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Great chromosomal variation among members of family Colubridae

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ABSTRACT

Cytological work was carried out on five common species of snakes of family Colubridae viz. *Lycodon aulicus*, *Boiga trigonata*, *Ptyas mucosus*, *Oligodon arnensis* and *Natrix stolata* collected from Botanical garden of Jammu Univ., Moh-Maya area and Bhaderwah Dist. of Jammu region (J&K). *Ptyas mososus* possesses 2n=34, *Natrix stolata* possess 2n=36, *Lycodon aulicus* have 2n=36, *Boiga trigonata* possess 2n=36 and *Oligodon arnensis* possess 2n=44. Except *Natrix* all the colubrid studied have no heteromorphic sex chromosomes. All the colubrids studied possess 2n=36 i.e typical ophidian karyotype except *Oligodon arnensis* which has 2n=44 and *Ptyas mucosus* which possess 2n=34 , number of macro and microchromosomes and thus NF varies among different members which can be attributed to para and pericentric inversions between macro and microcomplements.

Keywords:- Colubridae, Cytogenetics, Ophidian, Karyotype, Lycodon

INTRODUCTION

Snakes and Lizards are the most fascinating reptiles existing on the Earth at present. Cytological and biochemical data act as a supplement to taxonomical and morphological approach. Moreover data collected from morphological, karyological , taxonomical and biochemical analysis when combined help in relieving valuable information about the evolution of snakes. Family Colubridae includes the largest non poisoneous snakes with almost 255 genra and over 1000 species inhabiting whole world. Out of total only 38 genera and almost 130 species are reported from India (Sharma B.D, 1998) while in J&K this family is represented by 12 genra and 18 species only (Sahi, D.N, 1979). Family Colubridae exhibits great variation in chromosome number ranging from a highest of 2n=50 reported in *Cleilia occipitotutea* to lowest 2n=24 *Hydrodynastes bicinctus*, but 2n=36 with 16 macro and 20 micro is the most frequent number recorded in the family Colubridae showing great Karyotypic variation but NF is conserved. During the present course of study 5 species of family colubridae belonging to different subfamilies were studied for their cytogenetic and phylogenetic relationships.

MATERIAL AND METHODS

Live specimens of Lycodon aulicus, Boiga trigonata, oligodon arnensis, Ptyas mucosus and Natrix stolata of family Colubridae were collected from natural population of Jammu i.e samba, Kathua, sidhra, jammu university campus and Batote region of J&K with the help of hand net. In the laboratory they were injected intraperitoneally with 1.0% colchicines (1ml/100gm body weight) for 24 hrs before dissection. Both gonadial and somatic tissues (bone marrow taken out from ribs were extracted and hypotenised) in 1.0% sodium citrate for 40 minutes. Hypotenised tissues was then put in Carnoys fixative (3 methnol :1 acetic acid which is actually acting as fixating agent) for 30 minutes. Slides were then prepared by dabbing and stained in Giemsa stain.

RESULTS

Lycodon aulicus:- By examining somatic tissues we got well spread mitotic stages, (Fig 1). exhibiting a dioploid count of 36 chromosomes and comprises of 16 macrochromosomes and 20 microchromosomes. Macrochromosomes includes 8 metacentric, 6 submetacentrics and 2 acrocentrics. The microchromosomes are very small dot like, of variable ranging from 0.6 micron to 0.36 micron. The morphometric data of macrochromosome complement of male Lycodon aulicus is given in table 1. The sex chromosomes are homomorphic ZZ in male.

Boiga trigonata:- Spermatogonial metaphase complements exhibit a diploid count of 36 chromosomes (2n=36) comprising 18 macro- and 18 microchromosomes (fig 2). Macrochromosomes includes 5 pairs of metacentrics, 2 pairs of submetacentrics and 2 pairs of acrocentrics. The microchromosomes are Small dot like with size ranging from 0.72 micron to 0.45 micron. The morphometric data of macrochromosome complement of male *Boiga trigonata* is given in table 2. The sex chromosomes are homomorphic (ZZ) in male.

