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Development of Karyotyping and Chromosome Banding of *Osteobrama belangeri* (Pengba Fish)

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ABSTRACT

Cytogenetic characterization plays an important role in cataloguing and conservation of germplasm. *Osteobrama belangeri* is one of the important fish species in North East India and need urgent attention for conservation. Hence cytogenetic characterization was carried out for *Osteobrama belangeri*. Samples of the specimen were collected from Manipur, India and maintained in wet laboratory in live condition. Metaphase chromosome preparation was made using standard hypotonic solution, methanol acetic-acid fixation followed by Giemsa staining for karyotyping of the species. The diploid chromosome number was found to be 50 and the karyotype is composed of 14m+8sm+28 (st and t) with fundamental arm No. (FN) 72. Positive chromosome bands like C, G and NOR bands were developed on the metaphase chromosome spread. NOR bands were found on the constriction regions near centromeric, as well as, slightly away from terminal regions. While, G bands was at A, T-rich regions appearing to be away from centromere towards the end of the chromosome bodies. C bands were localized on the regions adjacent or immediate to the centromeres and on the constrictions as indicating the concurrency with constitutive hetrochromatin. The result showed the diploid chromosome number as 50 which is mostly seen in other cyprindis and the study could localize C, G and NOR bands for the first time on this species.

Key words: Cytogenetics, *Osteobrama belangeri*, metaphase, karyotype, banding

INTRODUCTION

Sustain ability is measured indispensable world wide as the objective of community development and environmental conservation (Al-Masroori and Bose, 2011). Chromosomal studies and karyotyping are the basic requisite for genetic improvement program and conservation of wild gene pool. Such studies were initiated in India by Sharma *et al.* (1960) and eventually Indian researchers reported the karyotype of about 125 species belonging to both inland and marine waters (Rishi, 1989). The genus *Osteobrama* belongs to Cyprinidae family and has 10 species. This fish is locally known as Pengba. It is a medium carp found in lakes and rivers of India (Manipur), Myanmar and China (Yunnan). The species was categorized as 'Extinct in wild' in the Conservation Assessment and Management Plan (CAMP, 1999) workshop conducted at National Bureau of Fish Genetic Research, Lucknow in 1997. It is present only in captive conditions. In the past, *Osteobrama belangeri* formed a big fishery in the Loktak Lake but now this species has become extinct in wild. The extinction of this species in Manipur was due to over exploitation,

habitat degradation and introduction of invasive species which caused fragmentation. It is the State fish of Manipur. Pengba, *Osteobrama belangeri* is considered to be one of the tastiest fishes in Manipur. Closely related with the history of the land and highly relished by the local people. The overall colour is golden yellow and it has six black bars evenly spaced along its flanks. The maximum size of pengba is 380 mm (14.96").

Cytogenetic investigation has taken a momentum in recent years in the field of fisheries which has lots of technical problem. The new source of data involving chromosome number and morphology, has been receiving increasing attention and shows high promise for interpreting the evolution of fishes.

Karyotype is the arrangement of chromosomes based on its size and shape. The other characteristics which are observed include the relative size of chromosomes, position of the centromeres, length of the arms, secondary constrictions and satellites (King *et al*., 2006). For studying various phenomena like chromosomal aberrations, cellular functions, taxonomic relationship and evolutionary events karyotypes can be used. Today, most karyotypes are stained with Giemsa dye which offers better resolution of individual bands, produces a more stable preparation and can be analyzed with ordinary bright-field microscopy (Caspersson *et al*., 1970). For karyotyping, chromosomes are grouped into metacentric (m), submetacentric (sm), subtelocentric (st) and telocentric (t) as per proposed by Levan *et al*. (1964).

Chromosome banding is the use of special staining procedure to deduce the pattern of longitudinal differentiation along chromosome, in the absence of any structural differentiation. However, for detailed study of the structure of chromosomes, specialized staining procedure need to be adopted where the particular stain binds with DNA and the pattern of bands can be discernible.

Cytogenetical techniques are potential tools for genetic characterization of germplasm resources, being a basic requisite for genetic improvement program and conservation of wild gene pool (Sharma, 2008). Chromosomal studies in fishes have not been as successful as those in other vertebrates because of relatively small size and large number of chromosomes found in many fish species and the limitations of the techniques employed (Klinkhardt *et al*., 1995). However, gradually the cytogenetical studies gained a considerable importance concerning species characterization, evolution and systematic (Gold *et al*., 1990; Barat *et al*., 2002). The cytogenetical studies in fishes are limited to just about 10% of the total fishes known taxonomically all over the world (Barat *et al*., 1996). Fish chromosome data have great importance concerning evolution, systematics, aquaculture and mutagenesis (Al-Sabti, 1991).

