

On the Chromosomes of two Cyprinid Fishes of the Subfamily Schizothoracinae from Kashmir.

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Abstract: Karyotypic study of two *Schizothorax* species viz. *Schizothorax plagiostomus* and *Schizothorax curvifrons* belonging to family Cyprinidae, from River Jhelum Kashmir, was carried out. Conventional KCl-acetomethanol air-drying protocol was followed for the chromosomal preparation. The diploid chromosome number in *S.plagiostomus* was 96 with a chromosomal formula of 24m+18Sm+54t and fundamental number (NF) =138. Diploid chromosome number in *S.curvifrons* was 94 with Karyotypic formula 26m+20Sm+20St=28t and fundamental arm number (NF) =140. The evolutionary significance of polyploidy and the role of chromosomal rearrangements was discussed. Both these fishes are new to cytological literature.

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Key words: *Schizothorax plagiostomus*, *Schizothorax curvifrons*, River Jhelum, Karyotype.

Introduction

Cytogenetic studies in recent years gained a considerable importance concerning species characterization, evolution and systematic (Gold *et al.*, 1990; Barat *et al.*, 2002). The cytogenetical studies in fishes are limited to just about 10% of the total fishes known taxonomically all over the world (Barat *et al.*, 1996). Fish chromosome data have great importance concerning evolution, systematics, aquaculture and mutagenesis (Al-Sabti, 1991). Chromosomal studies in fishes have not been as successful as those in other vertebrates because of relatively small size and large number of chromosomes found in many fish species and the limitations of the techniques employed (Klinkhardt, 1991). The air-drying technique, originally developed for mammalian organisms, is the most common procedure used for chromosome preparation in fish.

The increasing importance of chromosomal studies and the lack of data on fish karyotypes in Kashmir prompted us to examine the chromosomal content of *Schizothorax plagiostomus* and *Schizothorax curvifrons*. Both these fishes belong to subfamily Schizothoracinae, a widely distributed group in mountain streams, rivers and lakes around Himalayan Karakorum and Hindukush Ranges, The Tibet Plateau and Central Asia. These two fishes inhabit the River Jhelum, Lidder stream, Sindh Nallah of Kashmir Valley (Kullander *et al.*, 1999).

In this study, cytogenetic analysis of *S.plagiostomus* and *S.curvifrons* from River Jhelum was carried out with air-drying technique to determine their basic karyological structure. Both these species are new to cytological literature.

Materials and Methods

Ten live specimens (five each for *S.plagiostomus* and *S.curvifrons*) were collected from River Jhelum, near Chattabal Downtown Srinagar. The initial identification was made on the basis of morphology (Kullander *et al.*, 1999).

Chromosome and Karyotype analysis

All the samples were injected intraperitoneally with 0.05% Colchicine (Sigma, US) 1ml/100gm of body weight and kept alive for 2-3 hrs in fully aerated aquaria. Anterior kidney tissue was processed for chromosome preparation following conventional KCl-acetomethanol-air-drying protocols (Khuda-Bukhsh and Barat, 1987). The slides were stained with 2% Giemsa stain in phosphate buffer (PH 6.8). Leica DM LS2 trinocular microscope fitted with a camera and 100x×10x oil immersion lens combination was used to scan the cells and take the photographs. Fifty to sixty well spread metaphase complements were obtained for each species. The chromosomes of 5 well spread metaphase complements for each species 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 and Table III) an ideogram (Figure 3 and Figure 4) was prepared for each species in MS Excel 2007 software.

Results

Schizothorax plagiostomus: Chromosome number counts from 60 cells from five individuals varied between 94-100 (Table I). The modal value of $2n=96$ was seen in 60% of the cells examined. The diploid metaphase complements consisted of 96 chromosomes measuring between 2-8 μ m. Detailed karyotype analysis revealed that the 96 chromosomes comprised of 24 metacentric, 18 submetacentric and 54 telocentric chromosomes. The number of chromosome arms was determined as (NF) =138 and the chromosomal formula can be expressed as $29=24m+18Sm+54t$ (Figure 1).