Ptyas mucosus:- Both male and female individuals have been used for the chromosomal analysis which yielded 90 well spread metaphase plates. The karyotype analysis has revealed 34 as the diploid number (2n=34) for the species having 16 macro and 18 microchromosomes (fig 3). Five pair of macrochromosomes have their centromere in the median, 1 pairs in the submedian and two pairs in subterminal position. The 18 microchromosomes in this species form distinct size classes and their relative length percentage varies from 0.6 to 0.54 micron with NF=46 (table 3). No heteromorphic pair of chromosomes is detected in either sex of this species.

Natrix stolata:- After studying one male and one female and its 70 metaphase plates the organism exhibits2n=36 with 14 macro and 22 microchromosomes (Fig 4). The macrochromosomes can be classified into 2 groups, the first group consists of 5 pairs of chromosomes having median centromere the “Z” chromosome is included in this group, the second group includes one pair i.e. 6^{th} pair and “W” chromosomes having subterminal centromere. Microchromosoms form a distinct size group having terminal centromere except pair nimber 1 and 5 which have median centromere. The relative length percentage of microchromosomes of female ranges from 0.99 to 0.64micron with NF=47(table 4).

Oligodon arnensis:- When both male and female specimens were studied its observed to possess 2n=44 (Fig 5), with 22 acrocentric (macrochromosomes) and 22 microchromosomes. There exists a regular gradation in size of chromosomes from macro to microchromosomes, yet these are differentiated into macro and microchromosomes on the basis of fact that morphology of macrochromosomes is distinct but of microchromosomes is not quite distinct.

Table:1 Morphometric data of somatic karyotype of *Lycodon aulicus*

No.of Chromosome Pair	Mean Length of Short arm (P) in µ	Mean length of long arm (q) in µ	RL % $\frac{P^+q}{\text{Mean haploid length}}$*100	Arm ratio p/q	Centromeric Index CI=p/q+q	Nomenclature
1.	2.40	2.62	21.57	1.09	0.47	M
2.	1.24	2.10	14.35	1.69	0.37	sm
3.	1.23	1.42	11.38	1.15	0.46	m
4.	0.72	1.50	9.54	2.08	0.32	sm
5.	0.84	1.05	8.12	1.25	0.44	m
6.	0.82	0.82	7.30	1.00	0.50	m
7.	---	1.17	5.15	--	---	acro
8.	0.42	0.72	5.02	1.50	0.40	sm

Total length of haploid macrocomplement = 19.07 µ
Total Length of microcomplement = 4.20 µ
Total length of haploid set =23.27 µ

Table: 2 Morphometric data of somatic karyotype of *Boiga trigonate*

No. of Chromosome Pairs	Mean Length of Short arm (P) in µ	Mean length of long arm (q) in µ	RL % $\frac{P^+q}{\text{Mean haploid length}}$*100	Arm ratio p/q	Centromeric Index CI=p/q+q	Nomenclature
1.	1.95	2.10	16.71	1.07	0.48	m
2.	1.20	1.95	13.00	1.62	0.38	sm
3.	1.29	1.35	10.89	1.04	0.48	m
4.	1.05	1.20	9.28	1.14	0.46	m
5.	0.90	1.05	8.04	1.16	0.46	m
6.	--	1.50	6.19	--	--	acro
7.	0.48	0.72	4.95	1.50	0.40	m
8.	0.60	0.60	4.95	1.00	0.50	m
9.	--	0.80	3.30	--	--	acro

Total length of haploid macrocomplement = 18.74 µ
Total Length of microcomplement = 5.49 µ
Total length of haploid set = 24.23 µ