Genetic disorder or unique character identified through karyotyping and possible remedy may protest the loss of animals which in turn increase the total productivity (Khatun *et al*., 2011).

Karyotypic information can throw light on genetics characterization, phylogenetic relationships among species and karyotype evolution in fishes. *Osteobrama belangeri* is potential species for aquaculture in India, but So far, there is much less work on *Osteobrama belangeri* from cytogenetical aspects. In view of the above points, the present cytogenetic studies have been carried out in, *Osteobrama belangeri* to facilitate conservation and management of natural stock of this species.

MATERIALS AND METHODS

Materials used: Colchicine (Himedia), Giemsa stain (Himedia), sodium citrate solution (hypotonic solution), 3:1 methanol and glacial acetic acid (Carnoy's fixative), were used for chromosome preparation. The live fish specimen was collected from the wet laboratory where the

fishes were cultured at Central Institute of Fisheries Education. Hydrochloric acid, Barium Carbonate, Saline Sodium Citrate, Trypsin, Phosphate Buffered Saline, Silver Nitrate and Gelatin were used for banding techniques which were used of reagent grade and procured from local vendors. The experiment was conducted during the month of January to June, 2011.

Chromosome preparation: The fish specimen was treated with 0.01% Colchicine at 1 mL/100 g b.wt. and kept in an aerated tank for 2 and ½ h. Colchicine was used to arrest the cell division at metaphase. After 2 and ½ h the fish is sacrificed to dissect out the gills and kidney tissues. The extracted tissues were then placed in a hypotonic solution of 0.9% sodium citrate for 1 h. The tissues were chopped into smaller pieces and the hypotonic solution was changed every 15 min using pasteur pipettes. The swollen tissues were then placed into Carnoy's fixative for 45 min. The fixative was changed using pasteur pipette every 15 min. The tissues were then stored in refrigerator overnight. The tissues were brought back to room temperature and then placed in 45% acetic acid solution which was known as tissue suspension. The tissue suspension was then centrifuged 4-5 times at 27°C at 2000-3000 for 10 min. A little of the suspension was taken in a pasteur pipette and allowed to fall from a height on pre-warmed slides. The heat aids in bursting of the cells. The slides were then air dried and then stained using 8% giemsa stain for 45 min. Excess stain was removed by rinsing the slides with distill water. The slides were again air dried and then observed under microscope (Khuda-Bukhsh and Barat, 1987).

Development of C, G and NOR bands: C banding is the universal technique for demonstrating heterochromatin in chromosome. The slides with chromosomes should be allowed to age for a week before treating it for localization of C-bands. The slides are treated with dilute acid, warm $Ba(OH)_2$ solution, warm saline and giemsa stain (Sumner, 1972).

Dark and light bands can be seen over the chromosomes by treating the chromosomes with dilute Trypsin or hot Saline Sodium Citrate and staining with giemsa. The dark bands along the chromosomes contain facultative heterochromatin region popularly called as G band (Abe and Muramoto, 1974).

The Nucleolar Organizer Regions (NOR's) are those segments of chromosome that contains the genes for ribosomal RNA and on which the nucleoli are formed. The NOR's which contain hundred or even thousands of copies of ribosomal genes, commonly appear as constrictions of the chromosomes. The NOR's site can be stained specifically with silver. $AgNo_3$ staining was performed according to the method of Howell and Black (1980).

RESULTS

Several slides with metaphase chromosome spreads were prepared out of which some slides had appropriate chromosome spreads. The modal diploid chromosome number for *Osteobrama belangeri* was found to be 2n = 50 which is presented in Table 1. The karyotype comprises of 14 metacentric chromosomes, 8 submetacentric chromosomes and telocentric and subtelocentric chromosomes combining to be 28. Hence the karyotypic formula has been derived as 14m+8sm+28 (t and st) (FN = 72). The metaphase chromosome spreads and the karyotype are shown in Fig. 1 and 2, respectively.

Chromosome spreads of 20 fish specimens were prepared (Table 1). Around 200 slides were analyzed for the most appropriate metaphase chromosome spread. Cell count varied from 5-15.

