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Karyotype of a Bagrid catfish, *Mystus vittatus*, from the freshwater system of Chidambaram, Tamil Nadu, India

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ABSTRACT: Karyological characters of *Mystus vittatus* (Bagridae) in the freshwater system of Chidambaram were studied by examining metaphase chromosome spreads from the gill tissues. The examination of 149 metaphase spreads prepared from 25 fingerling specimens indicated that the chromosome number of this species was 2n=54 and the arm number was 12 for metacentric, 36 for submetacentric, and 30 for acrocentric type. The prepared karyotypes of this species consisted of 3 pairs of metacentric (m), 9 pairs of submetacentric (sm) and 15 pairs of acrocentric (a) chromosomes. The chromosome formula can be represented as 2n = 3m + 9sm + 15a. This karyotype is significantly different from same species reported by others. Karyological parameters showed that centrometric index, arm ratio, relative length, and length variation range of chromosome of this fish species are between 14.97–50.00, 1.00–5.68, 3.12–18.48, and 0.60–3.56, respectively. The largest chromosome in this species is a pair of submetacentric chromosomes. Considering the number of chromosomes, it seems likely that *M. vittatus*, is a diploid origin fish.

KEYWORDS: karyology, diploid chromosome, metaphase plates

INTRODUCTION

Studies on the chromosomes of fishes have not been successful or widespread as in other vertebrate groups. Fish karyotypes are generally characterized by a large number of small chromosomes, discouraging researchers from pursuing fish-karyotype analysis. Therefore karyological data on fish are available only for a small percentage (about 10%) of some 25 000 species taxonomically known so far.

The Bagridae family of fish is the richest and most important of the teleostei class and its members are distributed throughout the world[1]. In the Bagridae family, the fish *Mystus vittatus* (Smith, 1945) is economically important and distributed in the semitemporal freshwater system of south India[2]. Based upon the fish chromosome data, it seems that the chromosome number depends on the species in the Bagridae family, suggesting some major chromosome rearrangements which might have played a significant role during speciation and evolution of Bagridae[3]. The family of Bagridae have received special attention in Asia[4]; up to 40 species have been karyotyped so far. The number of chromosomes varies between species in genus, *Mystus*[5]. In *M. vittatus* the diploid chromosome number has been reported to be 2n = 58[6].

In this respect, the most important karyological studies of *Mystus* in India consist of *Mystus tengara*[5] and *Mystus gulio*[7]. Until now, there is no report about the freshwater species of *M. vittatus* in South India.

MATERIALS AND METHODS

Twenty five young fingerlings of *Mystus vittatus*, weight 1.8 ± 0.3 g and length 3.3 ± 0.4 cm, were collected from the freshwater system of Chidambaram, South India. The fish were transported live to the laboratory of the Department of Zoology, Annamalai University, and kept in a 100 l tank with well aerated chloride-free water at 25 ± 2 °C for acclimatization before experimentation.

The stock solution of colchicine was made by dissolving 50 mg colchicine in 100 ml of deionized distilled water. The colchicine was administered as an intramuscular injection of 0.1 ml at a dose of 0.1–0.5% of stock solution per gram of body weight. Then fish were left in a plastic trough at 25 ± 2 °C for 3–5 h before sacrificing. Then the fish were dissected out to isolate the gill tissue and placed in hypotonic solution (0.4% KCl) for 30 min. The swollen cells from hypotonic solution were fixed in 3:1 cooled Carnoy's

fluid (3 parts methanol and 1 part glacial acetic acid) for 20–30 min. Then, the tissues were minced with 2–5 drops of 50% fixative acetic acid in the well of a cavity slide for a minute to make a cell suspension.

The slides were prepared by letting a drop of the fixing solution containing the cell suspension fall onto the slide from heights of 30, 60, and 90 cm using a Pasteur pipette. The fixative was immediately burned off using the technique developed by Mellman[8]. The slides were stained in 4, 8, and 12% Giemsa Merk solution in distilled phosphate buffer (pH 6.8). They were assessed after 5–8 min exposure to determine optimum staining conditions. Slides were dipped into distilled water to wash off extra Giemsa solution and then were allowed to air dry at 25 °C for 1–2 h. Selected metaphases were photographed on a Nikon microscope (Model OPTIPHOT-2) using an oil immersion lens at 1000× magnification. In the course of the microscopic examinations, the chromosomes sets of 50 cells were counted and 10 of the best metaphases were used to measure the karyotype.

The average length of short and long arms and the centromeric index were calculated for each chromosome. The homologous chromosome pairs were classified according to increasing differences between the homologous chromosomes. The length recorded was converted into micrometers after the scale factor was calibrated with a stage micrometer. The chromosome pairs were classified following the method of Macgregor[9].

RESULTS AND DISCUSSION

In 149 metaphases from the cells of gill tissue of 25 fingerlings of *M. vittatus*, the frequency of diploid chromosome number was found to be 2n=54 which is valid over 85% of metaphase cells (Fig. 1). Giemsa stained metaphase spread of diploid set of 54 chromosomes is shown in Fig. 2. The karyotypic configuration comprises 3 pairs of metacentric, 9 pairs of submetacentric, and 15 pairs of acrocentric chromosomes. The number of chromosome arms was determined to be NF=39 and the chromosome formula can be expressed as 2n = 3m + 9 sm + 15a.

