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Full Length Research Paper

Karyoevolutionary and karyosystematic considerations on *Schizothorax curvifrons* and *Schizothorax niger* (Teleostei: Cyprinidae): Important hill-stream food fishes of Kashmir Himalaya

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Cytogenetic studies have helped in clarifying the problem of disagreement amongst taxonomists on the identity of a given species. Cytogenetic studies were performed on two fishes of the genus *Schizothorax* viz. *Schizothorax curvifrons* Heckel and *Schizothorax niger* Heckel (Cyprinidae: Schizothoracinae) obtained from Sindh Stream and Dal Lake Srinagar Kashmir, respectively. These fishes are considered to be the subspecies of the same species. The two species showed a diploid number of 98 in *S. niger* and 94 in *S. curvifrons*. The karyological data are analyzed in terms of the taxonomic aspects within this genus, and the validity of their existence as species chromosomally distinct from each other is emphasized.

Key words: Sindh stream, karyotype, cytotaxonomy, Kashmir Himalaya, chromosome.

INTRODUCTION

Cytotaxonomy, the correlation between cytology and taxonomy, originated during the second half of the 19th century when it was discovered that some animal and plant species may be classified according to their chromosomal characteristics (Bertollo et al., 1978). However, chromosomes began to be considered as useful tools in taxonomy only when comparisons between several species of the same genus were made on the basis of the number and morphology of chromosomes (Brown and Bertke, 1969).

Taxonomically, some groups of fish present serious difficulties leading to disagreements amongst classifiers on the identity of a given species. In many cases, cytogenetic studies may clarify this problem (Ojima et al., 1976). On account of phenotypical similarities, many biospecies may be grouped under the same name, that is, morphospecies with the possibility of demonstrating reproductive isolation in controlled experiments (Koswig, 1973). Thus, the study of fish cytogenetics and genetics is very promising in terms of solution to these problems.

Fishes of the subfamily Schizothoracinae are mostly hill-stream inhabitants and have a wide distribution in the freshwaters of Central Asian countries. The genus comprises 60 species and in Kashmir Himalaya, it is represented by five species viz. *Schizothorax niger* Heckel, *Schizothorax curvifrons* Heckel, *Schizothorax esocinus* Heckel, *Schizothorax plagiostomus* Heckel and *Schizothorax labiatus* (McClelland and Griffith). Since the time the *Schizothorax* fishes of Kashmir Himalaya were introduced to the world of science by Heckel in 1838, their specific status has been revised many times mainly based on the morphological features (Hora, 1936; Silas, 1960; Das and Subla, 1963; Saxena and Koul, 1966; Nath, 1986). Silas (1960) proposed that *S. curvifrons* is a subspecies of *S. niger* on account of overlap in most of their morphological features hence should not be considered as species levels. Kullander et al. (1999),

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Table 1. Showing percentage frequency of the metaphases.

Species	Number of chromosome	Number of cell	Frequency % of chromosome	Modal diploid number	FN
Schizothorax niger	94	2	3.3	98	154
	96	5	8.3		
	98	48	80		
	100	5	8.3		
Schizothorax curvifrons	92	4	8	94	140
	94	37	74		
	96	9	18		

based on principal component analysis of morphometric figures, conclusively stated that the two fishes are different species levels.

Hill-stream fishes constitute 3.5% of the total number of fish species in India and all of them can be easily put into the category of threatened species on account of increasing anthropogenic activities that lead to decline in their number (Rishi et al., 1998). The present study was undertaken to clarify the specific status of *S. curvifrons*, a hill-stream fish inhabiting the water bodies of Kashmir Himalaya, a poorly known region, so that it can be properly managed and conserved. The study is also important because biochemists can undertake comparative biochemical studies of the two distinct species to ascertain their nutritional value and subsequent culturing at large scale by aquaculturists.

MATERIALS AND METHODS

Ten live specimens, all females (five each for *S. curvifrons* and *S. niger*) were collected from Sindh stream and Dal lake Hazratbal Srinagar respectively. 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) 1 ml/100 g of body weight and kept alive for 2 to 3 h 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 trinoccular microscope fitted with a camera and 100× × 10× 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 (Tables 2 and 3), a karyogram (Figure 1a and b) was prepared for each species in decreasing order of length.

RESULTS

Schizothorax niger

The overwhelming majority (80%) of metaphase complements in the kidney tissue of *S. niger* contained 98 chromosomes, though a few plates had a range within 94 to100. The diploid metaphase complements consisted of 98 chromosomes measuring between 8 to 3.5 µm. *S. niger* revealed a karyotype (Figure 1a) formula of 24 metacentric + 32 submetacentric + 22 subtelocentric + 20 telocentric with a fundamental arm number (FN) as 154 (Table 1). The variation in the diploid numbers are usually the result of losses or additions during the karyotype preparation, including splashing due to their downfall from various heights from nearby cells, as reported in other studies (Suleyman et al., 2004; Esmaeli and Piraver, 2006).