Table 1: Chromosome set analysis of *Osteobrama belangeri* (Pengba)

	Chromosome No.					
Fish No.	48	50	52	2n modal	No. of fundamental arm	Total No. of cell counted
1	2	5	-	50	72	7
2	1	8	1	50	72	10
3	3	4	1	50	72	8
4	6	5	3	50	72	14
5	-	8	4	50	72	12
6	6	3	-	48	70	9
7	7	1	-	48	70	8
8	-	6	4	50	72	10
9	1	4	3	50	72	8
10	3	6	-	50	72	9
11	-	2	3	52	74	5
12	2	5	2	50	72	9
13	2	10	3	50	72	15
14	6	5	2	50	72	13
15	2	4	-	50	72	6
16	-	5	3	50	72	8
17	-	6	5	50	72	11
18	3	6	3	50	72	12
19	4	3	2	48	70	9
20	2	4	-	50	72	6

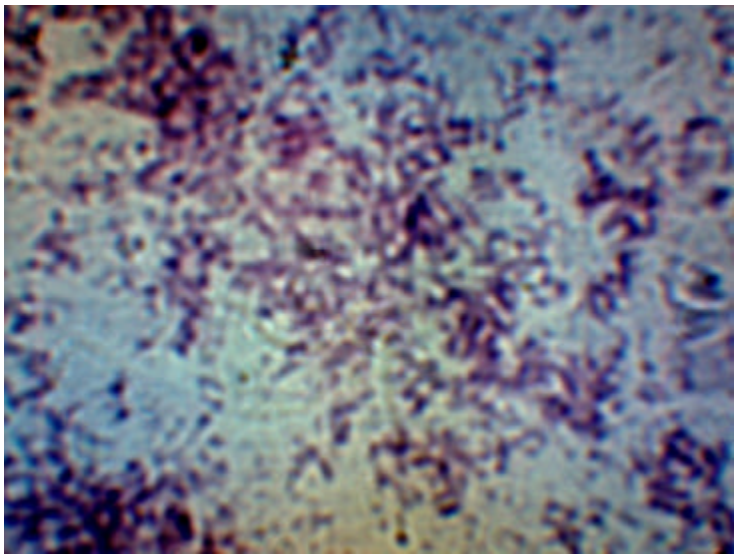

Fig. 1: Chromosome spread of *Osteobrama belangeri* (Pengba)

Diploid chromosome No. for some spreads was found to be 48 and for some were 52. Most of the chromosome spreads had the diploid chromosome No. as 50. Positive C, G and NOR bands were obtained for *Osteobrama belangeri* are shown in Fig. 3.

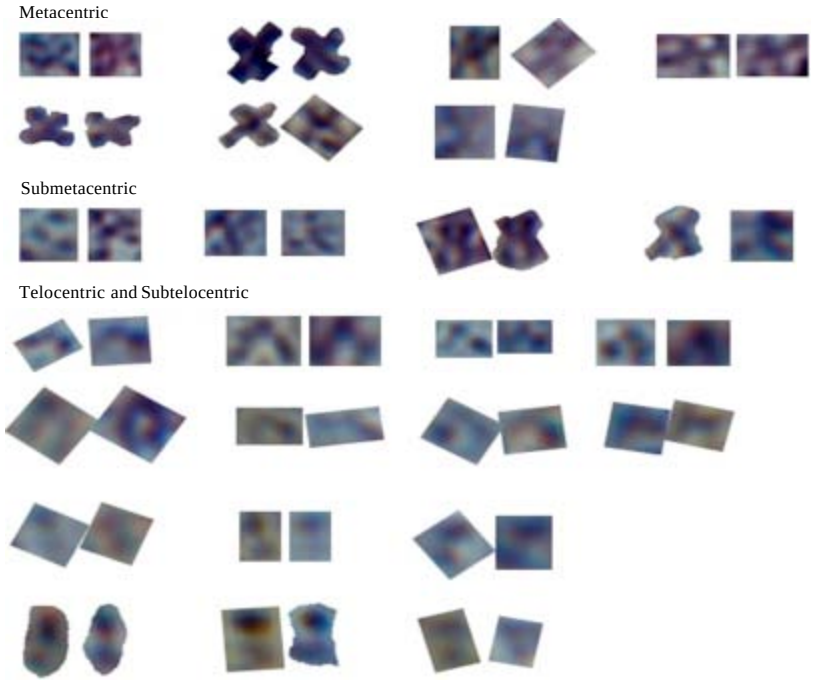

Fig. 2: Karyotype of *Osteobrama belangeri* stained with Giemsa (Pengba)

