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Karyological Analysis of an Endemic Indian Maharaja Barb (*Puntius Sahyadriensis*, SILAS-1953) from Northern Western Ghats, India

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Abstract: The family Cyprinidae, one of the largest freshwater fish families and genus *Puntius* is one of the group of barb fishes. Cytogenetic data of *Puntius* shows extensive variability in karyotypic formula. In this work karyological analysis of endemic fish species, Indian Maharaja Barb (*Puntius sahyadriensis*) from Northern Western Ghats of India. Gill cells of four male and four female fishes were used for the analysis. Conventional staining of both the cells showed diploid chromosome $2n=50$ and fundamental number (NF) was 66, Karyologically it showed 4 metacentric, 4 sub-metacentric, 8 Acrocentric and 34 Telocentric chromosomes. The Karyotype formula for the *Puntius sahyadriensis* is $(2n \text{ (diploid)} 50 = 4m+4sm+8a+34t)$

Keywords: Diploid Chromosome, India, Karyotype, *Puntius sahyadriensis*.

I. INTRODUCTION

Western Ghats of India is one of the major biodiversity hot spots in the world. Among all the organisms of freshwater fauna, fish is the most important, threatened and endemic taxonomic group in the Western Ghats and it contributes critical ecological role in the aquatic ecosystem [1]. The endemic fish *Puntius sahyadriensis* belongs to family Cyprinidae. It is native of India, particularly in Maharashtra and Karnataka. It is an endemic species of the Northern and Central Western Ghats [2]. It is recorded from the rivers Yenna, Koyana, Krishna at Wai [3], [4]; reported that, this species mainly occurs in hill streams and rivers. *Puntius sahyadriensis* is a popular ornamental fish collected and exported to the international aquarium for pet trade and also used as minor fish food in Maharashtra [5]. In the genus *Puntius* some of the species were cytogenetically studied. Each has near about same diploid chromosome number that is $2n=50$ excluding two species i.e. *Puntius sophore* and *Puntius stigma*.

Fig. no. 1 General characteristic of *Puntius sahyadriensis*

According to the various literatures on karyological studies on fishes, it showed that 5 species of fish from the genus *Puntius* have been studied. However, there is no record on *Puntius sahyadriensis* as far as the karyotypic study is concerned. Hence, in present study, we have reported first karyological analysis of *Puntius sahyadriensis* species, with standardization of Karyo type by using conventional staining (Giemsa) technique.

Table No.1. Review of Cytogenetic reports in the genus *Puntius* and *Puntius*

Sr. No.	Species	2n	Karyotype Formula	NF	Reference
1	<i>Puntius sahyadriensis</i>	50	$4m+4sm+8A+34t$	66	Present studies
2	<i>Pethia ticto</i> *	50	$14m+22sm+6st+8t$	86	Manna and Prasad, 1973

3	Pethia ticto*	50	28m+22sm-st	100	Taki and Suzuki, 1977
4	Pethia ticto*	50	28m+16sm+6st	94	Prabhathi and Prasanta, 2007
5	Puntius chola	50	2m+4sm-st+14t+30t	56	Taki and Suzuki, 1977
6	Puntius chola	50	2m+2sm+46t	54	Prabhathi and Prasanta, 2007
7	Puntius sophore	48	2m+46t	50	Biswal and Sahoo, 2010
8	Pethia conchonius*	50	17m+2sm+4st+2t	100	Flora and Raja, 2014
9	Puntius tambraparniei	50	12m+16sm+16st+6t	94	Muthukumarasamy and
10	Puntius arulius	50	10m+18sm+12st+10t	90	Manavalan,2007
11	Puntius stigma	48	4m+2sm+42T	54	Muthukumarasamy and
					Manavalan,2007
					Khuda and Barat, 1987

Note:-2n=diploid chromosome number, NF= fundamental number (number of chromosome arm),
m=metacentric, sm=submetacentric, a=acrocentric, t=telocentric.

* - Puntius genus is changed as Pethia.

II. MATERIALS AND METHODS

Live species of Puntius sahyadriensis were collected by using hand net from Saraswatirivernear Kode, Maharashtra, India. 08 fish species were transferred and acclimatized in the laboratory aquarium for 8 to 10 days with aeration and fed ad libitum. After acclimatization, Chromosomes were spread by using air dry technique and stain with 5% Giemsa described elsewhere[6] and observed under the ZEISS trinocular microscope with 100X magnification. The measurements or the length of the chromosomes were measured by using the ISIS-5.5 Karyotyping software. In present study, the length of p & q arm that is short arm (Ls) and long arm (L1) of chromosome, total length(LT) i.e.(LT= Ls+L1), Relative length (RL) and Centromeric index (CI) of chromosome were measured[7].

III. RESULTS AND DISCUSSION

The diploid chromosome number is 2n=50 were found in both the sexes. Chromosome set was distributed as Metacentric(4), Sub-metacentric (4), Acrocentric (8) and Telocentric (34)chromosomes and showed equal fundamental number (NF) i.e 66. The maximum length (1.521µm) and minimum length (0.666 µm) of chromosome was recorded, Table no. 2.

