Karyological Analysis of *Schizothorax labiatus* (Teleostei: Cyprinidae), a hill stream food fish of Kashmir Himalaya.

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Abstract: Karyotypic characterization of *Schizothorax labiatus* (Chush Snow trout) was carried out following Thorgaard and Disney (1990). The analysis of 80 metaphase plates revealed the chromosome number of this fish 2n=98 and a fundamental arm number (FN) =142. The diploid complement comprised 12 metacentric pairs, 10 submetacentric pairs, 1 subtelocentric pairs and 26 telocentric pairs (24m+20Sm+2St+52t). Total length of the haploid complement equalled 157.5µm with a range in the length of shortest and longest chromosome between 2-8µm. The arm ratio and the centromeric index ranged between 1-∞ and 0-50 respectively. The present study is the first to describe the chromosomal characteristics of *Schizothorax labiatus* from The Kashmir Valley.
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1. Introduction

Kashmir Himalayas have a great array of aquatic habitats ranging from high altitude tarns, crystal clear lakes, rural and urban lakes and ponds of different trophic status as well as springs, torrential streams and mighty rivers. All these water bodies contain some kind of fish. The work on fishes of Kashmir started by J. J. Heckel (1838) who for the first time reported about sixteen species of fishes in the valley, all new to science. Since that time many others have worked on the fish and fisheries of the valley. The prominent works in the field have been those of Mukerji (1936), Hora (1936), Silas (1960), Das and Subla (1963, 1964), Qadri *et al*. (1983), Yousuf (1996), Kullander *et al*. (1999) etc.

Fish species of Kashmir belong to *Cyprinidae, Cobitidae, Siluridae, Salmonidae and Poecilidae*, with the first one dominating the aquatic systems of the valley. The fishes of this family are distributed over Africa, Asia, Europe, and North America and live almost exclusively in freshwater. Characteristics of *Cyprinidae* include presence of "Pharyngeal teeth", lack of an adipose fin and the presence of barbels in many species.

Cytogenetic analysis in fish have allowed to determine sex chromosomes (Moreira-Filho *et al*., 1993; Devlin and Nagahama, 2002; Molina and Galetti, 2007), the characterization of vertebrate models, like the zebra fish (Sola and Gornung, 2001), the evaluation of genetically modified lineages (Porto-Foresti *et al*., 2004), and to perform inferences on cytotaxonomic (Bertollo *et al*., 2000; Bertollo *et al.,* 2004) and evolutionary issues (Demirok and Unlu 2001). Karyological studies have also provided basic information on the number, size and morphology of chromosomes (Tan *et al*. 2004) which is important to undertake chromosome manipulation in fish (khan *et al*., 2000). Since 1960 karyological studies in teleost fish have made noteworthy contributions in the field of genetics, taxonomy and environmental toxicology (Cucchi and Baruffaldi, 1990). Chromosomal analysis is important for fish breeding from the view point of genetic control, the rapid production of inbred lines and evolutionary studies (Kirpichnikov, 1981). Genetic divergences of populations and their local adaptations are a potential resource for breeding programs in aquaculture and for fishery management (Phillips and Rab, 2001). The study of karyotype is also important in aquaculture in connection with the use of chromosome manipulation techniques including induction of polyploidy, gynogenesis, androgenesis and inter or intra-specific hybridization (Wu *et al*., 1986; Diter *et al*., 1993). Karyological study can be useful for addressing a variety of evolutionary and genetic questions about animals (Macgregor, 1993) and may permit detection of changes that modified an ancestral karyotype as it evolved into new lines (Winkler *et al*., 2004) and chromosomal analysis is important for genetic control, taxonomy and evolutionary studies (Macgregor and Varly, 1993; Fister *et al*., 1999; Suleyman *et al*., 2004) and is widely use in various investigations (Pisano *et al*., 2007).

Despite these advancements, only a few fishes of Kashmir have been studied for their chromosomes viz. *Schizothorax curvifrons* and *S.plagiostomus* (Farooq *et al*., 2011), *S.esocinus* (Farooq *et al.,* 2011) and *Puntius conchonius* (Ganai and Yousuf,

2011).The present study was undertaken with the aim to investigate chromosomes and karyotype of *S.labiatus* to compare it to other members of the genus and generate information that can be utilized for its management and conservation.

