Karyotype Analysis of Some Fresh Water Fishes of Mula and Mutha River Pune, Maharashtra

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Abstract -

Karyological characters of 18 fishes from Mula and Mutha River were studied by examining metaphase chromosome spreads. Four species includes showed good chromosomal spreads. Diploid chromosome number of R. daniconius was 2n=75 whereas O. bimaculatus showed 2n=48. Similarly, P. sophore and M. cavasius showed 2n=50 and 2n=58 respectively. For other fish species, we faced technical difficulties which are discussed here with probable solutions on them.

Keywords – *Metaphase, karyotyping, Western Ghats, Mula-mutha, freshwater fishes*

I. Introduction

The Western Ghats of India is one among the 25 global biodiversity hot spots with a high degree of endemism with rare, endemic and threatened species of flora and fauna [1]. Such rich biodiversity and endemism is the outcome of unique geological, topographical and climatic conditions. Western Ghats harbor 11% of world's total ichthyofauna [2]. About 2000 freshwater fishes are known to occur in India, of which 288 are endemic to the Western Ghats [3], [4]. Studies dealing with compilation of the endemic fishes from various streams and rivers in the Western Ghats mountain ranges have been carried out several times [5-10]. While a great deal of attention has been given to the loss of biodiversity in tropical rain forests, or in coastal areas, the diversity of and within freshwaters has been widely neglected. Fresh water fishes represent the most threatened group of vertebrates[11]. In classifying the worlds' top 25 biodiversity hot spots; vertebrate group was considered excluding fish. This is mainly because of the poorly available data wherein the author predicts that there could be at least 5, 000 species waiting to be discovered among fish, which is more than all mammals [1].

The study of fish chromosome has become an active area of research in recent years [12]. Surprisingly, great variation in genome size has recently been recognized among fishes [13]. However, despite progress in genome size estimation in fishes, basic data such as chromosome number and/or karyotypes are not available for large number of species. This gap in chromosome studies has encouraged the collection and analysis of new datasets obtained from different sources, in which important progress can be observed. The first compilation on chromosome numbers in fishes was documented [14], who estimated the number of species studied around the world to that decade at 400 (ca. 1.6%). Numerous works have been done since then, and these were compiled in a review documented by [15]. Since 1960s, karyological studies in fish have made noteworthy contributions to increasing knowledge in the fields of genetics, taxonomy and environmental toxicology [16]. The progress in increasing such knowledge has been closely related to the evolution of application methodologies [17]. Studies of the chromosomes of fish have not been as successful or widespread as in other vertebrate groups. Standard karyotypes are reported for less than 10% of more than 20, 000 extant species of fish [18].

Most of the fishes possess relatively high number of small chromosomes that can be easily viewed with a light microscope at the meta phase stage of mitosis. Unfortunately, studies on the karyotyping of endemic freshwater fishes of Western Ghats are scarce. Although morphological and anatomical characteristics of these fishes from Mula and Mutha River have been studied extensively, application of non-morphological methods, such as cytogenetics studies, may provide a framework for the correct species identification of this fish because the rate at which the new species of fishes describing from the Western Ghats is very high. Therefore, along with the morphological, osteological and molecular techniques, karyotyping studies could be one additional tool for species discovery. Karyotypical studies are still meager for several fresh water fishes of Mula and Mutha River Pune, Maharashtra. Therefore, the objective of current

study was to compile cytogenetic data (Karyotyping) for freshwater fishes found in Western Ghats more specifically from the Mula –Mutha River, the taxonomic representation and to suggest its possible use in conservation of endemic freshwater fish fauna.

II. Materials And Methods

Animal collection and rearing

In present study, total 18 fish species were collected from 5 different sites (Table 1) of northern Western Ghats, Maharashtra and studied for their cytogenetic study. These 18 species belongs from 10 families and 5 orders. Out of the 18 species studied, 6 species belongs from the family Cyprinidae, 3 species belongs from the family Nemachilidae, 2 species from family Ambassidae, 1 species each from family Channidae, Cobitidae, Gobidae, Bagaridae, Siluridae, Notopteridae and Mastacembelidae. The fishes were directly collected through streams in live condition by using hand net. Some freshly collected fish specimens were procured from local fisherman. Fishes were collected in large plastic bottles with declorinated tap water and were brought to the laboratory. In laboratory, fishes were maintained in the large aquariums (120 X 60 X 60 cm) equipped with the aerator and moderate intensity of light source. Fishes were fed with the fish food as per their need *ad libitum*. Every day fishes were checked for their growth and mortality. No mortality has been observed during the study period. After experimentation, remaining fishes were released at their natural habitat following precautionary measures.

