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CHROMOSOMAL STUDIES ON FOUR CYPRINID FISHES

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Karyotype analysis of four cyprinids *Cirrhinus reba* (Hamilton), *Amblypharyngodon mola* (Hamilton), *Esomus danricus* (Hamilton) and *Chela bacaila* (Hamilton) from rivers and ponds of Orissa, India were studied. The diploid chromosome numbers in these four species were ranged from 48-52. The karyotypes were observed to be 2n=50, 10 metacentric (m) + 8 sub-metacentric (sm) + 6 sub-telocentric (st) + 26 telocentric (T) in *C. reba*; 2n = 52, 8 m + 8 sm + 36 T; 2n=48, 12 m + 12 sm + 24 T and 2n=52, 14 m + 8 sm + 12 st + 18 in *A. mola*, *E. danricus* and *C. bacaila*, respectively. Species specific karyotypes were also observed among all these species. No sex chromosomes could be found in the form of heteromorphic pair. The evolutionary significance of these four karyotypes was also discussed.

INTRODUCTION

The study of fish chromosome has been an active area of research over the years owing to its importance in conservation of fish population, cytotaxonomy, phylogeny research and evolutionary studies (Kirpichnikov, 1981; Luca *et al.*, 2010). Cytogenetical studies in fishes are limited to about 10% of the total fishes taxonomically known all over the world (Barat *et al.*, 1996). The basic information on the number, size and morphology of chromosomes in fish has been of immense help to undertake chromosome manipulations (Khan *et al.*, 2000). The smaller cell size with relatively large number of chromosomes crowded together at metaphase, compared to the other vertebrate groups, however, has been major hurdles for the study on fish chromosomes (Nayar, 1964; Cucchi and Baruffaldi, 1990). The existence of morphological variation even between homologous chromosomes in the same nucleus has been a major difficulty. Study on karyotyping for different fish becomes necessary not only for their differences between the species but also occurrence of the polymorphism often within the same fish species (Al-Sabti, 1991).

Among the freshwater fishes, the family Cyprinidae is known to be the richest and most important family that has got extensive distribution all over the world (Al-Sabti, 1991; Kalbassi, 2008). Without exception, the India and the state Orissa too possess a rich and diverse population of cyprinids in both lotic and lentic water systems. Studies on karyotype of cyprinids have shown very low variability (Rab and Collares-pereira, 1995),

which are generally characterized by comparatively small chromosomes with centromere placed from median to virtually terminal position (Luca *et al.*, 2010). The present study was an attempt to study the karyotype of four cyprinids *viz.*, *Cirrhinus reba, Amblypharyngodon mola, Esomus danricus* and *Chela bacaila,* those are widely distributed in several water bodies of Orissa, India.

MATERIAL AND METHODS

Live samples of four identified fish species were collected from Naraj barrage of the River Mahanadi, near Cuttack, the River Daya, near Kausalyaganga and rural ponds of Khurda and Puri districts of Orissa using cast net and fry net (Table 1).

Table 1. Specification of fishes used for analysis

Name of the species	Collection site	No of specimen
C. reba	River Daya, Khurda	05
A. mola	Village -Velurhat, Khurda	08
E. danricus	Village – Joypur, Puri	06
C. bacaila	Naraj barrage, River Mahanadi	07

Fishes were brought to the laboratory of Directorate of Research for Women in Agriculture, Bhubaneswar, Orissa in oxygen-filled polythene bags and subsequently were acclimatized in FRP tanks with provision of aeration for 2-3 days. The initial species identification was made on the basis of morphology (Talwar and Jhingran 1992; Nath and Dey, 2000).

The fishes were injected intramuscularly with 0.05% colchicine (Sigma, US) @ 1 ml/100 g body weight and kept alive for 2-3 hrs with proper aeration. The live fishes were operated after two hours and the gill and kidney tissues were collected and thoroughly washed in distilled water to remove clotted blood and other artifacts. The tissue samples were minced to make cell suspension in 0.56% KCl and kept for 30 minutes at room temperature. The cell suspension thereafter was centrifuged at 2000 rpm for 10 min. The supernatants were discarded and cell pellets were further processed following fixation in 1:3 Glacial Acetic acid and Methanol. The fixation and centrifugation stages were repeated two times. The slides were prepared following 'Flame drying method' (Khuda Bukhsh and Barat, 1987). The slides were stained with 4% Giemsa solution for two hours and air-dried. 41-62 metaphase spreads were screened in the above four species. The well-spread metaphases were observed and photographed under compound microscope (Zeiss-Axiostar plus). Three karyotypes from three well spread metaphases were prepared for each species and chromosome morphology was determined following Levan *et al.* (1964).

RESULTS AND DISCUSSION

All the homomorphic pairs were arranged linearly according to length of chromosome from highest to lowest. The details of each chromosome pair of each species are presented in Table 2. Species specific pattern of karyotype was observed in each species though all the four species belonged to same family under cyprinidae.