Schizothorax curvifrons: The somatic metaphase complements contained 94 chromosomes in 40 out of 50 cells studied (Table I). Therefore, the diploid chromosome number in this species was ascertained to be 94 and the karyotype (Figure 2) consisted of $2n=26m+20Sm+20St+28t$ with the fundamental arm number (NF) of 140. The size of the chromosome varied between 10.4-1 μ m. Cells not showing modal counts were probably caused by loss during preparation or by chromosomes being obscured by surrounding cell nuclei.

Discussion

Fishes are the most speciose vertebrate group, with an estimated 24618 recognised species; more than half the total number of living vertebrate species (Nelson, 1994) and exhibit a bewildering range of diversity in their ecology, morphology, life history, behaviour and physiology (Comber and Smith, 2004). Despite their diversity, they have remained cytologically neglected as standard karyotypes are reported for less than 10% of the total extant species of fish (Gold *et al.* 1990).

Both the species of *Schizothorax* analysed cytologically in the present study revealed a high number of chromosomes ranging from 94-96. Species with high numbers are considered to have resulted through polyploidy from ancestral $2n=48$ or 50 (Rishi *et al.*, 1998). Large-scale genomic expansions or whole-genome duplication events have been documented in early vertebrate evolution (Friedman and Hughes, 2001; Ohno, 1970; Wang and Gu, 2000), near the base of the phylogenetic tree of teleost fishes (Christoffels *et al.*, 2004; Meyer and Schartl, 1999; Robinson-Rechavi *et al.*, 2001; Wittbrodt *et al.*, 1998), and near the basal roots of several major teleostean clades [such as salmonids (Allendorf and Thorgaard, 1984), catostomids (Ferris, 1984; Uyeno and Smith, 1972), acipenserids (Vasil'ev, 1999) and some cyprinids (Larhammer and Risinger, 1994)]. Such genomic enlargements have been hypothesised as key factors that enable or even drive diversification in various vertebrate groups (Holland *et al.*, 1994; Meyer and Malaga-trillo, 1999; Navarro and Barton, 2003a,b; Ohno, 1970; Stephens, 1951). 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). Our results suggest that these fishes are tetraploid. 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).

Schizothorax plagiostomus revealed a diploid number of $2n=96$ ($24m+18Sm+54t$) and $NF=138$ while as *S.curvifrons* showed a diploid number of $2n=94$ ($26m+20Sm+20St+28t$) and $NF=140$. Decrease in the $2n$ and NF may be attributed to the Robertsonian arrangements and pericentric inversion (Choudhury *et al.*, 1982).

The chromosomes of *S.curvifrons* were categorized into four groups (Fig.2) viz. Metacentric, submetacentric, subtelocentric and telocentric but none of the chromosomes could be recognised as subtelocentric in *S.plagiostomus* because cyprinid fishes are characterised by presence of relatively small chromosomes with their centromeric positions ranging

gradually from median to nearly terminal, making it difficult to assign some chromosomes to particular chromosomal categories and thus making correct identification of individual chromosomes nearly impossible (Rab and Collares-Pereira, 1995). And the karyological study of teleost fish presents technical difficulties which are not encountered in the study of other vertebrates and these difficulties are due to small size and high number of chromosomes (Cucchi and Baruffaldi, 1990). Further, differential arm contraction can alter a chromosome classification in a karyotypic formula (Joswiak *et al.*, 1980).

The overall dissimilarity in the $2n$, Karyotypic configuration and NF points to the role of almost all types of chromosomal rearrangements in the karyological evolution of these two fishes. Both these fishes are new to the cytological literature.

Table I: Showing percentage frequency of the metaphases.

