 3). Pair no. 2, 3 and 4 of group A and 9, 10 and 11 of group B was found to be submetacentric while rest of all the chromosomes in both the groups were found to be metacentric type. Haploid formula for the complement was calculated as n=7M+6SM (Figure 13) and the corresponding fundamental arm number was calculated as FN=52. No heteromorphic sex chromosomes were identified in the karyotype of either male or female. Morphometric measurement of the chromosomes in male karyotype showed mean haploid length=19.33µm and total complement length=38.66µm. Similarly for female karyotype, mean haploid length=13.69µm and total complement length=27.38µm. Other details of chromosomal morphometry are given in the Table 1 and Table 2 respectively for male and female karyotypes. Idiograms prepared for the male and female karyotypes (Figure 7 and 8).

C-banding showed presence of centromeric C-bands in all the chromosomes of karyotype (Figure 5). Ag-NOR banding showed presence of a pair of NORs on the long arm of 10th pair (Figure 6).

Meiotic stages observed included leptotene characterized by the presence of a ball of coiled chromatin material (Figure 9). Second stage was zygotene in which chromatin was condensed and appeared as thick threads lying side by side. The chromatin condensation followed by synapsis which was obvious in this stage (Figure 10). Third stage was diakinesis which comprised of thirteen ring shaped bivalents (n=13). Five were larger and eight bivalents were smaller bivalents. Chiasma terminalization was there and each ring possessed two terminal chiasma (Figure 11). Metaphase-I showed thirteen well condensed bivalents. Five larger and eight smaller bivalents were found as homomorphic pairs (Figure 12). In metaphase-II, thirteen chromosomes were observed. Out of five larger chromosomes, three were submetacentric and two were metacentric. Smaller chromosomes were eight in number and three of these were submetacentric (Figure 13).