2. Materials and Methods.

Live fish were obtained (8 specimens, all females) from local fishermen in the River Jhelum and transported live to the Limnology and Fisheries Laboratory of Centre of Research for Development University of Kashmir and placed into 50 l fully aerated aquarium for several days. For karyological preparation the protocol of Thorgaard and Disney (1990) was followed. Fish received two doses of phytohemagglutinin (PHA) injections (4µgg^{-1} bw), in a 20-h interval at 20˚c . Fishes were pre-treated by jntraperitoneal injection of colchicine (0.05% @ 1ml/100g bw) eight hours the second dose of PHA to arrest cell division at the metaphase stage and kept alive for 2-3 hours before sacrificing. For the preparation of smears, their cephalic kidney was removed, homogenized and hypotonised simultaneously by potassium chloride 0.56% for 35 minutes at room temperature. Because of their tiny tissues, they were well mixed. Suspensions were spun at 1000 rpm for 10 minutes. Supernatant was discarded and the cells were fixed by cold fresh Carnoy (3:1 methanol and glacial acetic acid) and refrigerated for 30 minutes. This process was repeated three times and smears were prepared on cold lamellae using splash method from 1m height and air dried for 24 h, then stained with 2% Giemsa.

2.1. Chromosomal analysis

Leica DM LS2 trinoccular microscope fitted with a camera and 100x×10x oil immersion lens combination was used to scan the cells and take the photographs. Eighty well spread metaphase complements were obtained for chromosomal analysis. The chromosomes of 5 well spread metaphase complements were individually measured from photomicrographs with precision dial callipers and their centromeric indices and arm ratios were determined in order to ascribe the morphology as suggested by Levan *et al*. (1964). Using chromosomal indicators (Table II) an ideogram (Fig.2) was prepared in MS Excel 2007 software.

Table 1. Percentage frequency of the metaphases (where f % = Frequency % of chromosomes).

Species	No. of chromosomes	No. of cells	f %	modal No.
S.labiatus	94	8	10	**98**
	96	6	7.5	
	98	64	80	
	100	2	2.5	

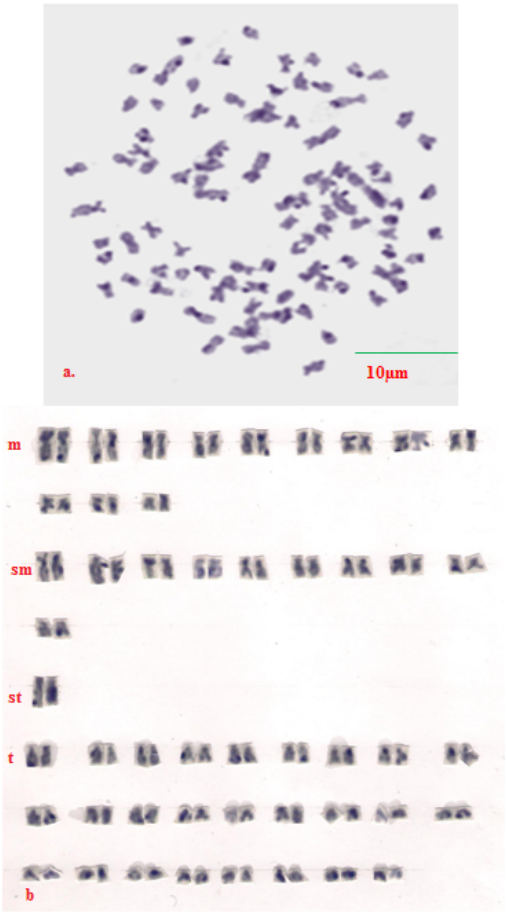

Fig. 1a-b: ***a.*** Chromosome preparation of *Schizothorax labiatus*. ***b.*** Karyotype of *S.labiatus* (m=metacentric;sm=sub-metacentric;st=subtelocentric; t=telocentric).

3. Results

A high number of small chromosomes were observed in *Schizothorax labiatus*. Eighty cells from the anterior kidney tissue were analysed in total. The overwhelming majority (80%) of the metaphase complements contained 98 chromosomes, though the count varied between 94-100 in a few cells (Table I). Cells not showing modal counts were probably caused by loss during preparation or by chromosomes being obscured by surrounding cell nuclei. The diploid complement (Fig.1a) comprised 12 metacentric pairs, 10 submetacentric pairs, 1 subtelocentric pairs and 26 telocentric pairs (Fig.1b). Total length of the haploid complement equalled 157.5µm with a range in the length of shortest and longest chromosome between 2-8µm (Table II). The arm ratio and the centromeric index ranged between 1-∞ and 0-50 respectively. The chromosomal formula can be represented as: K (2n) = 98 = 24m+20Sm+2St+52t.