Pair No.	Length of short arm (μ m) 'S'	Length of long arm (μ m) 'L'	Total length(μ m) L+S	Arm ratio (L/S)	Centromeric index	Category
1	5.2	5.2	10.4	1	50	M
2	5	5	10	1	50	M
3	5	5	10	1	50	M
4	4	4.5	9.5	1.12	42.1	M
5	4	4.5	9.5	1.12	42.1	M
6	3	3.5	6.5	1.16	46.1	M
7	3	3.5	6.5	1.16	46.1	M
8	3	3	6	1	50	M
9	2.5	2.5	5	1	50	M
10	2.3	2.3	4.6	1	50	M
11	2	2	4	1	50	M
12	2	2	4	1	50	M
13	2	2	4	1	50	M
14	3	5.5	8.5	1.83	35.2	Sm
15	2.9	5.4	8.3	1.86	34.9	Sm
16	2.5	5.3	7.8	2.12	32.0	Sm
17	2.2	5	7.7	2.27	28.5	Sm
18	2	5	7	2.50	28.5	Sm
19	1.8	4.1	5.9	2.27	30.5	Sm
20	1.5	3.8	5.3	2.53	28.3	Sm
21	1.2	3.3	4.5	2.75	26.6	Sm
22	1.2	3.3	4.5	2.75	26.6	Sm
23	1	2.8	3.8	2.80	26.3	Sm
24	1.3	4.8	6.1	3.69	21.3	St
25	1	4.5	5.5	4.5	18.1	St
26	1	4.2	5.2	4.2	19.2	St
27	1	4	5	4	20	St
28	1	3.8	4.8	3.8	20.8	St
29	1	3.8	4.8	3.8	20.8	St
30	1	3.7	4.8	3.8	20.8	St
31	1	3.6	4.6	3.6	21.7	St

32	1	3.6	4.6	3.6	21.7	St
33	1	3.6	4.6	3.6	21.7	St
34	0	6	6	0	0	T
35	0	6	6	0	0	T
36	0	6	6	0	0	T
37	0	5	5	0	0	t
38	0	5	5	0	0	t
39	0	5	5	0	0	t
40	0	5	5	0	0	t
41	0	5	5	0	0	t
42	0	5	5	0	0	t
43	0	4	4	0	0	t
44	0	4	4	0	0	t
45	0	4	4	0	0	t
46	0	3	3	0	0	t
47	0	1	1	0	0	t

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

Species	No. Of chromosomes	No. Of cells	Frequency % of chromosomes	Modal diploid No.
<i>Schizothorax plagiostomus</i>	94	5	8.33	96
	96	36	60	
	98	12	20	
	100	7	11.66	
<i>Schizothorax curvifrons</i>	92	4	8	94
	94	40	80	
	96	6	12	

Table III: Chromosome morphometry of *Schizothorax curvifrons* (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	4	4	8	1	50	m
3	3	4	7	1.3	42.8	m
4	3	3	6	1	50	m
5	3	3	6	1	50	m
6	3	3	6	1	50	m
7	2	3	5	1.5	40	m
8	2	3	5	1.5	40	m
9	2	3	5	1.5	40	m
10	2	3	5	1.5	40	m
11	2.5	2.5	5	1	50	m
12	2	2	4	1	50	m

13	2	5	7	2.5	28.5	Sm
14	2	5	7	2.5	28.5	Sm
15	2	5	7	2.5	28.5	Sm
16	2	4	6	2	33.3	Sm
17	1	3	4	3	25	Sm
18	1	2.5	3.5	2.5	28.5	Sm
19	1	2.5	3.5	2.5	28.5	Sm
20	1	2	3	2	33.3	Sm
21	1	2	3	2	33.3	Sm
22	0	4	4		0	t
23	0	4	4		0	t
24	0	4	4		0	t
25	0	4	4		0	t
26	0	4	4		0	t
27	0	4	4		0	t
28	0	4	4		0	t
29	0	4	4		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	3	3		0	t
37	0	3	3		0	t
38	0	3	3		0	t
39	0	3	3		0	t
40	0	3	3		0	t
41	0	3	3		0	t
42	0	3	3		0	t
43	0	2.5	2.5		0	t
44	0	2.5	2.5		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