Sr.	Name of place	Latitude	Longitude
1.	Sangarun, Khadakwasla	18°23’45.49”N	73°41’8.01”E
2.	Aundh bridge, Aundh	18°34’2.61”N	73°48’36.42”E
3.	Adarwadi, Tamhini	18°27’14.71”N	73°26’0.76”E
4.	Dasave, Lavasa	18°23’12.18”N	73°30’8.15”E
5.	Khadaki, Pune	18°34’26.89”N	73°50’57.60”E

Table 1: Showing collection sites and their GPS coordinates

Preparation of karyotype

Fishes were segregated into two groups (large and small) based on their size. Different methodology was used for obtaining their chromosome spread.

a) Large size fish:

It is well established that colchicine arrest the cell division at metaphase. Therefore, fish specimens were treated with 0.01% Colchicine at 1 mL/100 g body weight. Individual fish was kept in an aerated tank for 90 min. After which fish was sacrificed to dissect out the gills tissues after a deep anesthesia using MS-222. The extracted tissues were then placed in a hypotonic solution of 0.9% sodium citrate for 1 hr. The tissues were chopped into smaller pieces and the hypotonic solution was changed after every 15 min using Pasteur pipettes. The swollen tissues were then fixed using Carnoy’s fixative for 45 min. The fixative was also changed using Pasteur pipette after 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 (Fig. 1). The temperature difference between suspension (chilled) and the pre-warmed slides leads to the 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 distilled water. The slides were again air dried and then screened for chromosomal spread under the light microscope.

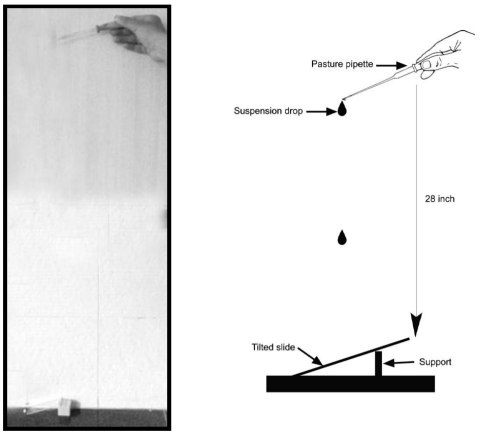

Fig. 1: *Photograph showing the methodology for obtaining chromosome spread. A Pasture pipette containing suspension placed exactly above the slide and drop is added onto the slide.*

b) Small sized fishes:

Small size individual fish was transferred to a beaker containing 200 ml of 0.05% Colchicine so as to pass colchicine through gill lamellae and reach internal body tissue. After 5 hr the fish was 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 60 min. The tissues were chopped into smaller pieces and the hypotonic solution was

changed every 15 min using centrifuge at 2000 rpm for 2 mins. The swollen tissues were then placed into Carnoy's fixative for 45 min. The fixative was changed using centrifuge at 2000 rpm for 2 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 3-4 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 (Fig. 1). The temperature helps to burst open 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 distilled water. The slides were again air dried and slides were photographed using compound microscope.

III. Results

In current study, total 18 different species were studied for their karyotypic analysis. These species were *Cirrhinus fulungee, Puntius sophore*, *Osteobrama vigorsii, Garra mullya*, *Devario aequipinnatus*, *Rasbora daniconius*, *Schistura denisoni, Acanthocobitis mooreh*, *Indoreonectes evezardi*, *Lepidocephalichthys thermalis*, *Chanda nama*, *Parambassis ranga*, *Channa gachua, Glossogobius giuris, Mystus cavasius, Ompok bimaculatus, Notopterus notopterus* and *Mastacembelus armatus.*

Out of 18 species studied, chromosomal spreads were only obtained for the 4 species. These 4 species were *P. sophore, R. daniconius, M. cavacius* and *O. bimaculatus.* Success rate for obtaining the chromosomal spread for *P. sophore, R. daniconius, M. cavacius* and *O. bimaculatus* was found to be 57.89 %, 88.88 %, 92 % and 72 % respectively (Table 2). Among the successful chromosomal spread we observed huge variation in terms of number of chromosomes. *R. daniconius* (Fig. 2A) showed the highest number of the chromosomes whereas *O. bimaculatus* showed the lowest number of the chromosomes. Number of diploid chromosome of *R. daniconius* was 2n=75 (Fig. 2B), whereas *O. bimaculatus* (Fig. 2G) showed 2n=48 (Fig. 2H). Similarly, *P. sophore* (Fig. 2C) and *M. cavasius* (Fig. 2E) showed 2n=50 (Fig. 2D) and 2n=58 (Fig. 2F) respectively.