Table: 3 Morphometric data of somatic karyotype of *Ptyas mucosus*

No. of Chromosome Pairs	Mean Length of Short arm (P) in µ	Mean length of long arm (q) in µ	RL % P+q ____*100 Mean haploid length	Arm ratio p/q	Centromeric Index CI=p/q+q	Nomenclature
1.	1.80	1.80	15.90	1.00	0.50	m
2.	1.20	1.80	13.30	1.50	0.40	m
3.	1.20	1.20	10.60	1.00	0.50	m
4.	0.90	1.20	9.30	1.30	0.42	m
5.	0.44	1.45	8.38	3.20	0.28	st
6.	0.90	0.90	7.98	1.00	0.50	m
7.	0.21	1.11	5.85	5.20	0.18	st
8.	0.42	0.90	5.85	2.09	0.31	sm

Total length of haploid macrocomplement = 17.43 µ
Total Length of microcomplement = 5.10 µ
Total length of haploid set = 22.53 µ
%age relative length of haploid macrocomplement =77.4% of total haploid length
%age relative length of haploid microcomplement = 22.6% of total haploid length

Table: 4 Morphometric data of somatic karyotype of *Natrix stolata*

No. of Chromosome Pairs	Mean Length of Short arm (P) in µ	Mean length of long arm (q) in µ	RL % P+q ____*100 Mean haploid length	Arm ratio p/q	Centromeric Index CI=p/q+q	Nomenclature
1.	1.80	1.80	15.48	1.00	0.50	M
2.	1.26	1.26	10.83	1.00	0.50	m
3.	1.20	1.20	10.32	1.00	0.50	m
4.	0.99	0.99	8.51	1.00	0.50	m
5.	0.75	0.75	6.45	1.00	0.50	m
6.	--	1.05	4.51	--	--	t
7.	0.90	0.90	7.74	1.00	0.50	m
8.	---	1.80	7.74	--	--	t

Total length of haploid macrocomplement including one "Z" chromosome = 14.85 µ
Total Length of microcomplement = 8.40 µ
Total length of haploid set = 23.25 µ
%age relative length of haploid macrocomplement =63.9 % of total haploid length
%age relative length of haploid microcomplement = 36.1 % of total haploid length

Table: 5 Morphometric data of somatic karyotype of *Oligodon arnensis*

No. of Chromosome Pairs	Mean Length of Short arm (P) in µ	Mean length of long arm (q) in µ	RL % $\frac{P^{+}q}{\text{Mean haploid length}}$ *100	Arm ratio p/q	Centromeric Index CI=p/q⁺q	Nomenclature
1.	--	1.5	7.35	--	--	a
2.	--	1.5	7.35	--	--	a
3.	--	1.5	7.35	--	--	a
4.	--	1.2	5.8	--	--	a
5.	--	1.2	5.8	--	--	a
6.	--	1.2	5.8	--	--	a
7.	--	1.2	5.8	--	--	a
8.	--	0.9	4.41	--	--	a
9.	--	0.9	4.41	--	--	a
10.	--	0.9	4.41	--	--	a
11.	--	0.9	4.41	--	--	a
12.	--	0.9	4.41	--	--	a
13.	-	0.6	2.94	--	--	a
14.	--	0.6	2.94	--	--	a
15.	--	0.6	2.94	--	--	a
16.	--	0.6	2.94	--	--	a
17.	--	0.6	2.94	--	--	a
18.	--	0.6	2.94	--	--	a
19.	--	0.6	2.94	--	--	a
20.	--	0.6	2.94	--	--	a
21.	--	0.6	2.94	--	--	a
22.	--	0.6	2.94	--	--	a
23.	--	0.6	2.94	--	--	a

Total length of haploid macrocomplement = 13.86 µ
Total Length of microcomplement = 6.60 µ
Total length of haploid set = 20.46 µ
%age relative length of haploid macrocomplement =63.9 % of total haploid length
%age relative length of haploid microcomplement = 36.1 % of total haploid length

DISCUSSION

Nearly 80% of all living snakes belong to family Colubridae. Many attempts have been made to break this huge family into recognisable small phyletic subdivisions. This group is divided by different workers like Smith, 1943; Underwood, 1967; as well as classification of this group is given by British and American museum of Natural history, but cytological data do not coaccord completely with any of these classification.