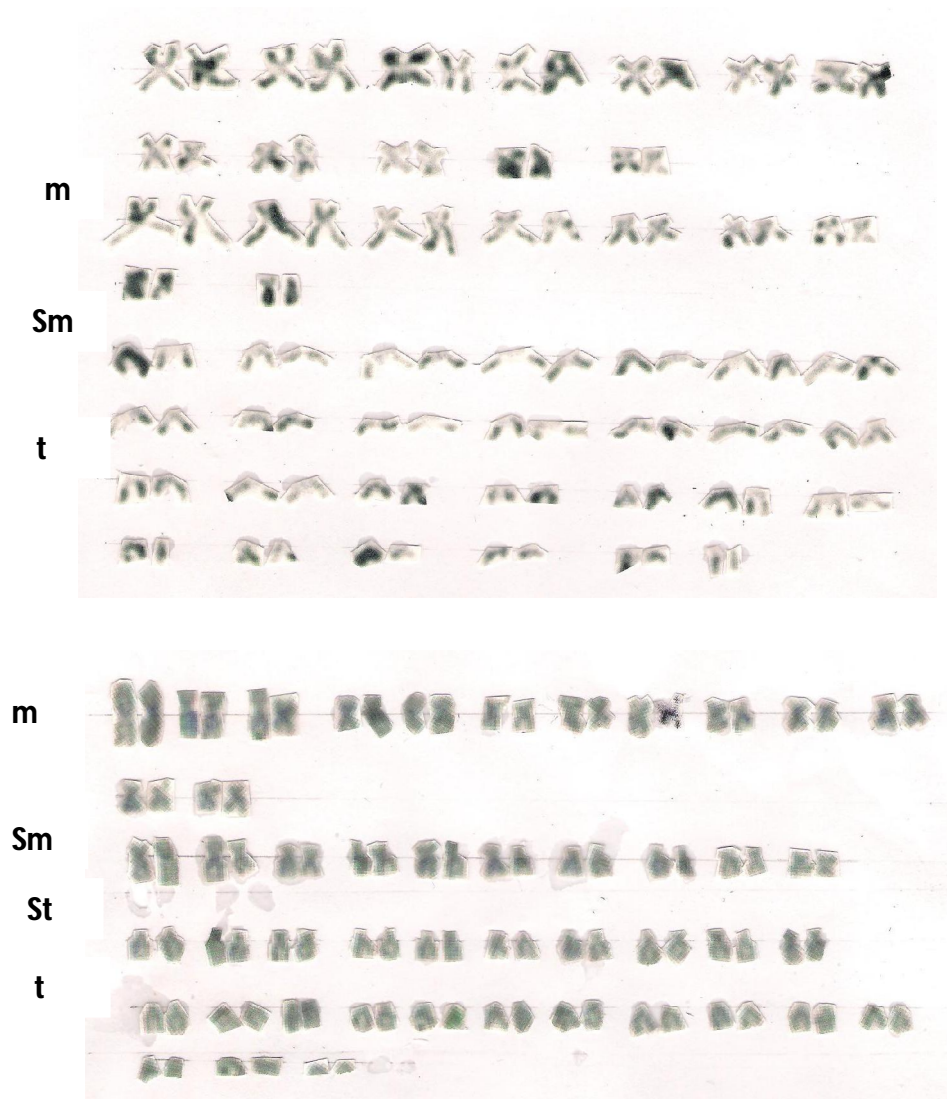


Figure 1: Karyotype of *Schizothorax plagiostomus*

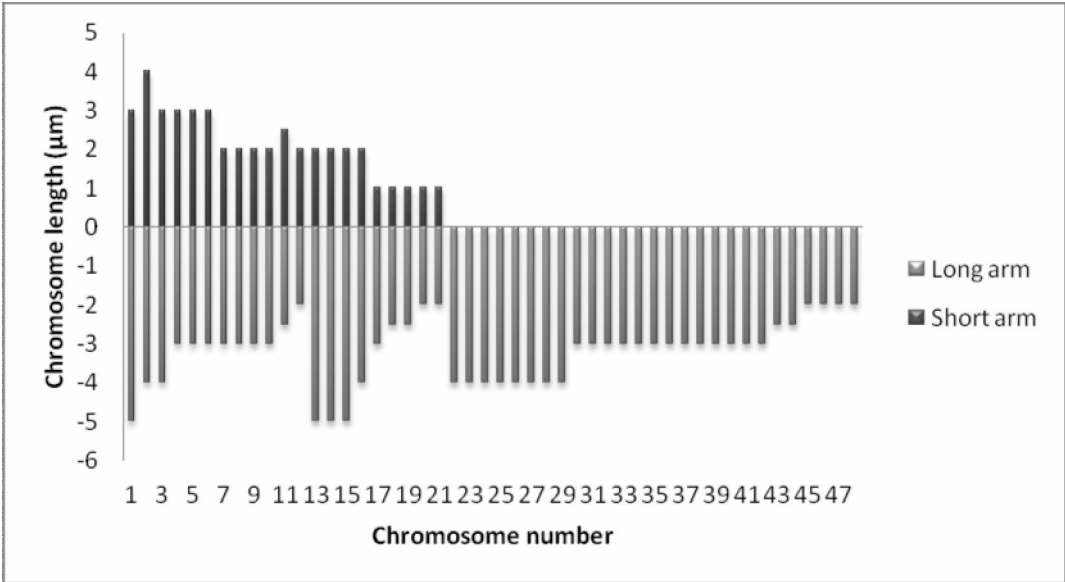


Figure 2: Karyotype of *Schizothorax curvifrons*