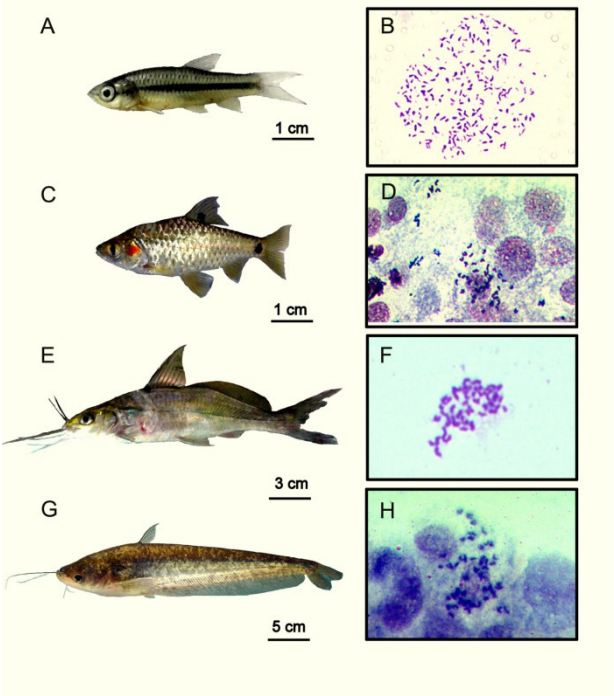

Fig. 2: *Showing the 4 different fish species with their chromosomal spreads*

(A) *Rasbora daniconius*; (B) Chrmosomal spread of *R. danicomius*; (C) *Punctius sophore*; (D) Chrmosomal spread of *P. sophore; (E)Mystus cavicius;* (F) Chrmosomal spread of *M. cavacius;* (G) *Ompak bimaculatus;* (H) Chrmosomal spread of *O. bimaculatus*

In *R. daniconius*, chromosomes in the karyotype had a homologous pair except chromosome 75 and 76 (Fig. 3A), which were arranged in decreasing size. The investigation of metaphases showed notable difference in size of chromosomes as well as remarkable difference between chromosomal type. In addition, the sex chromosomes could not be distinguished without banding techniques in this species. Chromosome number 75 and 76 could be sex chromosome since these are the two chromosomes that were found singly (Fig. 3A). Ideogram of *R. daniconius* showing characteristics of chromosomes based on centromere position. Ideogram showed that, the *R. daniconius* has 9 metacentric, 9 submetacentric and 9 acrocentric, 31 telocentric chromosomes (Fig. 3B; Table 3). Remaining 18 pairs of the chromosome remain unidentified from the current karyotype due to poor resolution if the image. Due to the clumps formed by the chromosomes of the *P. sophore, M. cavacius* and *O.bimaculatus* we could not able to segregate them based on size.

Sr. No.	Fish species studied	Total fish specimens collected from all the study sites	Total number of attempts for obtaining good chromosomal spread	Number of successful attempts	% success
1	*Cirrhinus fulungee*	15	12	0	0
2	*Puntius sophore*	40	38	22	57.89
3	*Osteobrama vigorsii*	12	5	0	0
4	*Garra mullya*	14	9	0	0
5	*Devario aequipinnatus*	10	6	0	0
6	*Rasobora daniconius*	16	9	8	88.88
7	*Schistura denisoni*	24	15	0	0
8	*Acanthocobitis mooreh*	26	20	0	0
9	*Indoreonectes evezardi*	21	16	0	0
10	*Lepidocephalichthys thermalis*	32	25	0	0
11	*Chanda nama*	9	6	0	0
12	*Parambassis ranga*	13	8	0	0
13	*Channa gachua*	6	3	0	0
14	*Glossogobius giuris*	5	3	0	0
15	*Mystus cavasius*	28	25	23	92.00
16	*Ompok bimaculatus*	18	16	12	75.00
17	*Notopterus notopterus*	4	2	0	0
18	*Mastacembelus armatus*	3	2	0	0

Table 2: *Showing the details of the total number of fish specimens collected, used, number of successful attempts in obtaining good chromosomal spread with its percent success*