Schizothorax curvifrons

The diploid chromosomal complement of this fish contained 94 chromosomes in 37 out of 50 cells scanned. Therefore, the diploid chromosome number in this fish was ascertained to be 94 and the karyotype (Figure 1b) consisted of 2n= 26m+20sm+20st+28t with a fundamental arm number (FN) of 140. The size of the chromosomes varies between 10.4 to 1 µm.

DISCUSSION

Both the species of *Schizothorax* analysed cytologically in the present study revealed a high number of chromosomes ranging from 94 to 98. All the *Schizothorax* species studied karyologically till date - *S. richardsonii* Gray and *S. kumaonensis* Menon (Lakara et al., 1997), *S. zarudnyi* Nikolskii (Kalbassi et al., 2008), *S. plagiostomus* (Farooq et al., 2011) and *S. esocinus* (Farooq et al., 2011) show a high chromosome number

Table 2. Chromosome morphometry of *Schizothorax niger* (m= metacentric; Sm= sub-metacentric; St= sub-telocentric; t= telocentric).

Pair number	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	5	8	1.6	37.5	m
3	4	4	8	1	50	m
4	3	4	7	1.3	42.8	m
5	3	4	7	1.3	42.8	m
6	3	4	7	1.3	42.8	m
7	3	3	6	1	50	m
8	3	3	6	1	50	m
9	2	3	5	1.5	40	m
10	2	3	5	1.5	40	m
11	2	3	5	1.5	40	m
12	2	3	5	1.5	40	m
13	2	6	8	3	25	Sm
14	2	6	8	3	25	Sm
15	2	5	7	2.5	28	Sm
16	2	4	6	2	33.3	Sm
17	2	4	6	2	33.3	Sm
18	2	4	6	2	33.3	Sm
19	2	4	6	2	33.3	Sm
20	1	3	4	3	25	Sm
21	1	3	4	3	25	Sm
22	1	3	4	3	25	Sm
23	1	3	4	3	25	Sm
24	1	3	4	3	25	Sm
25	1	3	4	3	25	Sm
26	1	3	4	3	25	Sm
27	1	3	4	3	25	Sm
28	1	2	3	2	33.3	Sm
29	1	5	6	5	16.6	St
30	1	5	6	5	16.6	St
31	1	5	6	5	16.6	St
32	1	5	6	5	16.6	St
33	1	4	5	4	20	St
34	1	4	5	4	20	St
35	1	4	5	4	20	St
36	1	4	5	4	20	St
37	1	4	5	4	20	St
38	0.5	3	3.5	6	14.2	St
39	0.5	3	3.5	6	14.2	St
40	0	6	6	∞	0	t
41	0	6	6	∞	0	t
42	0	6	6	∞	0	t
43	0	5	5	∞	0	t
44	0	5	5	∞	0	t
45	0	5	5	∞	0	t
46	0	5	5	∞	0	t
47	0	5	5	∞	0	t
48	0	5	5	∞	0	t
49	0	5	5	∞	0	t

Table 3. Chromosome morphometry of *Schizothorax curvifrons* (m= metacentric; Sm= sub-metacentric; St= sub-telocentric; t= telocentric).

Pair number	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	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	t
35	0	6	6	∞	0	t
36	0	6	6	∞	0	t
37	0	5	5	∞	0	t
38	0	5	5	∞	0	t
39	0	5	5	∞	0	t
40	0	5	5	∞	0	t
41	0	5	5	∞	0	t
42	0	5	5	∞	0	t
43	0	4	4	∞	0	t
44	0	4	4	∞	0	t
45	0	4	4	∞	0	t
46	0	3	3	∞	0	t
47	0	1	1	∞	0	t