Figure 1: An Adult Male Euphlyctis cyanophlyctis

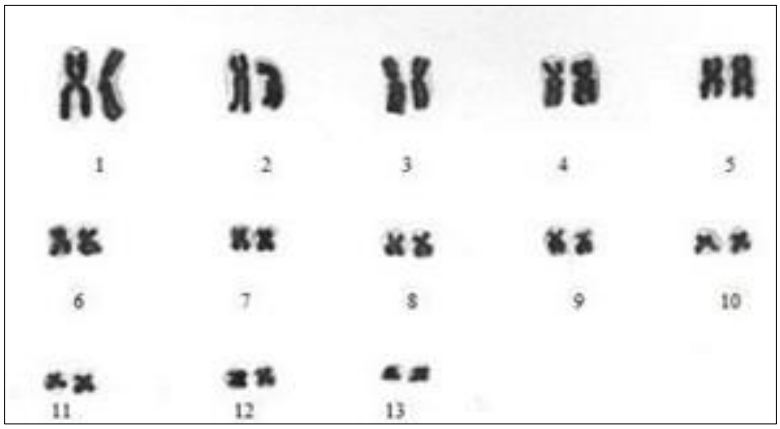

Figure 2: Karyotype of Male Euphlyctis cyanophlyctis

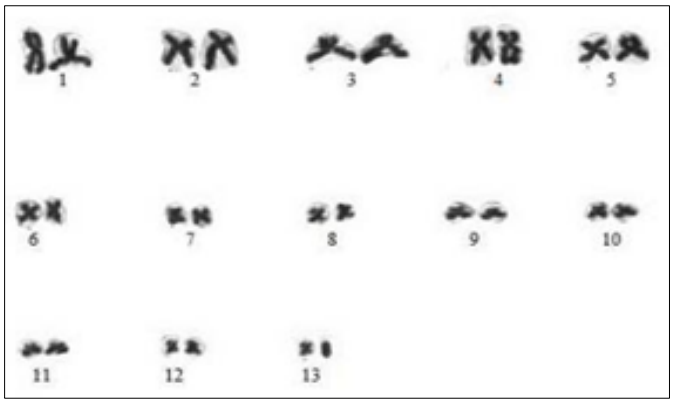

Figure 3: Karyotype of Female Euphlyctis cyanophlyctis

Figure 4: An Adult Male Euphlyctis cyanophlyctis

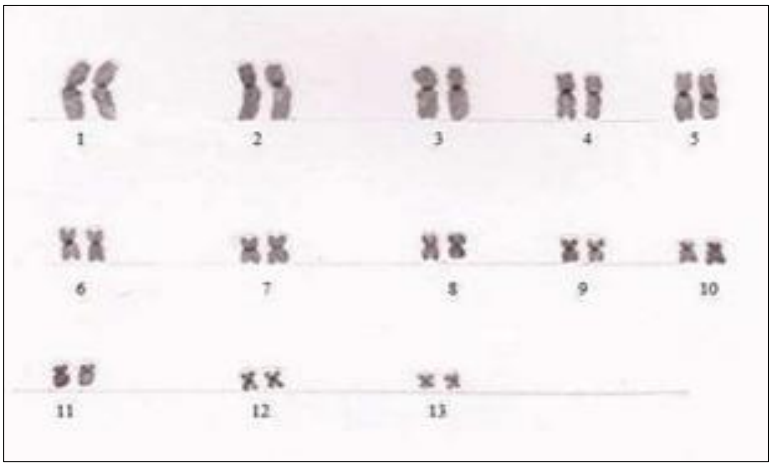

Figure 5: C-Banded Karyotype of Euphlyctis cyanophlyctis

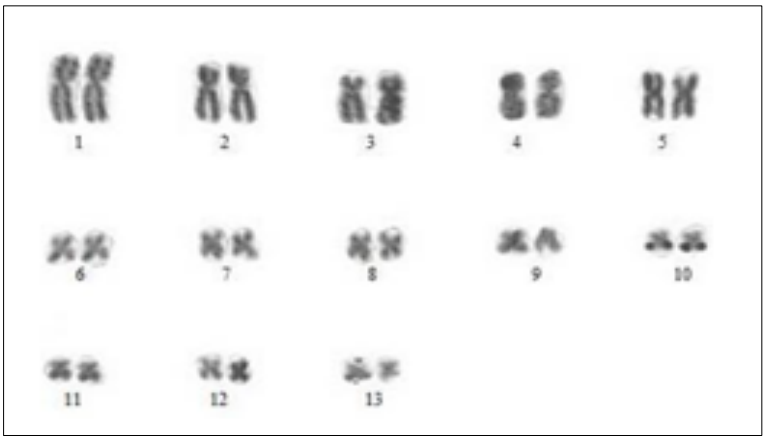

Figure 6: NOR-Banded Karyotype of Euphlyctis cyanophlyctis

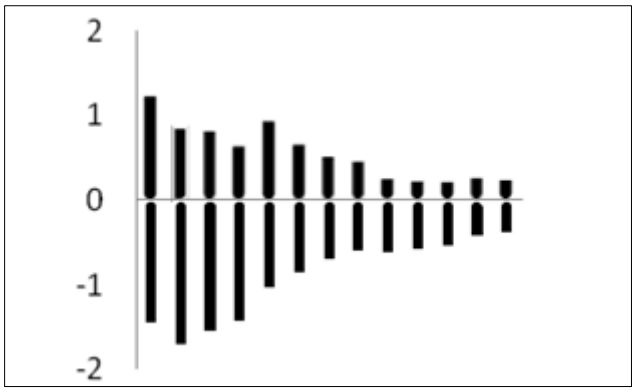

Figure 7: An Idiogram of Male Euphlyctis cyanophlyctis Constructed on the Basis of Chromosome Numbers and Position of the Centromere

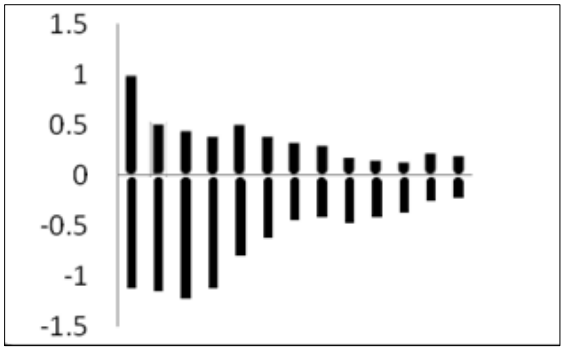

Figure 8: An Idiogram of Female Euphlyctis cyanophlyctis Constructed on the Basis of Chromosome Numbers and Position of the Centromere