The 300 genera and 2500 species reported till date have variously been divided among 6 to 10 poorly defined subfamilies but cytologically only 4% of these species have been worked out. Colubrid reveals great variability in number and morphology of chromosome in different generas including in this family are the species with the highest diploid number i.e *Clelia occipitolutea* with 2n=50 chromosomes (Becak 1965, Becak et al, 1966) and the one with smallest diploid number i.e. *Hydrodynastes bicinctus schultzi* and *H. gigas* both having 2n=24 chromosomes (Becak and Becak 1969) but in general 2n=36 having 16 macro and 20 microchromosomes is considered as typical ophidian Karyotype shown by most species.

During the present investigation 5 species of Colubridae belonging to 5 different subfamilies viz. *Ptyas mucosus* (Subfamily Colubrinae), *Oligodon arnensis* (Subfamily Xenodontinae), *Natrix stolata* (Subfamily Natricinae), *Lycodon aulicus* (Subfamily Lycodontinae) and *Boiga trigonata* (Subfamily Boignae) has been studied. It is observed that out of these only *L.aulicus* exhibits typical ophidian karyotype with 2n=36 having 16 macro and 20 microchromosomes, though 2n=36 is also exhibited by *Natrix stolata* but it possess different number of micro and macrochromosomes (14 macr and 22 microchromosomes), *L. aulicus* belongs to subfamily Lycodontinae, only 5 species of this subfamily have been worked out by Bhatnagar (1961) and Singh, 1972) and recorded 2n=36 with 12 metacentric\submetacentric , 4 acrocentric(macro) and 20 microchromosomes, while during present study 2n=36 has been found to comprise of 8 metacentric, 6 submetacentric and 2 acrocentric(macrochromosomes) and 20 microchromosomes(Sharma and Kour, 2004). These differences in the morphology of microchromosomes in the two population can be attributed to pericentric inversion.

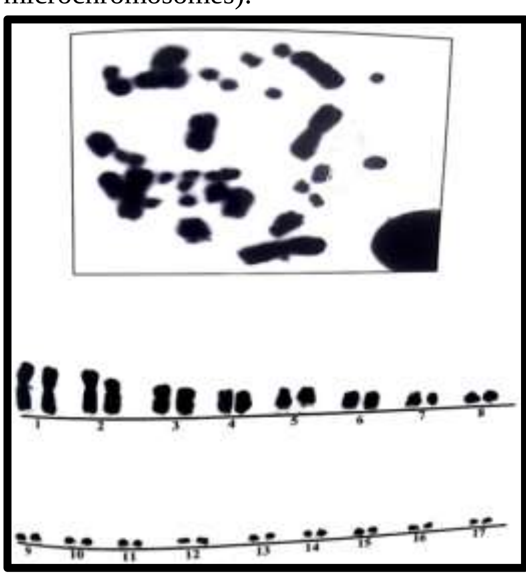
Sub family Boinae to which *Boiga trigonata* belongs shows highly conserved karyotype. All the species worked out thus far possess 2n=36 with little variation from typical ophidian karyotype *Boiga trigonata* has already been investigated by Smith 1972 reported 2n=36 having 18 macro (10 meta, 4 submetacentric and 4 acrocentric) and 18 microchromosomes and 18 microchromosomes . The present data on *Boiga trigonata* coaccords completely with that of Singh (1972). According to Sharma and kour (2004) Since both *L.aulicus* and *B.trigonata* belong to same family they show conservatism in the diploid number since they belong to different subfamily and genera they show difference in the number of both macro and microchromosomes, the number of biarmed macrochromosomes remaining the same. The difference in the number of metacentric and submetacentric in 2 species may be accounted assuming pericentric inversion involving centromeric shift thus converting 2 submetacentric in L.aulicus to metacentric in *Boiga trigonata* and vice versa. The presence of 2 acrocentric more in the macrocomplement of the *Boiga trigonata* compared to *L.aulicus* that may be due to translocation involving the macro and microcomplement in *B.trigonata* or due to duplication experienced by 2 microchromosomes converting them to macro chromosomes. The second assumption is further strengthened from the value of total complement length which is greater in *Boiga trigonata* (48.46%) than *L.aulicus* (46.54%).