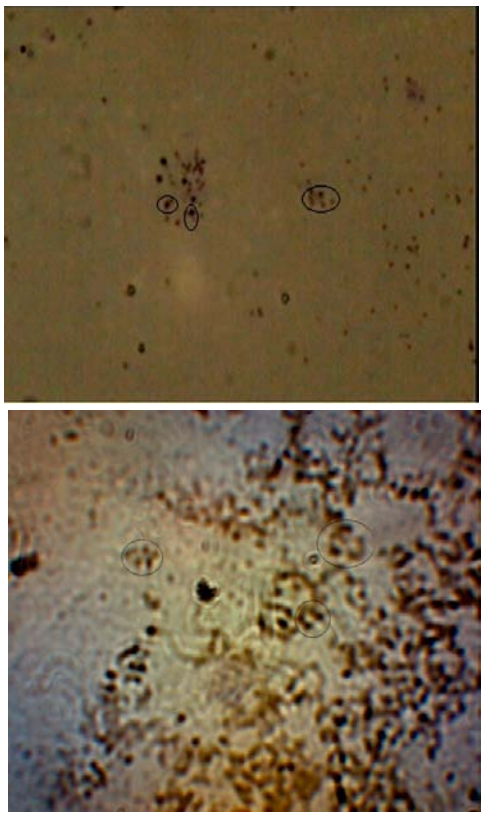

Fig. 3: C, G and NOR bands generated bands

DISCUSSION

In *Osteobrama belangeri*, the diploid chromosome number was found to be 50. The diploid chromosome number of the species is in conformity with Kumar *et al.* (2009). However, the karyotype formula has been found to be different from that of the reported one. The karyotype formula for this species was derived as 14 metacentric, 8 submetacentric, 28 telocentric and subtelocentric. Kumar *et al.* (2009) have found it as 6 met acentric, 16 submetacentric, 12 subtelocentric and 16 telocentric. The difference in the karyotype formula may be due to the overdose of colchicines which causes contraction of chromosomes. Physical mapping of rRNA gene in the species *Osteobrama belangeri* was also carried out by these workers.

Similarly in other species of the genus *Osteobrama* i.e., *Osteobrama cotio cotio,* two different studies showed different diploid chromosome number and the chromosome formula. The diploid chromosome number was reported to be 48 with karyotype formula as 18 m+24 sm+6 st by Arkhipchuk (1999) while Biswal *et al.* (2010) reported it as 2n = 54 with chromosome formula 10m+8sm+8st+28t (FN = 72). However, there is not much work on these species of *Osteobrama*.

Although, fishes represent 43% of the living vertebrates, even today many of the fish species still remain to be karyotyped. Fishes show a great diversity in karyotypes, the diploid No. ranging between as low as 16 and as high as 240.

Study on chromosomes of fish has become a priority area of research in recent years. Chromosome analysis can be useful for addressing a variety of evolutionary and genetic questions about fishes. The global fish fauna consists of about 28, 900 species of fishes of which 2, 200 species are cytogenetically studied. India's contribution so far was around 220 species (Biswal *et al.*, 2010). In most of the cyprinid fishes such as *Catla catla, Labeo rohita, Cirrhinus mrigala* and *Labeo fimbriatus*, the diploid chromosome number has been recorded as 50 (Sharma and Lakra, 1999; John *et al.*, 1993; Manna and Prasad, 1971; Biswal *et al.*, 2010). Cyprinid fishes are characterized by the presence of relatively small chromosomes with their centromere positions ranging gradually from median to nearly terminal, making it difficult to assign some chromosomes to particular chromosomal categories and thus making correct identification of individual chromosomes nearly impossible (Rab and Collares-Pereira, 1995). It has been observed that there is no scientific study on *O. belangeri* so far. Due to lack of management practices, the species is on the verge of extinction. Therefore, it is very much necessary to initiate the conservation measures which include detailed understanding of its biology and genomics which include the cytogenetic profiling of the species. It has already been stated that in most of the countries, rivers which are natural habitat of the fish germplasm are being polluted (El-Shahaby *et al.*, 2003). Work should be carried out to find out the occurrence of intra-species or inter-species hybrids in the nature since the chromosome numbers of hybrids are normally found to be same as that of the parents which was reported in tilapia by Manosroi *et al.* (2003). Genotoxicity studies also need to be carried out in polluted water bodies as it has been carried out in tilapia (Mohamed *et al.*, 2008). The present study is a pioneering in this aspect and need further studies for genetic characterization of the species.

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