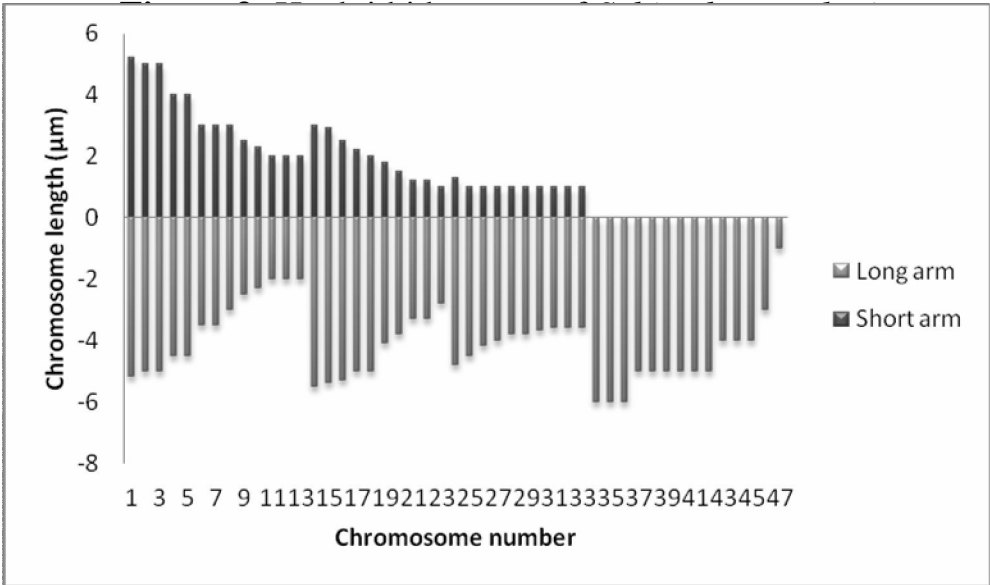


Figure 4: Haploid ideogram of *Schizothorax curvifrons*.

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