CYTOGENETICS AND CYTOTAXONOMIC CONSIDERATION OF TWO ENDANGERED ORNAMENTAL FISHES *PUNTIUS ARULIUS* AND *P. TAMBRAPARNIEI* (CYPRINIFORMES: CYPRINIDAE) FROM WESTERN GHATS, INDIA

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Abstract

Puntius arulius and *Puntius tambraparniei* are two closely related endangered ornamental fishes of Western Ghat mountain ranges in peninsular India. Diploid chromosome of both the species is 2n = 50 with NF values of 90 and 94 respectively. Results on the topotype specimens revealed them to be distinct genotypes.

Keywords

Endangered fishes, genotype, karyotype, ornamental fish, *Puntius arulius*, *P. tambraparniei*, Western Ghats

Puntius arulius (Jerdon, 1849) was described from Cauvery river at Srirangapatnam. Recently this species was recorded from Nilgiri and Wynaad hills (Shaji 2000; Arunachalam & Manimekalan, 2000) from various streams of Kabini river. It is a rare barb having restricted distribution in the Cauvery river basin. The occurrence and range extension of this species from various streams in Tunga and Bhadra rivers have been recently documented (Arunachalam *et al*., In press). *Puntius tambraparniei*, a rare and endemic species (Molur & Walker, 1998) restricted to Tamiraparani river basin was described by Silas (1953) and the occurrence of this species from upstreams of Gadana river (Arunachalam & Sankaranarayanan, 2000), Manimuthar river (sub basins of Tamiraparani river) and Tamiraparani main stream have been reported. In order to distinguish the two closely related species that have high ornamental value a karyotypic study has been aimed at from topotypes (Cauvery river at Srirangapatnam and Tamiraparani river at Kallidaikurichi).

Materials and Methods

Fishes were captured using cast nets and 10 specimens were used for karyotyping. For each species, cytological preparations were made from part of kidney after injecting the fishes with 0.5% colchicine (1ml/100g of body weight) and allowing them to swim in a well aerated water pond. Fishes were sacrificed after 210min. Hypotonic solution air drying, 0.5% Giemsa's stain technique was employed for chromosome preparation. Numbers of metaphase spreads were scanned and photographed using 100x Nikon microscope. Karyotype was prepared following the classification of Levan *et al*. (1964).

Results and Discussion

Puntius tambraparniei: Among the 63 metaphase spreads (Table 1), the diploid chromosome number was 2n = 50 (12m+16sm+16st+6t) and the NF value = 94. The spread and idiogram are shown in Fig. 1 (C & D). The maximum length of chromosome was 10.54 µm and minimum length was 5.4µm and the adjacent chromosome length difference ranged from 0.0-5.14µm (Table 2).

Puntius arulius: Among the 68 metaphase spreads (Table 3), the diploid chromosome number was 2n = 50 (10m+18sm+12st+10t) and the NF value = 90. The spread and idiogram are shown in Fig. 1 (A & B). The maximum and minimum lengths of the chromosome were 13µm and 7µm respectively, the adjacent chromosome length difference ranged from 0.0 - 2.8µm (Table 4).

The model number of chromosome in the family Cyprinidae is 50 (Rishi, 1989). Eight species of the genus *Puntius* have 2n = 50, in which, the *conchonius* and *arulius* group consisted of more biarmed chromosomes which reveal a close phylogenic relationship between *arulius* and *conchonius* group. On the basis of NF value the various species of *Puntius*, irrespective of their distribution area, can be categorized into two groups, *viz*., one with a range of arm number NF = 52-80 and another with a range of arm number NF = 80-100 (Sharma *et al*., 1990), and these two species of *Puntius* in the present study fall into the second category.

Puntius arulius in the Cauvery river basin and *Puntius tambraparniei* in Tamiraparani river basin both are east flowing might have supported polytypic species including *Puntius filamentosus* which has a wide distribution now in peninsular rivers. There are reports available on the occurrence of *Puntius arulius* from Kallada river in Thenmalai mountain ranges (Gopi, 2000), western side of Agastyamalai hill ranges and from upstreams of Manimuthar river (personal collection) on the eastern side of Agastyamalai. These two populations of *Puntius filamentosus* and *Puntius arulius* might have exchanged their genes freely before. Based on the biological concept or biospecies concept (Bernardi 1980), there will be an intermediate taxonomic category between *Puntius arulius* and *Puntius filamentosus*. Recently, taxonomists Pethiyagoda & Kottelat (2005) examined the topotype specimens of *Puntius tambraparniei*, from the Zoological Survey of India, Southern Regional Station, Chennai, and based on their extensive studies they elevated the subspecies to a separate new species. It is distinguished by several morphological characters such as, by its inferior mouth, by having lower lip medially absent and rays of the dorsal fin rays 3-5 elongated into filament-like extensions (Pethiyagoda & Kottelat, 2005). According to the present study differences in metacentric and teleocentric chromosomes between *Puntius arulius* and *Puntius tambraparniei* reveal that these two species can be considered as separate genotypes. The concept of identification of species based on biochemical methods (Colombera, 1982; Moynihan & Mahon, 1983) also supports our view. Based on the karyotyping and