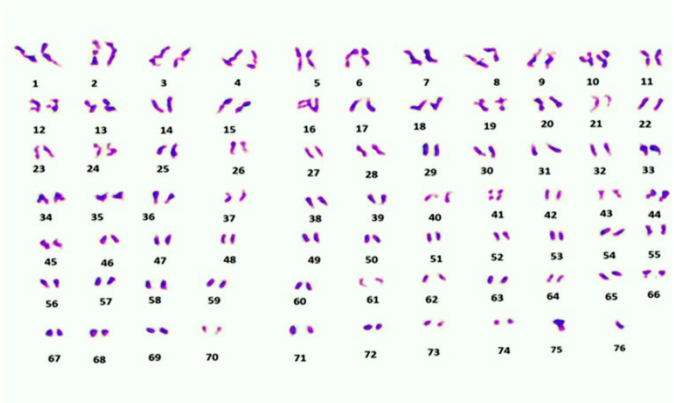

Fig. 3 A: *Sized based segregation of the chromosomes of the Rasbora daniconius*

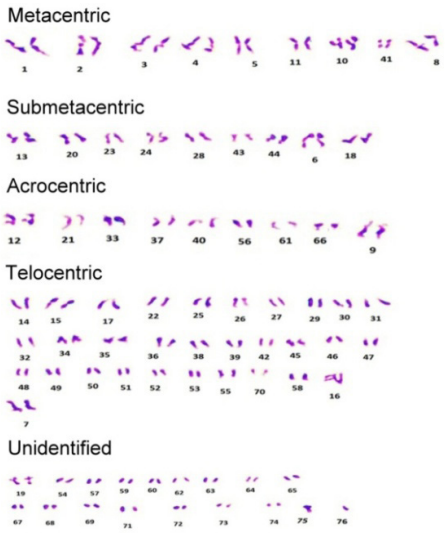

Fig. 3 B: *Ideogram of the Rasbora daniconius*

IV. Discussion

In the present study, karyological investigation of 18 different fish species from three different localities was carried out. Out of 18 different species studied, *Rasbora daniconius, Puntius sophore, Mystes cavasius, Ompok bimaculatus* showed relatively good chromosomal spreads. For other fish species we were unable to obtain good chromosomal spread. This could be due to the fact that, karyological study of fish presents technical difficulties, which are not encountered in the study of other vertebrates, and these difficulties are due to the small size and very high number of chromosomes [16]. Most of the fishes that were used in the current study were very small in size like *Indoreonectes evezardi, Schistura denisoni, Acanthocobitis mooreh* etc. and it was very difficult to treat them with colchicine. Moreover, several incomplete metaphases were encountered in the preparation, and these probably resulted from hypotonic overtreatment. It is the common problem encountered by other workers in the field of karyotyping [19]. A few studies have used fish standard karyotypes to examine taxonomic or systematic problems [20]. The major difficulty encountered is the morphological variation existing even between homologous chromosomes in the same nucleus [21-22].

Sr. No	Position of centromere	Chromosome pair numbers	Total no.
1	Metacentric	1, 2, 3, 5, 8, 10, 11, 28, 41	9
2	Submetacentric	4, 6, 13, 18, 20, 23, 24, 43, 44	9
3	Acrocentric	9, 12, 21, 33, 37 , 40, 56, 61, 66	9
4	Telocentric	7, 16, 14, 15, 17, 22, 25, 26, 27, 29,30, 31, 32 34, 35, 36, 38, 39, 42, 45, 46, 47, 48, 49, 50, 51, 52, 53, 55, 70, 58	31
5	unidentified	19, 54, 57, 59, 60, 62, 63, 64, 65, 67, 68, 69, 71, 72, 73, 74, 75, 76	18

***Table 3: Showing the ideogram of* R. daniconius**

Sometimes, it could happen that some chromosomes are more contracted than others, so chromosome measurements of very small chromosomes compared to those of man and mammals is difficult. Another problem is that fish karyotypes are not identical, as in human being or other animal species, so we cannot have a standard karyotype for fish. Moreover, not only their differences between species, but polymorphism often occur within the same fish species [22].

Difference in the diploid number of the chromosome in each species indicates that, each species is characterized by a specific chromosome complement. Presence of very high number of chromosomes (2n=75) in the *R. daniconius* could be used as a key character for its identification. The number of chromosomes per cell is rather conservation characteristic and may be used as an indicator of closeness of species, within families. The number and position of the arms of the chromosomes is even more conservative than chromosome number and is often equally useful in taxonomic studies. Fish which have several chromosome series (2n>50) are called polyploids. Presence of chromosomes (2n=75) in the *R. daniconius* indicates that it is polyploid fish. Other polyploidy fish species are *Cyprinus carpio* [23] and Barbus species from Southern Africa with 2n = 98 to 100 and 2n = 148 or 150 chromosomes respectively (Oellerman and Skelton, 1990) since they also have very high (2n > 50) number of chromosomes. The role of polyploidy in evaluation and survival of fish is very important because it provides from natural selection pressure [24]. In *Schizothorax nigar*, [25] also noticed the polyploidy. Other polyploidy fish species of cyprinids are *Tor putitora*, *Tor khudree* and *Tor tor* [26]. Therefore with respect to the number of *R. daniconius* chromosomes and their resistance to the environmental conditions, it seems to be polyploid fish.