Table II: Chromosome morphometry of *Schizothorax labiatus* (m= metacentric; Sm=sub-metacentric; St=sub-telocentric; t=telocentric)

Pair No.	Length of short arm (µm) 'S'	Length of long arm (µm) 'L'	Total length(µm) L+S	Arm ratio (L/S)	Centromeric index	Category
1	3	5	8	1.6	37.5	m
2	3	3	6	1	50	m
3	2	3	5	1.5	40	m
4	2	3	5	1.5	40	m
5	2.5	2.5	5	1	50	m
6	2	2.5	4.5	1.2	44.4	m
7	1.5	2	3.5	1.3	42.8	m
8	1.5	1.5	3	1	50	m
9	1.5	1.5	3	1	50	m
10	1	1	2	1	50	m
11	1	1	2	1	50	m
12	1	1	2	1	50	m
13	2	4	6	2	33.3	Sm
14	2	4	6	2	33.3	Sm
15	1	3	4	3	25	Sm
16	1	2.5	3.5	2.5	28.5	Sm
17	1	2	3	2	33.3	Sm
18	1	2	3	2	33.3	Sm
19	1	2	3	2	33.3	Sm
20	1	2	3	2	33.3	Sm
21	1	2	3	2	33.3	Sm
22	1	2	3	2	33.3	Sm
23	1	4	5	4	20	St
24	0	4	4	∞	0	t
25	0	4	4	∞	0	t
26	0	3	3	∞	0	t
27	0	3	3	∞	0	t
28	0	3	3	∞	0	t
29	0	3	3	∞	0	t
30	0	3	3	∞	0	t
31	0	3	3	∞	0	t
32	0	3	3	∞	0	t
33	0	3	3	∞	0	t
34	0	3	3	∞	0	t
35	0	3	3	∞	0	t
36	0	2	2	∞	0	t
37	0	2	2	∞	0	t
38	0	2	2	∞	0	t
39	0	2	2	∞	0	t
40	0	2	2	∞	0	t
41	0	2	2	∞	0	t
42	0	2	2	∞	0	t
43	0	2	2	∞	0	t
44	0	2	2	∞	0	t
45	0	2	2	∞	0	t
46	0	2	2	∞	0	t
47	0	2	2	∞	0	t
48	0	2	2	∞	0	t
49	0	2	2	∞	0	t