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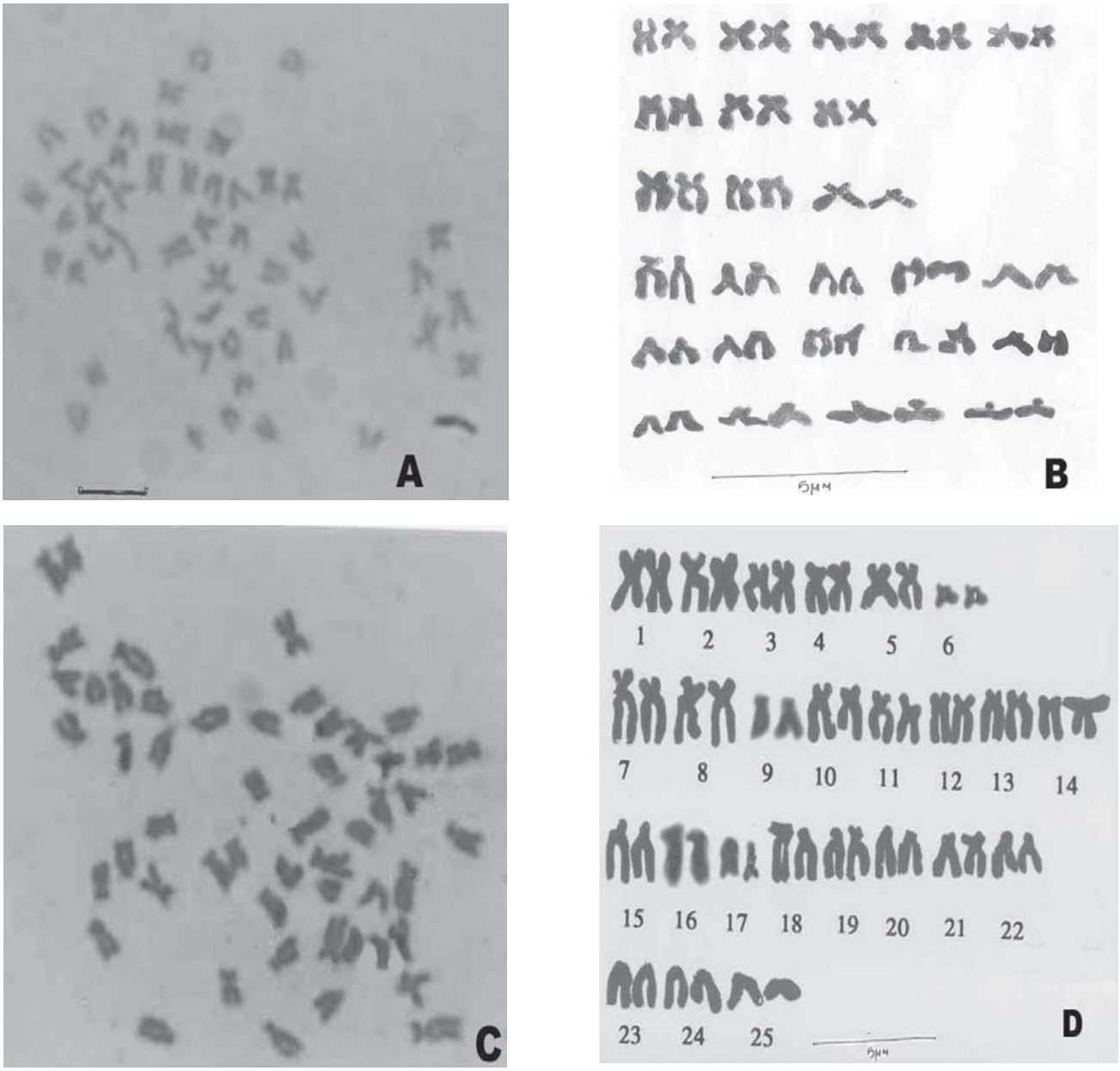

Figure 1. Chromososme spread and idiogram
A* - Puntius arulius *spread (100X); B* - Puntius arulius *karyotyping (scale 5µm);
C* - Puntius tambraparniei *spread (100X); D* - Puntius tambraparniei *karyotyping (scale 5µm)

morphometric data it can be concluded that *Puntius tambraparniei* is a distinct species and not a subspecies of *P. arulius.*

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Table 1. Chromosome complement of* Puntius tambraparniei *2n = 50

	Chromosome Number				
	48	**49**	**50**	**51**	**52**
1	-	1	6	-	-
2	1	-	5	-	-
3	-	-	4	-	-
4	-	-	5	1	-
5	-	-	6	-	1
6	-	-	5	-	-
7	1	-	7	-	-
8	-	-	8	-	-
9	-	-	5	1	-
10	-	-	6	-	-
Total no. of fish	2	1	**57**	2	1