Fig. No. 2

Fig. No. 3

Fig. No. 2 & 3:-The image of Metaphasic chromosome spreading and Karyotype of *P. sahyadriensis* species capture under the ZEISS trinocular microscope with 1000X Magnification, were observed ($2n$ (diploid) $50 = 4m+4sm+8a+34t$), by using conventional staining technique (Giemsa). The scale bar indicates $2\ \mu\text{m}$. Earlier literature reports that the *Puntius chola* species have two records, $2n=50$ in both but, here also variation in karyotpe formula and Fundamental number (NF), shown in table no. 1. Variation in chromosome size and type is due to intraspecific centric fusion, in that two telocentric chromosomes fuse together and form metacentric chromosome. The result of $2n$ changes due to the centromeric fusion but fundamental number (NF) remains constant [8]. *Puntius tambraparniei* and *Puntius arulius* also shows $2n = 50$, but show variation in karyotypic formula [9]. However *Puntius sophore* and *Puntius stigma* showed one less chromosome pair i.e $2n=48$ [10], [6]. The diploid chromosome ($2n=50$) was also recorded in *Pethia conchoni* [11]. *Pethia ticto* has diploid chromosomal set $2n=50$ with variation in type and size of chromosomes as shown in Table No. 1. [12], [13], [14]. Chromosome number and morphological variations can be found between different population of the same species or among different individuals of the same population or even in different cells of the same species [15].

Table No.2. The mean of short arm chromosome length (Ls), length of long arm chromosome (L1), total length of chromosome (LT), Relative length (RL), Centromeric index (CI) and standard deviation (S.D.) of RL, CI from the 20 cells of the *Puntius sahyadriensis* $2n=50$ CI classifies the type of the chromosomes and RL shows the size of chromosomes in relation to other chromosomes in the same set (Chaiyasut 1989).

Chromosome Pair	LS	L1	LT	RL $\pm$ SD	CI $\pm$ SD	Chromosome Size	Chromosome Type
1	0.7314	0.812	1.4360	0.0559 $\pm$ 0.00	0.5303 $\pm$	Large	Metacentric
2	0.5800	7	1.1979	5	0.018	Large	Metacentric
3	0.5106	0.709	1.3814	0.0464 $\pm$ 0.00	0.5513 $\pm$	Large	Sub-metacentric
4	0.5102	1	1.3369	3	0.048	Medium	Sub-
5	0.3120	0.945	1.2374	0.0526 $\pm$ 0.00	0.6485 $\pm$	Medium	metacentric
6	0.3225	9	1.2308	8	0.052	Large	Acrocentric
7	0.3168	0.826	1.0796	0.0513 $\pm$ 0.00	0.6400 $\pm$	Large	Acrocentric
8	0.2903	6	1.0537	6	0.038	Large	Acrocentric
9	0.0000	0.978	1.3533	0.0462 $\pm$ 0.00	0.7231 $\pm$	Large	Acrocentric
10	0.0000	2	1.2900	6	0.054	Large	Telocentric

11	0.0000	0.889	1.2200	0.0456±0.00	0.6810 ±	Large	Telocentric
12	0.0000	9	1.1892	3	0.180	Large	Telocentric
13	0.0000	0.805	1.5217	0.0403±0.00	0.7167 ±	Large	Telocentric
14	0.0000	5	1.1100	3	0.025	Large	Telocentric
15	0.0000	0.806	1.0925	0.0394±0.00	0.7334 ±	Large	Telocentric
16	0.0000	6	1.0483	4	0.038	Medium	Telocentric
17	0.0000	1.353	1.0208	0.0486±0.00	1.000 ±	Medium	Telocentric
18	0.0000	3	0.9883	4	0.000	Medium	Telocentric
19	0.0000	1.290	0.9617	0.0463±0.00	1.000 ±	Medium	Telocentric
20	0.0000	0	0.9406	2	0.000	Medium	Telocentric
21	0.0000	1.220	0.9133	0.0438±0.00	1.000 ±	Medium	Telocentric
22	0.0000	0	0.8425	1	0.000	Small	Telocentric
23	0.0000	1.189	0.8217	0.0426±0.00	1.000 ±	Small	Telocentric
24	0.0000	1	0.7642	1	0.000	Small	Telocentric
25	0.0000	1.151	0.6667	0.0413±0.00	1.000 ±	Small	Telocentric
		6		0	0.000		Telocentric
		1.110		0.0398±0.00	1.000 ±		
		0		0	0.000		
		1.092		0.0390±0.00	1.000 ±		
		5		0	0.000		
		1.048		0.0376±0.00	1.000 ±		
		3		1	0.000		
		1.025		0.0366±0.00	1.000 ±		
		8		1	0.000		
		0.971		0.0353±0.00	1.000 ±		
		6		1	0.000		
		0.961		0.0344±0.00	1.000 ±		
		6		2	0.000		
		0.940		0.0336±0.00	1.000 ±		
		8		2	0.000		
		0.913		0.0326±0.00	1.000 ±		
		3		2	0.000		
		0.842		0.0302±0.00	1.000 ±		
		5		1	0.000		
		0.821		0.0294±0.00	1.000 ±		
		6		1	0.000		
		0.764		0.0274±0.00	1.000 ±		
		1		1	0.000		
		0.666		0.0239±0.00	1.000 ±		
		6		1	0.000		

IV. CONCLUSION

Near about all records in genus *Puntius* showed diploid chromosome number $2n=50$, so it can be concluded that the chromosome number in this genus is conservative and the number of chromosome in *Puntius sahyadriensis* species is similar to the other species of the genus *Puntius*. Present karyological data of *Puntius sahyadriensis* also helpful for taxonomic study. Our results showed that the largest chromosome observed is Sub-metacentric and is about 1.521 μm , while the smallest chromosome is telocentric having 0.666 μm . The largest chromosome is near about 3 times larger than the smallest chromosome. By considering above results following Karyotype formula will be given for *Puntius sahyadriensis* species.

$$2n \text{ (diploid) } 50 = 4m+4sm+8A+34t$$

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