Oligodon arnensis belongs to subfamily Xenodontinae which includes only 3 genera and 18 species of this Subfamily have been worked out cytologically so far. This family shows widest range in diploid number from 2n=24 in *Hydrodynastes* (the lowest no in serpents) to 2n=46 in *Oligodon arnensis*. Out of 18 species only 4 shows typical ophidian karyotype while rest all shows deviation from normal pattern. *Oligodon arnensis* has already been worked out by Bhatnagar 1959. According to him it has 2n=44 with 22 meta and 22 acrocentric macrochromosome the present study completely coaccords with Bhatnagar (1959) so no change is due come of time.

Subfamily Colubrinae is largest subfamily of Colubrinae consisting of most terrestrial and arborial snakes. This subfamily is highly conserved Karyologically i.e out of 40 cytologically investigating species, 33 species possess 2n=36. The diploid number in this subfamilies ranges from 2n=34 to 2n=42. The genera *Malpolon, Pseustes* and *Ptyas* are some of the colubridae that do not depict 2n=36 karyology. *Ptyas* has been studied by Bhatnagar (1959) and Singh *et al* (1976) reporting 2n=34 with 16 macro (8 meta and 6 submetacentric) and 18 microchromosomes while during the present study 2n=34 with 16 macro chromosomes (10 meta, 2sub meta and 4 subtelocentric) and 18 microchromosomes have been observed. This variation in karyotype is attributed to pericentric inversion since both studies were conductrd on species from 2 different geographical region.

Subfamily Natricine is highly specialised and evolved group among Colubrid with diploid number ranging from 2n=34 to 2n=46. This subfamily includes few genera which lack microchromosomes i.e. New World Natricinae though Old World Natricine possess microchromosomes thus its believed that both Old and New World *Natrix* might have originated along different lines of evolution. *Natrix stolata* has already been worked out by Bhatnagar, 1959 and Singh, 1972 reported 2n=36 with 14 macro and 22 microchromosomes with metacentric Z and acrocentric W . The present results completely coaccords with that of Singh, (1972).

Morphometric data of Karyotype of *Ptyas mucosus* is observed to be more closely related to *Eryx johni* than that of other Colubrids in having firstly 2n=34 with 16 macro- and 18 microchromosomes, secondly the genome size of *Eryx johnii* is almost same as that of *Ptyas musosus*, furthermore having absence of sex chromosome heteromorphism which is prevelant in other colubrids. In addition, the macrocomplement of *E. johnii*(22.2%) and *Ptyas musosus* (22.6%) contribute same to the total haploid set. *N.stolata*, on the other hand possess well differentiated sex chromosomes i.e metacentric ‘Z’ and acrocentric “W’ which according to Becak and Becak (1969) is the first towards differentiation of sex chromosomes in colubrids. The genome size of *N. stolata* is close to that of *Ptyas mucosus* with slight variations i.e here the microcomplement contributes 36% rather than 22.6% as in *P.mucosus* which is infct contributed by additional 4 microcomplement in *N.stolata*. The relative length percentage of different chromosome pairs of both the species is nearly same excepting 2nd pair of *P.mucosus* has split by centric fission thus resulting in 14 macrochromosomes (loss of 2 macrochromosomes and addition of 4 microchromosomes).