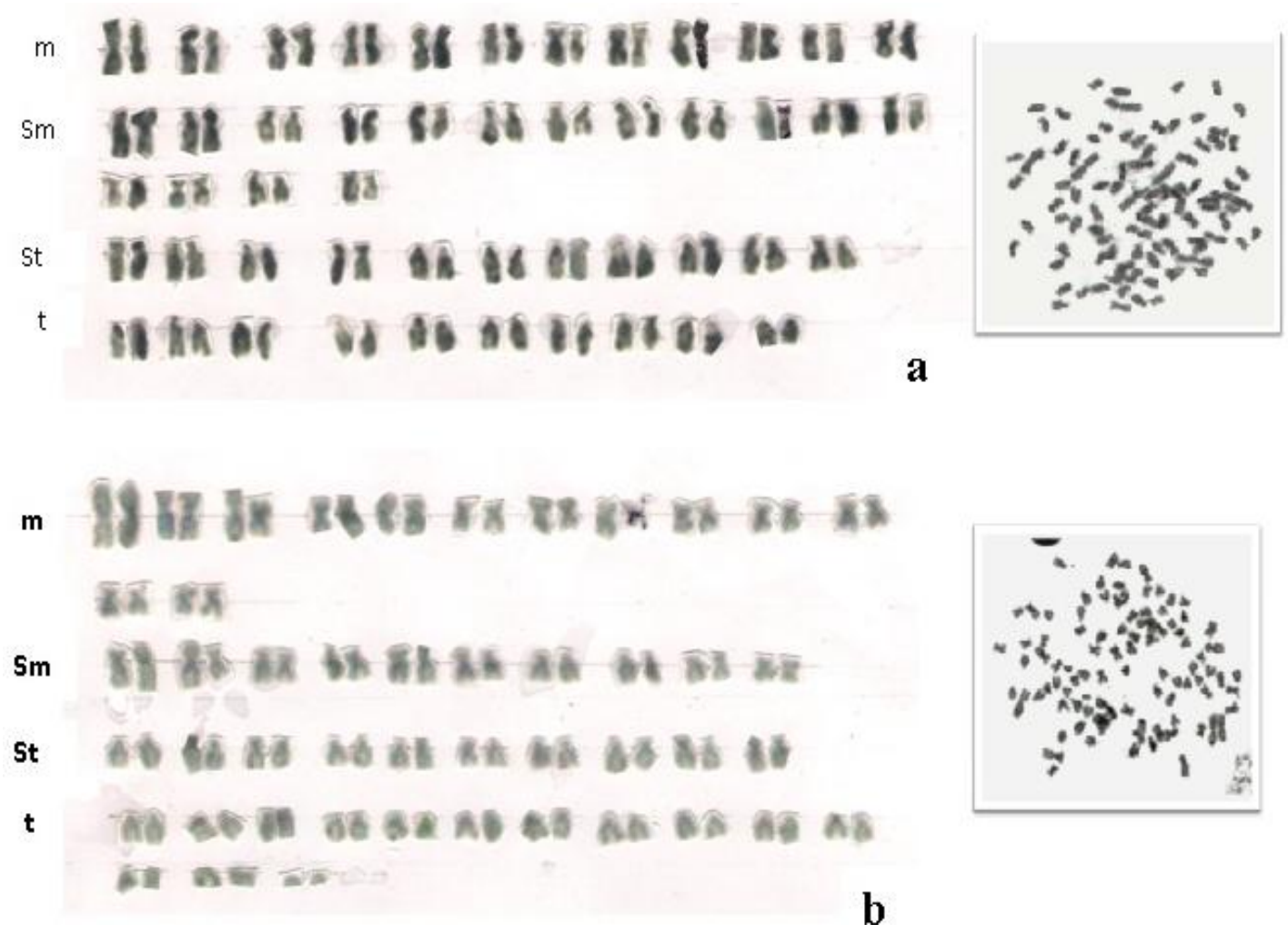

Figure 1. a) Karyotype of *S. niger*; b) karyotype of *S. curvifrons*.

ranging from 96 to 98. Species with high numbers are considered to have resulted through polyploidy from ancestral 2n= 48 or 50 (Rishi et al., 1998). 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). 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 this assertion.

Variation in the karyotypic configuration of *S. niger* (24m+ 32sm+22st+20t and FN=154) and *S. curvifrons* (26m+20sm+20st+28t) and FN=140) can easily be explained by centric fusion and fission events. Both centric fission and fusion probably provide important mechanisms to explain the diverse range of chromosome numbers observed in many mammalian and non-mammalian animal taxa (Todd, 1970; Imai et al., 1986, 1988, 2001; Kolnicki, 2000). Decrease in 2n and FN in *S. curvifrons* may be attributed to Robertsonian arrangements and pericentric inversion (Choudhury et al., 1982).

Cyprinid karyotypes have not been without systematic implications (Joswiak et al., 1980) because comparative karyology has become a useful tool in fish systematic studies (Arai, 1982; Buth et al., 1991) as chromosome number and morphology shows changes that modified an ancestral karyotype as it evolved into new lines (Winkler et al., 2004) and are useful for addressing a variety of evolutionary, genetic and cytotaxonomic questions about animals (Kirpichnikov, 1981; McGregor, 1993). The present study was also undertaken with the same objective to generate the information regarding the species status of the *S. curvifrons* and *S. niger* in Kashmir Valley. The study reveals that despite overlap in the general

morphological features, the two species of *Schizothorax* investigated are genetically different and hence definite species as the chromosomal differentiation in animal species usually precedes strong morphological differentiation (Howell and Villa, 1976). Most morphologic features of fishes have been shown to have the potential of being modified by the environmental conditions (Svardson, 1965; Fowler, 1970). Therefore, a morphologically based classification should be tested by the features not likely to be environmentally plastic and chromosome structure is best suited for this purpose as it reflects genetic divergence and is least affected by environmental distortion (Campos, 1972).

The present study conclusively confirms the specific status of the two species of *Schizothorax* on the basis of their genetic material. The study negates the proposition of Silas (1960) regarding the taxonomic status of *S. niger* and *S. curvifrons*, who had combined these two species into a single species *S. niger* treating *curvifrons* and *niger* as two varieties or subspecies. The chromosome study has clearly shown that these two species of fish be treated as distinct species and not varieties or subspecies of the same species.

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