Table 2. The standard Chromosome morphometry data (in micro meter) for* Puntius tambraparniei *2n = 50

	LA	**SA**	**TL**	**AL**	**RL**	**A/R**	**CI (%)**
1	4.05	3.78	7.83	3.92	5.95	1.07	48.28
2	4.57	3.43	8	4.00	6.08	1.33	42.88
3	3.24	3.51	6.75	3.38	5.13	0.92	52.00
4	4.57	2.16	6.73	3.37	5.12	2.12	32.10
5	4.57	2.16	6.73	3.37	5.12	2.12	32.10
6	3.51	1.89	5.4	2.70	4.11	1.86	35.00
7	7.84	2.7	10.54	5.27	8.02	2.90	25.62
8	5.68	2.97	8.65	4.33	6.58	1.91	34.34
9	5.95	2.16	8.11	4.06	6.17	2.75	26.63
10	5.95	1.89	7.84	3.92	5.96	3.15	24.11
11	4.87	1.89	6.76	3.38	5.14	2.58	27.96
12	4.6	2.16	6.76	3.38	5.14	2.13	31.95
13	4.6	2.16	6.76	3.38	5.14	2.13	31.95
14	4.4	1.89	6.29	3.15	4.78	2.33	30.05
15	6.95	2.16	9.11	4.56	6.93	3.22	23.71
16	6.95	2.1	9.05	4.53	6.88	3.31	23.20
17	6.95	1.62	8.57	4.29	6.52	4.29	18.90
18	6.95	1.62	8.57	4.29	6.52	4.29	18.90
19	6.14	1.62	7.76	3.88	5.90	3.79	20.88
20	5.95	1.08	7.03	3.52	5.35	5.51	15.36
21	4.6	1.06	5.66	2.83	4.30	4.34	18.73
22	4.87	1.02	5.89	2.95	4.48	4.77	17.32
23	6.49	-	6.49	3.245	-	-	-
24	5.95	-	5.95	2.975	-	-	-
25	5.68	-	5.68	2.84	-	-	-

LA - Long Arm; AL - Average length; CI % - Centromeric Index Percentage; SA - Short Arm; RL - Relative Length; TL - Total Length; A/R - Aspect Ratio

Table 3. Chromosome complement of* Puntius arulius *2n = 50

	Chromosome Number				
Number of Fish	**48**	**49**	**50**	**51**	**52**
1	-	-	8	-	-
2	1	-	5	1	-
3	-	1	7	-	-
4	-	-	6	-	1
5	-	-	7	-	-
6	-	-	5	1	-
7	2	-	8	-	-
8	-	-	4	-	-
9	-	1	5	-	-
10	-	-	6	-	-
Total no. of fish	3	2	**60**	2	1

Table 4. The standard Chromosome morphometry data (in micro meter) for* Puntius arulius *2n = 50

	LA	**SA**	**TL**	**AL**	**RL**	**A/R**	**CI (%)**
1	7.00	5.00	12.00	6.00	9.13	1.40	41.67
2	5.20	4.00	9.20	4.60	7.00	1.30	43.48
3	5.00	3.70	8.70	4.35	6.62	1.35	42.53
4	4.00	3.00	7.00	3.50	5.32	1.33	42.86
5	7.00	4.00	11.00	5.50	8.37	1.75	36.36
6	8.50	4.50	13.00	6.50	9.89	1.89	34.62
7	7.00	4.50	11.50	5.75	8.75	1.56	39.13
8	6.00	3.00	9.00	4.50	6.84	2.00	33.33
9	8.00	3.00	11.00	5.50	8.37	2.67	27.27
10	6.20	3.00	9.20	4.60	7.00	2.07	32.61
11	5.00	2.00	7.00	3.50	5.32	2.50	28.57
12	6.00	2.00	8.00	4.00	6.08	3.00	25.00
13	5.00	2.00	7.00	3.50	5.32	2.50	28.57
14	6.00	2.00	8.00	4.00	6.08	3.00	25.00
15	6.00	2.50	8.50	4.25	6.46	2.40	29.41
16	6.50	3.00	9.50	4.75	7.22	2.17	31.58
17	6.00	3.00	9.00	4.50	6.84	2.00	33.33
18	5.00	2.00	7.00	3.50	5.32	2.50	28.57
19	6.50	2.00	8.50	4.25	6.46	3.25	23.53
20	7.00	1.50	8.50	4.25	6.46	4.67	17.65
21	6.50	-	6.50	3.25	-	-	-
22	6.00	-	6.00	3.00	-	-	-
23	6.00	-	6.00	3.00	-	-	-
24	5.50	-	5.50	2.75	-	-	-
25	5.00	-	5.00	2.50	-	-	-

LA - Long Arm; AL - Average length; CI % - Centromeric Index Percentage; SA - Short Arm; RL - Relative Length; TL - Total Length; A/R - Aspect Ratio

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