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Full Length Research Paper

A karyological analysis of *Puntius conchonius* (Hamilton, 1822) (Pisces, Cyprinidae), a new cytotype from Dal Lake Srinagar Kashmir, J&K, India

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The karyotypic and cytological characteristics of Rosy barb, *Puntius conchonius* (Hamilton, 1822) (cyprinidae) were investigated by examining metaphase chromosomes spreads from the anterior kidney following Thorgaard and Disney (1990). The fishes were obtained from the local fishermen and transported live to the Limnology and Fisheries Laboratory of Centre of Research for Development, University of Kashmir. Ten fish were injected intraperitoneally with two doses of phytohemagglutinin (PHA), 4 µg g^{-1}body weight with a 20 h interval to induce cell division. After 8 h in 20°C water, the fish were injected intraperitoneally with colchicine 0.05% @ 0.5 ml/50 g body weight to depress the cell division in the metaphase stage and left for 2 to 3 h before sacrificing. Kidney and gill epithelia were used for karyotype analysis. The diploid chromosome number of the fish was 2n = 50, consisting of 11 pairs of metacentric, 8 pairs of submetacentric and 6 pairs of telocentric chromosomes respectively. Centromeric index, arm ratio and Fundamental Number were determined as 0 to 50, 1-∞ and 88 respectively. No heteromorphic sex chromosomes were cytologically detected.

Key words: Chromosome, *Puntius conchonius*, Dal Lake, karyotype.

INTRODUCTION

Cyprinid fishes have been favourite subjects of extensive cytogenetic investigations with particular reference to evolutionary aspects (Taki et al., 1977). Polyploidization has been demonstrated in the course of chromosomal evolution of this family (Ohno et al., 1967). The increasing knowledge of chromosomes is now providing reliable information on the phyletic relationship in the cyprinidae to a certain extent (Taki et al. 1977).

The genus *Puntius* Hamilton represents the species of the largest number among all species of cyprinid genera in Asian tropics. Fishes of this genus occur throughout the region from Pakistan to southern China inhabiting various types of freshwaters. *Puntius conchonius* is found in north eastern India (Taki et al. 1977) and in Kashmir it is found in Dal Lake and Mansbal Lake (Kullander et al., 1999).The karyotype of various Indian *puntius* species has been worked out earlier but there has been no report from the valley of Kashmir. The analysis of chromosomes is important for genetic control, taxonomy and evolutionary studies (MacGregor and Varly, 1993; Fister et al., 1999; Unlu and Killic-Demirok, 2001; Suleyman et al., 2004; Kalbassi et al., 2006) and is widely used in various fish investigations (Pisano et al., 2007). In addition, the trend of karyological changes and fixation in various new species can be studied by this method (Winkler et al., 2004). The aim of this study was to investigate the chromosomes and karyotype of *P. conchonius* from Dal lake Srinagar Kashmir and compare it with other species from taxonomic point of view.

MATERIALS AND METHODS

Live fish were obtained from local fishermen in the Dal Lake and

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Table 1. Frequency of chromosomes in the counted metaphase plates.

Number of chromosome in each metaphase plate	47	48	49	50
Number of plates	4	16	2	58
Frequency (%)	5	20	2.5	72.5

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 at 20°C for several days. Air dried chromosome preparation method with some modifications was used as described by Thorgaard and Disney (1990). Fish received two doses of phytohemagglutinin (PHA) injections (4 $\mu g g^{-1}$ bw), in a 20 h interval at 20°C. Eight hours after the second PHA injection, 0.05% colchicine was injected intraperitoneally (0.5 ml/50 g body weight) to depress the mitotic division at the metaphase stage and left for 2 to 3 h before sacrificing. The fish were anesthetized by 300 ppm clove oil for 40 s, their anterior kidney and gill filaments were removed, homogenized and hypotonised simultaneously by tri-sodium citrate 1% for 35 min at room temperature. Because of their tiny tissues, they were well mixed. Suspensions were centrifuged at 1300 rpm for 10 min. Supernatant was removed and the cells were fixed by cold fresh Carnoy (3:1 methanol and glacial acetic acid). This process was repeated three times and the cold fresh Carnoy was replaced at 30 min intervals. Smears were prepared on cold lamellae using splash method from 1 m height and air dried for 24 h, then stained with 2% Giemsa.

Chromosomal analysis

Leica DM LS2 microscope with 100x magnification lens immersion oil was used for taking the photographs and analysing the chromosomes. Eighty metaphase plates were counted and a proper plate was selected to obtain karyotype formulae. Microsoft Excel 2007 software was used to calculate the centromeric indices and to draw ideogram. For each chromosome Centromeric index, arm ratio and total length were calculated as described by Levan et al. (1964) and the fundamental number was also calculated. Chromosomes were classified into metacentric, sub-metacentric and telocentric following the method of Levan et al. (1964).