Karyological investigation of species from genus *Punctius* was also carried out. According to [27], *P. chola* has 2n=50 with karyotype formula (KF) 2m + 4sm + 2st + 42t in. Similarly, *P. conchonius* has also 2n=50 and with KF as 14m + 28sm + 8st and 2n=50 & 14m + 24sm + 8st + 4t as KF in *P. ticto*. Our results are in harmony with the result of the other studies since *Puntius sophore* also showed 2n=50. Moreover, the diploid chromosome number of all three major carps, *Labeo rohita*, *Catla catla* and *Cirrihinus mrigala* was reported to be 50, indicating that majority of cyprinid species have 2n = 50 chromosome [22, 28]. Most of the members of Cyprinidae have 2n of chromosome ranges from 44 to 100. High diploid chromosome number 2n=98-100 are thought to have resulted by polyploidy of 2n=48 or 50. Chromosomal analysis in the present study as well as [28] on three Indian major carps reveled that, most of the cyprinids shared the same diploid number which is 2n=50. The results are also reinforced by the karyological study of *C. catla* and *L. rohita* done by [29-33]. Karyotype studies on *C. mrigla* have been performed by [30, 32]. All these studies have shown the diploid number as 50, confirming the present results. The most commonly

occurring diploid number in family Cyprinidae is 50 [35], considered to be the modal number of this species. Presence of same modal number in the present studies reinforces the hypothesis that in cyprinids, chromosome number is much conserved and represents plesiomorphic condition.

Fishes of the genus *Mystus* are small to medium sized stripe catfishes with 34 valid species [36]. Out of this 34 species of *Mystus* only five species were cytogenetically studied. It has been reported that the number of chromosomes varies between species in genus *Mystus*. Previous studies with present investigations suggest that diploid count of 2n=56±2 to be the modal number of the genus *Mystus* and it is in confirmation with the reports [37] of the Bagridae family. As per the karyotypic data already available on 5 species of the genus *Mystus* the diploid chromosome number ranges from 54-58 [38]. The diploid count (2n=58) of the present species shows similarity to that of *Mystus vittatus*, from West Bengal, India [39]. The apparent modal diploid number of 2n=56±2 in the genus *Mystus* is also similar to some other fishes of Bagridae family such as, *Hemibagrus wyckii* [40] previously described as *Mystus wyckii* with 2n=54 from Thailand, Nakhon Phanom Province [41], *Hemibagrus menoda* [40] previously described as *Mystus menoda* with 2n=56 from Cachar, India [42] and *Mystus corsula* with 2n=58 from West Bengal, India [43]. This finding suggests the close relationship between the two genera, *Mystus* and *Hemibagrus* of Bagridae family and also support the conservative nature of the karyotype macrostructure within the group, especially regarding the diploid chromosome number 2n=56, which the ancestors of all Siluriformes [44]. The number of chromosomes varies between species in genus *Mystus* at various geological regions of India [38]. In the current study, we did not found such evidences for the *M. cavasius* variation in the 2n number of the chromosomes. *M. cavasius* from four different regions of the India (Jammu, Bihar, Maharashtra and Orissa) showed exactly same number of the diploid chromosomes i.e. 2n=58 [38].

Ompok bimaculatus is commonly found in natural water bodies such as rivers and floodplains. *Ompok* is an important genus of this family that retains four freshwater fish species in India namely: *O. bimaculatus* (Indian butter catfish), widely distributed in India and other countries of Southeast Asia. About 12 species, belonging to family Siluridae, have cytogenetically been studied in past. Results of present investigation, showed that the diploid chromosome number of *O. bimaculatus* was 2*n*=48. This is in agreement with the earlier studies conducted in Thailand [45]. Interestingly, it differs from the report of [46] showing a 2n=40 for *O. bimaculatus* in India, which differs from studies [47] who showed that this same species in India had 2n=41 and 2n=42 in males and females, respectively. Various workers have reported different diploid number in *O. bimaculatus* (2n = 40, 41, 42). Many workers have reported 42 diploid numbers in *O. bimaculatus* [48, 49]. This may be due to inappropriate identification of the subject species. Further investigation with the use advanced molecular technique would aid in better understanding of the variation in diploid number of the chromosome in *O. bimaculatus*.

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