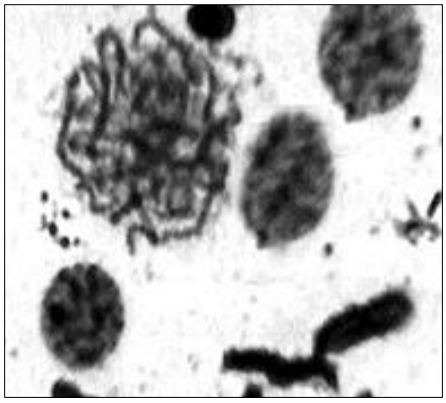

Figure 9: Leptotene

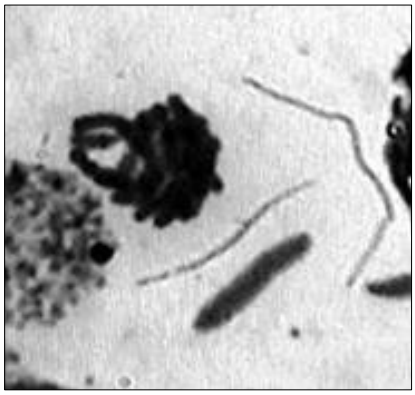

Figure 10: Zygotene

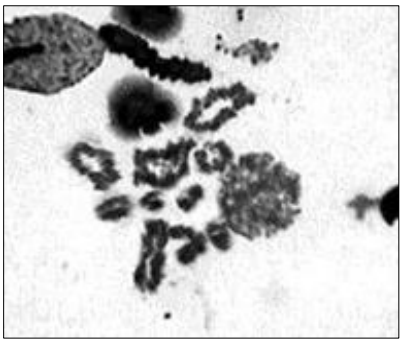

Figure 11: Diakinesis

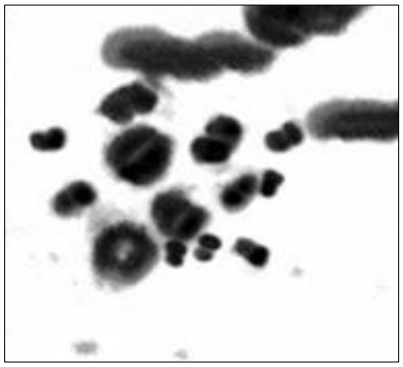

Figure 12: Metaphase-I

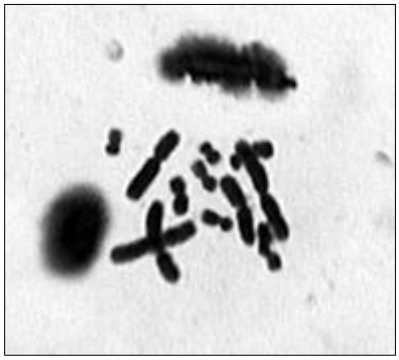

Figure 13: Metaphase-II

Table 1: Chromosomal Morphometric Data of Male *Euphlyctis cyanophlyctis* (2n=26) 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	1.46	2.69	13.91	1.18	0.45	Metacentric
2	0.84	1.73	2.57	13.29	2.05	0.32	Sub-Metacentric
3	0.82	1.56	2.38	12.31`	1.90	0.34	Sub-Metacentric
4	0.64	1.44	2.08	10.76	2.25	0.30	Sub-Metacentric
5	0.94	1.05	1.99	10.29	1.12	0.47	Metacentric
6	0.66	0.87	1.53	7.91	1.31	0.43	Metacentric
7	0.51	0.71	1.22	6.31	1.39	0.41	Metacentric
8	0.46	0.62	1.08	5.58	1.35	0.42	Metacentric
9	0.25	0.64	0.89	4.60	2.56	0.28	Sub-Metacentric
10	0.22	0.60	0.82	4.24	2.85	0.26	Sub-Metacentric
11	0.21	0.56	0.77	3.98	2.67	0.27	Sub-Metacentric
12	0.26	0.43	0.69	3.56	1.69	0.37	Metacentric
13	0.`23	0.39	0.62	3.20	1.69	0.37	Metacentric