The diploid metaphase complements of *C. reba* consisted of 50 chromosomes measuring between 7.33 to 2.0 µm. *C. reba* showed a karyotype (Fig. 1B) formula of 10 metacentric (m) + 8 sub-metacentric (sm) + 6 sub-telocentric (st) + 26 telocentric (T) with fundamental arm number (FN) as 68 (Fig. 1A&B).

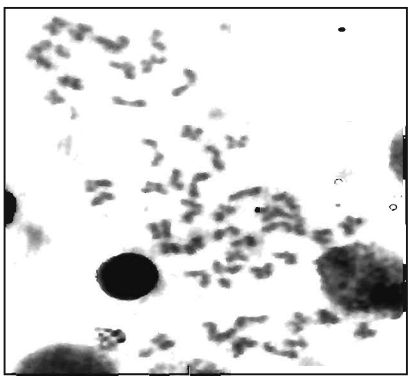

Fig. 1A Metaphase Plate of *C. reba*

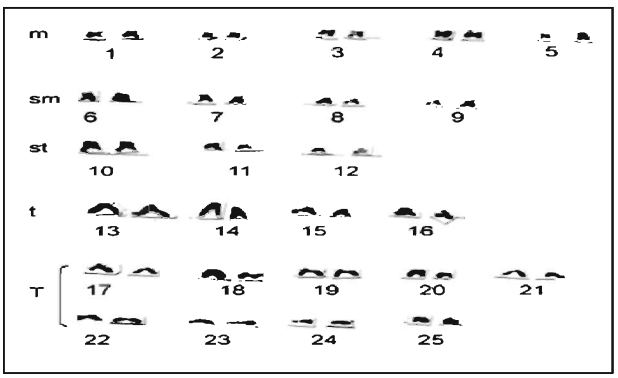

Fig. 1B Karyotype of *C. reba*

The study in case of a minor carp, *A. mola* revealed 2n=52 chromosomes with a karyotype (Fig. 2A&B) formula of 8 m + 8 sm + 36 T and a fundamental arm number of 68. The size of the chromosomes varies between 5.35 to 1.6 µm.

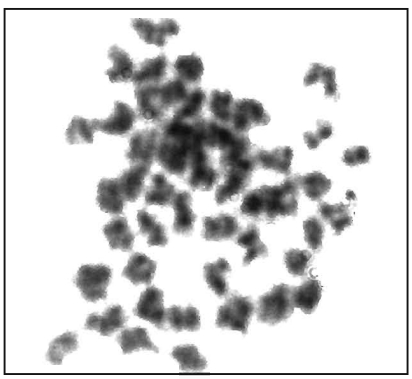

Fig. 2A Metaphase Plate of *A. mola*

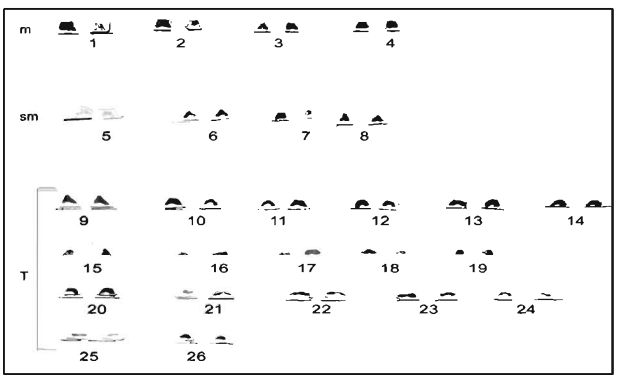

Fig. 2B Karyotype of *A. mola*

With diploid chromosome number as 48, the size of the chromosomes of *E. danricus* varies between 9.0 to 2.25 µm. Fig. 3A&B presents the karyotype formula of 12 m+12 sm+24 T and fundamental arm number of 72.

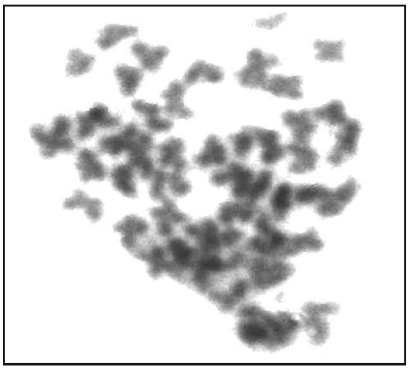

Fig. 3A Metaphase Plate of *E. denricus*

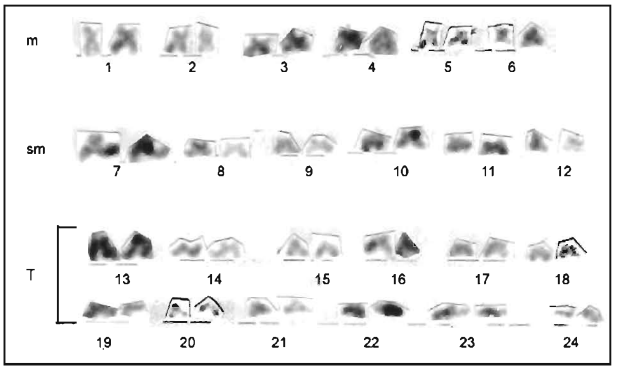

Fig. 3B Karyotype of *E. danricus*

The diploid metaphase complements of *C. bacaila* consisted of 52 chromosomes measuring between 8.02 to 3.0 µm. *C. reba* showed a karyotype (Fig. 4A&B) formula of 14 m + 8 sm + 12 st + 18 T with fundamental arm number (FN) as 74. The size of the chromosomes varies between 5.0 to 1.7 µm. No sex chromosomes could be identified in the form of heteromorphic pair in size or staining difference in any of the species.