RESULTS

Eighty metaphases of the ten specimens of *P. conchonius* were counted. The observed diploid number per metaphasic plate ranged from 47 to 50. A modal diploid number of 2n = 50 constituted 72.5% (22 m+16 Sm+12 t) and 2n = 48 constituted 20% of the counted metaphase plates (Table 1). Other diploid numbers other than 2n = 50 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; Nasri et al., 2010). Using a proper metaphasic plate (Figure 1) chromosomal indicators were calculated (Table 2) and the chromosomal formulae obtained as 2n = 50 including eleven metacentric, eight sub-metacentric and six telocentric pairs respectively and fundamental number as FN = 88. Based on chromosomal indicators (Table 2) an ideogram (Figure 2) was drawn in MS excel 2007. Comparison with already worked out species of *P. conchonius* in Jammu and other parts of the country (Nayyar, 1964; Sharma and Agarwal, 1981; Tripathi and Sharma, 1987) reveals that it is a new cytotype, inhabiting Dal lake Kashmir.

DISCUSSION

Because of their smaller size and usually abundant and more contracted structures, studying and measuring fish chromosomes is somewhat more difficult than those of mammals (Suleyman et al., 2004). Besides, identification of fish chromosomes is difficult due to the lack of any standard karyotype for fishes, polymorphism exists not only among various fish species but also within species (Al-Sabti, 1991).

The most commonly occuring diploid number in family cyprinidae is 50, considered to be the modal number in case of this family (Manna, 1984; Rishi, 1989). According to the studies performed by various workers on *Puntius* species of India (Nayyar, 1964; Taki et al., 1977; Tripathi and Sharma, 1987), it seems that 2n = 50 in the genus *Puntius*, as in many other cyprinids. The present study also revealed the same diploid number. Despite the similarity of the diploid number in species of *Puntius*, there are differences in their karyotype formulae. Whereas Nayyar (1964) reported the presence of all acrocentric chromosomes in *P. conchonius*, the present study confirms the presence of both biarmed and acrocentric chromosomes. The primitive teleost karyotype is thought to have consisted of 46 to 48 acrocentrics (Nayyar, 1966; Ohno et al., 1968; Ohno, 1970; Fitzsimons, 1972; LeGrande, 1975). Karyotypes with biarmed chromosomes are generally regarded to represent a derived condition (Ohno et al., 1968; Ohno, 1970; Denton, 1973; Gold, 1979). Therefore *P. conchonius* investigated in the present study shows a derived karyotype configuration. No heteromorphic sex chromosomes were found and the fish appears to be a cytotype of *P. conchonius* found in Jammu region of the State as that species has only 48 chromosomes.

The present study is the first to describe the chromosomal characteristics of *P. conchonius* from Kashmir.