Table 2: Chromosomal Morphometric Data of Male *Euphlyctis cyanophlyctis* (2n=26) 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	0.99	1.13	2.21	16.14	1.14	0.45	Metacentric
2	0.51	1.26	1.77	12.92	2.40	0.28	Sub-Metacentric
3	0.44	1.23	1.67	12.19	2.70	0.26	Sub-Metacentric
4	0.39	1.13	1.52	11.10	2.80	0.25	Sub-Metacentric
5	0.51	0.81	1.32	9.64	1.58	0.38	Metacentric
6	0.39	0.63	1.02	7.45	1.62	0.38	Metacentric
7	0.33	0.45	0.78	5.69	1.21	0.42	Metacentric
8	0.30	0.42	0.72	5.25	1.40	0.41	Metacentric
9	0.18	0.48	0.66	4.82	2.60	0.27	Sub-Metacentric
10	0.15	0.43	0.58	4.23	2.86	0.25	Sub-Metacentric
11	0.13	0.39	0.52	3.79	3.00	0.25	Sub-Metacentric
12	0.22	0.27	0.49	3.57	1.22	0.44	Metacentric
13	0.19	0.24	0.43	3.14	1.26	0.44	Metacentric

DISCUSSIONS

The karyotypic characters of *Euphlyctis cyanophlyctis* are conserved like other members of ranoid stock. Family Dicroglossidae basically belongs to ranoid anuran stock. Cytogenetic studies on ranoid anurans began in early twentieth century (Witschi, 1929 and 1933; Iriki, 1932; Makino, 1933; Wickbom, 1945 and 1949). As the family comprises of the largest number of species, there is an extensive amount of cytogenetic data available, but presently there is a taxonomic chaos in the ranid stock. The data available is still confusing and needs to be supplemented further with more conclusive information. Studies have revealed the basic diploid chromosome number 2n=26 in almost all of the ranoids studied till date (Kawamura, 1939a and b; Wickbom, 1945; Mathey, 1951; Seto, 1965; Nishioka, 1972; Ivanov and Madiyanov, 1973; Bardhan *et al*., 1978; Duda and Koul, 1973; Morescalchi, 1973; Haertel *et al*., 1974; Schmid *et al*., 1978; Chakrabarti *et al*., 1983; Nishioka *et al*., 1987; King, 1990; Kuramoto, 1992; Mohammad *et al*., 1997; Vences *et al*., 2000; Joshy *et al*., 2006; Shanthi *et al*., 2010; Arslan *et al*., 2010; Jazayeri *et al*., 2012; Saba *et al*., 2013; Saba and Balwan, 2013). The modal number of 2n=26 is conserved in ranids and represents most of the ancestral species (Morescalchi, 1968 and 1973; king, 1990; Kuramoto, 1992). Almost all of the ranids have symmetrical karyotypes with all biarmed karyotypes. Karyotype comprises of two groups of chromosomes distinguished on the basis of their sizes, first group of five pairs of larger chromosomes and second group of eight pairs of smaller chromosomes. All chromosomes are either metacentric or submetacentric in nature.

Karyotypic details are in confirmation with the general cytogenetic trends in ranoid anurans. Cytogenetic analysis of *Euphlyctis cyanophlyctis* carried out in this study is in partial agreement with the earlier studies (Bardhan *et al*., 1978; Duda and Koul, 1973; Shanthi *et al*., 2010; Alam *et al*., 2012). Duda and Koul (1973) reported metacentric, submetacentric and subtelocentric chromosomes in *Rana cyanophlyctis* (former name of *Euphlyctis cyanophlyctis*) from Kashmir. But in present work no subtelocentric chromosomes were observed. Meiotic studies showed the presence of thirteen bivalents at diakinesis and metaphase-I stages. Similar rings were seen in present study also. Since this is the preliminary chromosomal investigation of the species from Jammu, there is a lot of scope regarding further DNA based RAPD, AFLP, or sequencing etc. for the extensive taxonomic evaluation of the species from the state of Jammu and Kashmir.

Conflict of Interest: Authors declare that there is no conflict of interest.

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