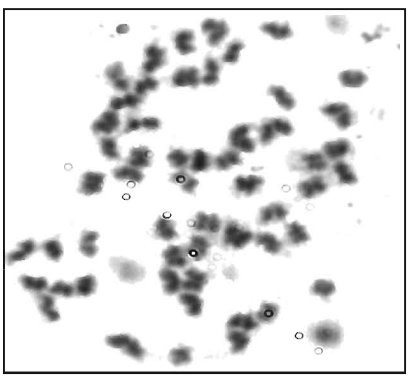

Fig. 4A Metaphase Plate of *C. bacaila*

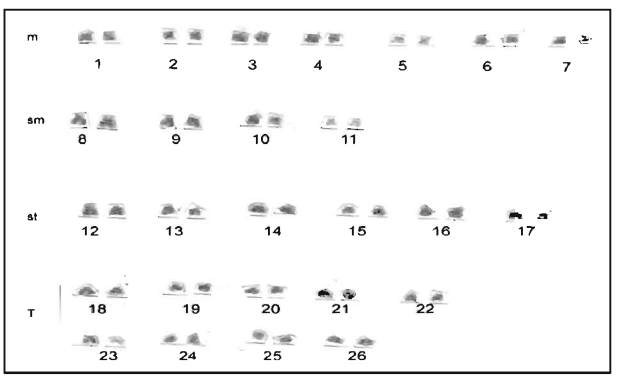

Fig. 4B Karyotype of *C. bacaila*

The present cytogenetical studies on these four species under family Cyprinidae exhibited the variability in diploid chromosome number, which ranged from 48-52. The majority of the species under this family has 2n=50 for which the modal chromosome number was detected as 50. The chromosomal data on *C. reba* was reported earlier by Manna and Khuda-Bukhsh (1977) with a karyotypic formula of 2n=48, 36m+2sm+6st+4T

and fundamental arm number (FN) as 86. The present observation was different in both the chromosome numbers (2n=50) as also the karyotype (10 m+8 sm+6 st+26 T). The karyotype of another congeneric species i.e., *C. mrigala* was also reported with 2n=50 and karyotype formula as 6 m+8 sm+14 st+22 t/T (Prasad, 1971). The similarities of karyotype are observed more closely in these two species including the present study. Though the diploid chromosome number of *A. mola* under present observation was in conformity with earlier report by Manna and Khuda Bukhsh (1977), but karyotype pattern was different. In the present study, the number of bi-armed chromosomes was less as compared to the previous report of 12 m+20 sm+8 st+10 T by Manna and Khuda Bukhsh (1977). Such variation is attributed to some Robertsonian rearrangements that might have occurred in different geographically isolated populations, if any. The variations also have been observed in other two species *E. danricus* and *C. bacaila*. The chromosomal studies on *E. danricus* had been reported earlier by Manna and Khuda Bukhsh (1977) and Sharma and Tripathi (1986) and in both the studies the diploid chromosomal number was same i.e. 50, but all the three karyotypes including the present one were different. However, the karyotype in case of *C. bacaila* in the present study, for both the diploid chromosome numbers as also the chromosome formula was different (Table 3).

Table 2. Karyotype analysis of four fish species

Species	No of cells	Chromosome no (2n)	Chromosome formula				FN
			m	sm	st	T	
C. reba	55	50	10	8	6	26	68
A. mola	62	52	8	8	-	36	68
E. danricus	47	48	12	12	-	24	72
C. bacaila	41	52	14	8	12	18	74

Table 3. Comparative analysis of the karyotypes of four species reported earlier

Species	Reference	Chromosome no. (2n)	Chromosome formula				FN
			m	sm	st	T	
C. reba	Manna and Khuda Bukhsh (1977)	48	36	2	6	4	86
A. mola	Manna and Khuda Bukhsh (1977)	50	12	20	8	10	82
E. danricus	Manna and Khuda Bukhsh (1977)	50	12	16	10	12	78
	Sharma and Tripathi (1986)	50	10	18	18	4	78
C. bacaila	Manna and Khuda Bukhsh (1977)	50	10	12	10	18	72

The broad-spectrum variation in the karyotypes of same species indicates a need of proper attention for morphological identification of the species and then with the aid of molecular DNA marker assay. However, possible change in the genotype with the change in environment after few decades as also in different geographical locations cannot be ruled out. It is therefore necessary for further work for confirmation in this area using a comparative analysis of ancient and present samples with the molecular DNA marker assistance.

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