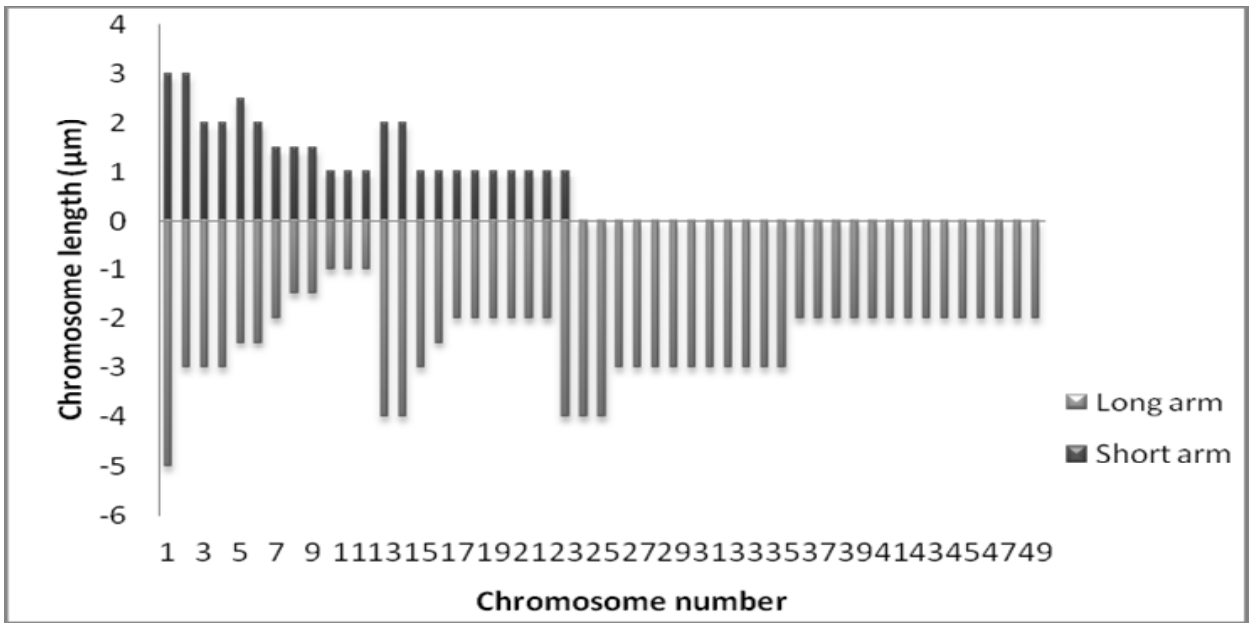

Fig. 2: Haploid ideogram of *Schizothorax labiatus.*

Table III: Showing different *Schizothorax* species worked out thus far.

S. No.	Name of the species	2n	Chromosome morphology m	Sm	St	t	NF value	Author and Year
1	*Schizothorax kumaonensis*	98	24	6	68		128	Rishi *et al*., 1998
2	*Schizothorax kumaonensis*	98	18		70	10	126	Lakara *et al*., 1997
3	*Schizothorax progastus*	98	16	20	12	50	134	Rishi *et al*., 1983
4	*Schizothorax richardsoni*	98	16		42	40	154	Lakara *et al*., 1997
5	*Schizothorax esocinus*	98	30	22	10	36	150	Farooq *et al*., 2011
5	*Schizothorax labiatus*	98	24	20	2	52	142	Present work

4. Discussion

Schizothorax labiatus analysed cytologically in the present study revealed a high number of chromosomes 2n=98. Species with high numbers are considered to have resulted through polyploidy from ancestral 2n= 48 or 50 (Rishi *et al.,* 1998). Chromosome counts in nearly all cyprinid polyploids occur in multiples or combinations of the most common karyotype (48-50) and tetraploids (96, 98 or 100) and hexaploids (148-150) have arisen through hybridisation (Dowling and Secor, 1997). This is well illustrated by a number of species of fish belonging to diverse orders. Buth *et al*., (1991) noted 52 such taxa most of which belong to cyprinidae identified through karyological analysis (Dowling and Secor, 1997) and such forms are ancestral polyploids (Ohno *et al*., 1969). Polyploidy in fishes has been associated with traits including large body size, fast growth rate, long life and ecological adaptability (Uyeno and Smith, 1972; Schultz, 1980). Since *Schizothorax* fishes are hill stream fishes, it may be that polyploidy may have resulted on account of cold temperature of their habitat. The use of thermal shocks to eggs for induction of polyploidy (Chourrout, 1988) provides support to the above assertion. The role of polyploidy in evolution and survival of fish is very important because it prevents from natural selection pressure (Oellerman and Skelton, 1990).

Interestingly *Schizothorax labiatus* showed diploid number similar to that recorded for other species inhabiting different geographical locations (Table III) e.g.,*S.esocinus*, 2n=98 (Farooq *et al*., 2011) *Schizothorax richardsonii*, 2n=98 (Sharma *et al*., 1992; Lakara *et al*., 1997), *Schizothoracichthys prograstus*, 2n= 98 (Rishi *et al*., 1983), *S. kumaonensis*, 2n=98 (Rishi *et al*., 1998; Lakara *et al*., 1997) but different fundamental arm number which may be attributed to the intra-chromosomal changes involving pericentric and paracentric inversion, suggesting origin from the same primitive ancestor. The overall similarity in the chromosome number and morphology implies that *Schizothorax* species are very closely related in that they have not been isolated as evolving entities long enough for random

chromosome changes to have taken place and become fixed, and that a particular karyotype would be selected implies an adaptive advantage for that particular configuration.

Cells lacking normal value (2n=94-100) were also encountered in the preparations and these probably resulted from losses during the preparation or addition from the neighbouring cells or hypotonic overtreatment (Nanda *et al*., 1995).

The present study is the first to describe the chromosomal characteristics of *Schizothorax labiatus* from The Kashmir Valley. The results of the study can be used for the genetic manipulation and management and conservation of the species.

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