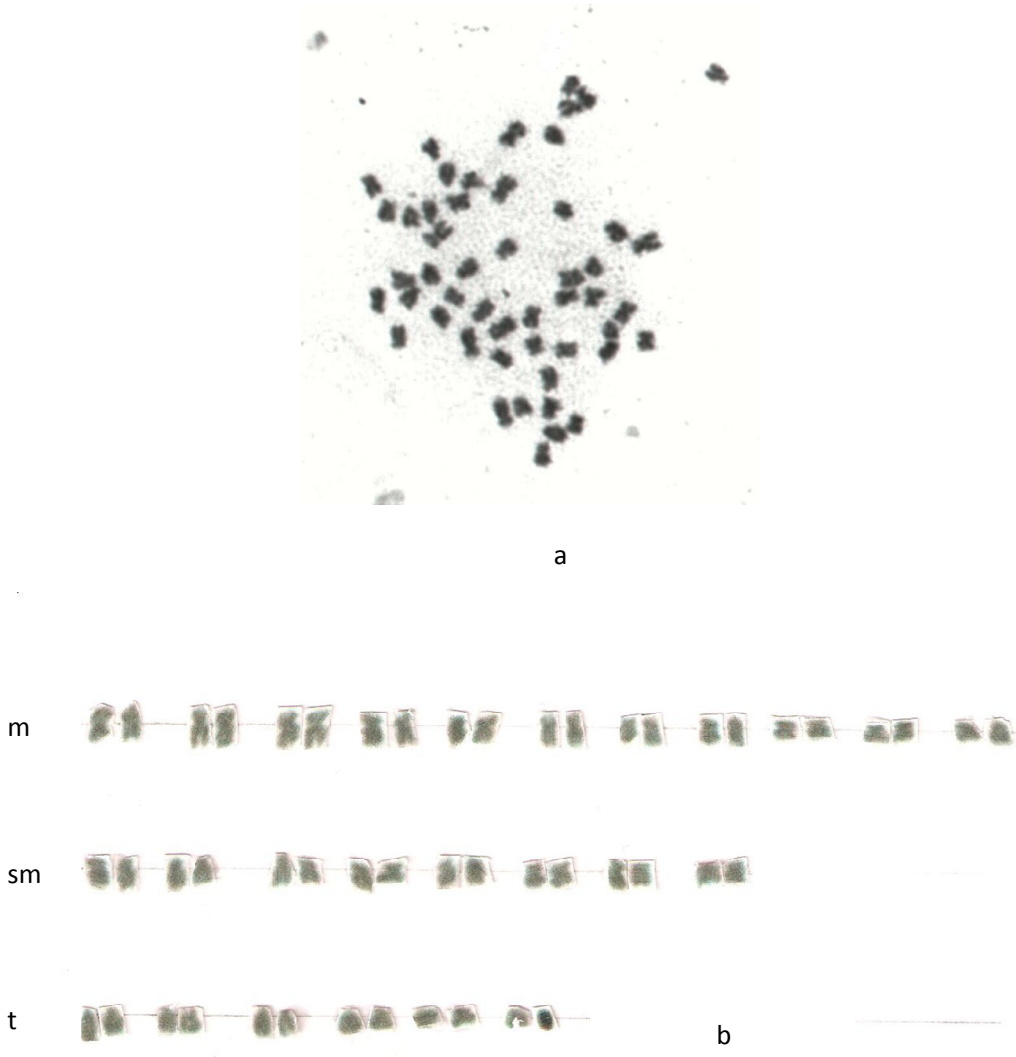

Figure 1. (a) Chromosome preparation and (b) karyotype.

Table 2. Somatic chromosomes morphometery of *Puntius conchonius.*

Serial no.	Short arm	Long arm	Total length	L/S	Centrolmeric index	Category
1	1.25	1.25	2.5	1	50	Metacentric
2	1	1.5	2.5	1.5	40	Metacentric
3	1	1.5	2.5	1.5	40	Metacentric
4	1	1.5	2.5	1.5	40	Metacentric
5	1	1	2	1	50	Metacentric
6	1	1	2	1	50	Metacentric
7	1	1	2	1	50	Metacentric
8	0.75	1	1.75	1.33	42.85	Metacentric
9	0.75	0.75	1.5	1	50	Metacentric
10	0.5	0.75	1.25	1.5	40	Metacentric
11	0.5	0.75	1.25	1.5	40	Metacentric
12	0.5	1.5	2	3	2.5	Sub-metacentric
13	0.5	1.5	2	3	2.5	-do-
14	0.5	1.5	2	3	2.5	-do-

Table 2. Contd.

15	0.5	1	1.5	2	33.33	-do-
16	0.5	1	1.5	2	33.33	-do-
17	0.5	1	1.5	2	33.33	-do-
18	0.5	1	1.5	2	33.33	-do-
19	0.5	1	1.5	2	33.33	-do-
20	0	2.5	2.5	∞	0.00	Telocentric
21	0	2	2	∞	0.00	-do-
22	0	2	2	∞	0.00	-do-
23	0	2	2	∞	0.00	-do-
24	0	1.5	1.5	∞	0.00	-do-
25	0	1.5	1.5`	∞	0.00	-do-

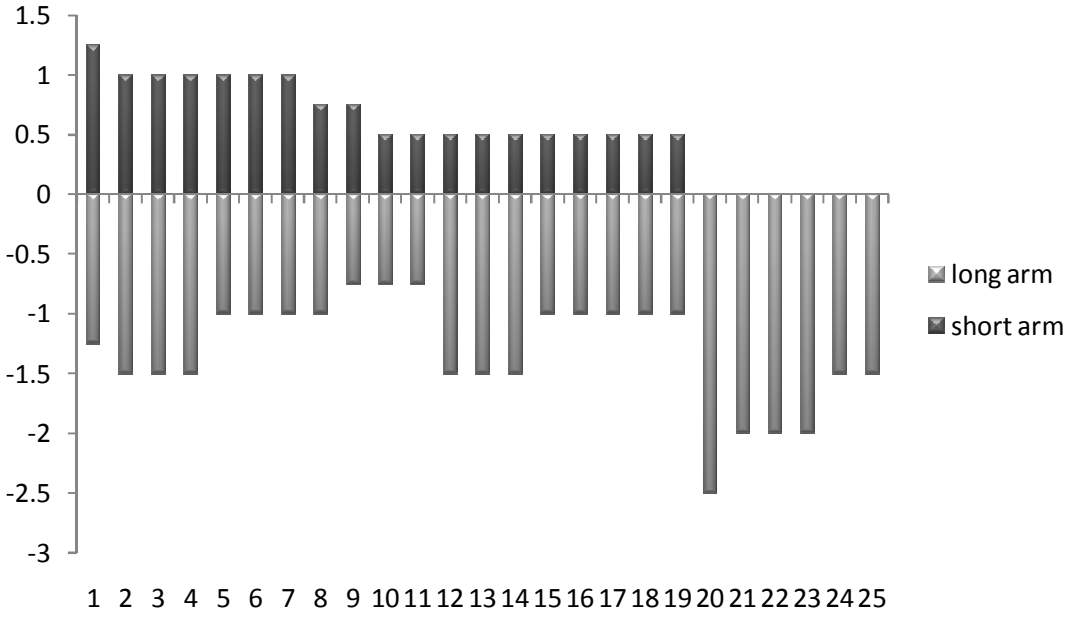

Figure 2. Haploid ideogram of *Puntius conchonius* showing chromosomes arranged in decreasing order of length.

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