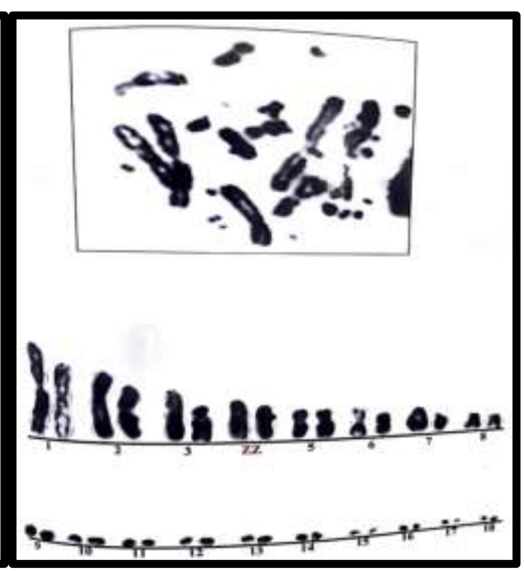
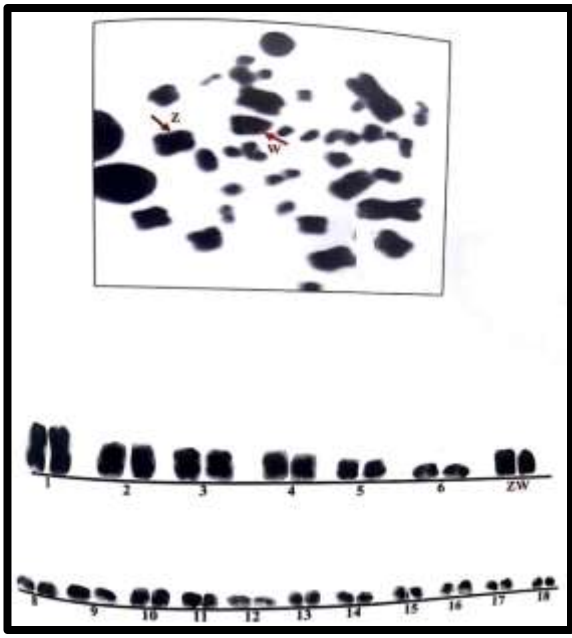


Fig 3 Showing complement and karyotype of *Ptyas mucosus* Fig 1 Showing complement and karyotype of *Lycodon aulicus*



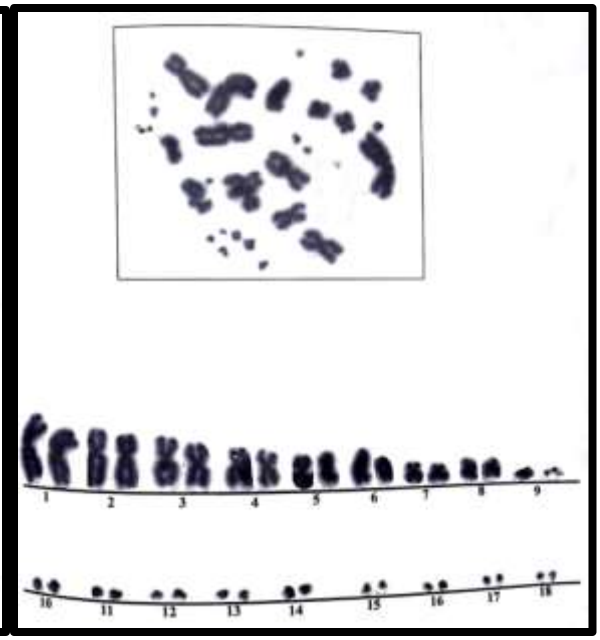


Fig 4 Showing complement and karyotype of *Natrix stolata* Fig 2 Showing complement and karyotype of *Boiga trigonata*

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