The morphological and numerical data are summarized in Table 1. According to this table, relative length, arm ratio, centromeric index, and length variation range of chromosomes are between 3.12–18.48, 1.00–5.68, 16.67–50.00, and 0.60–3.56, respectively. The largest chromosome is a pair of submetacentric chromosome. The morphometry ideogram for a chromosome of *M. vittatus* is represented in Fig. 3. The optimum colchicine concentration for *M. vittatus* was determined to be 0.3% per gram body weight of

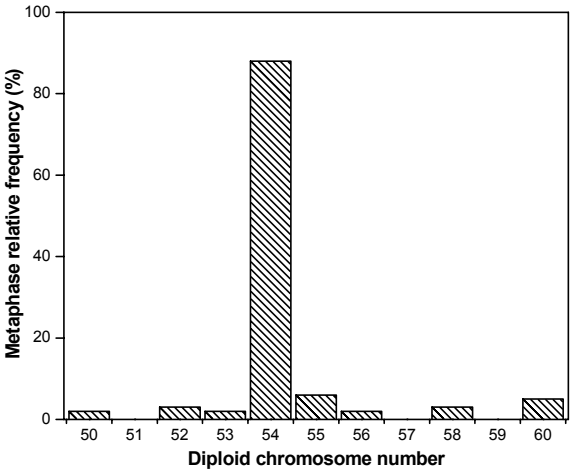

Fig. 1 Relative frequency of diploid chromosome number recorded in 149 metaphases of *M. vittatus*.

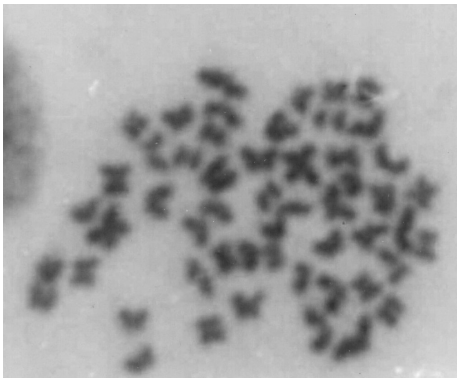

Fig. 2 Metaphase spread from gill tissue of *M. vittatus* from freshwater system of Chidambaram (1000×), 2n=54.

colchicine solution for 2 h. This concentration has effectively arrested dividing cells in metaphase.

Several techniques have been developed to examine the somatic chromosomes of adult fish. Experience has shown that somatic chromosomes prepared from colchicine treated tissue by air drying method provide better results for studying morphology and classification of chromosomes than those obtained from other techniques. Technical difficulties for karyological study in the fish are not encountered in the study of other vertebrates, which have much larger chromosomes[10].

The chromosomal study was conducted in several steps. The first step in the procedure was treatment of the cells with colchicine, which arrests cell division at the metaphase[11]. High concentration and long period of colchicine treatment have an effect on chromo-

Table 1 Morphometry of the karyotype of *M. vittatus* showing the mean values of measurements from the 10 best mitotic metaphases.

Pair No.	Length of chromosomes (μm)			Relative length (%)	Arm ratio (%)	Centromeric index (%)	Classification of chromosome[a]
	Long-arm [x-macron] ± SD	Short-arm [x-macron] ± SD	Total length [x-macron]				
1	1.83 ± 0.08	1.73 ± 0.08	3.56	18.48	1.08	48.60	SM
2	1.43 ± 0.07	1.43 ± 0.07	2.86	14.85	1.00	50.00	M
3	1.81 ± 0.06	0.60 ± 0.04	2.41	12.51	3.02	24.90	SM
4	1.30 ± 0.04	0.54 ± 0.03	1.84	9.55	2.41	29.35	SM
5	1.40 ± 0.05	0.42 ± 0.05	1.82	9.45	3.33	23.08	SM
6	1.41 ± 0.04	0.41 ± 0.02	1.82	9.45	1.00	22.53	A
7	1.35 ± 0.06	0.42 ± 0.06	1.77	9.19	3.21	23.72	A
8	1.42 ± 0.03	0.25 ± 0.05	1.67	8.67	5.68	14.97	A
9	1.25 ± 0.02	0.30 ± 0.03	1.55	8.04	4.17	19.35	A
10	1.21 ± 0.03	0.29 ± 0.01	1.49	7.74	4.17	19.46	A
11	1.15 ± 0.01	0.30 ± 0.04	1.45	7.53	3.83	20.70	SM
12	1.09 ± 0.04	0.32 ± 0.07	1.42	7.37	3.44	22.53	SM
13	0.61 ± 0.05	0.30 ± 0.09	1.39	7.22	3.63	21.58	SM
14	0.95 ± 0.09	0.61 ± 0.05	1.22	5.33	1.00	35.24	M
15	0.95 ± 0.06	0.25 ± 0.04	1.20	6.23	3.80	20.83	A
16	0.56 ± 0.09	0.56 ± 0.02	1.13	5.87	1.00	49.56	M
17	0.90 ± 0.04	0.20 ± 0.09	1.10	5.71	4.50	18.18	A
18	0.79 ± 0.05	0.30 ± 0.04	1.09	5.66	2.39	27.52	A
19	0.65 ± 0.06	0.43 ± 0.06	1.08	5.61	1.97	39.81	A
20	0.71 ± 0.02	0.33 ± 0.03	1.04	5.40	2.15	31.73	A
21	0.70 ± 0.03	0.25 ± 0.08	0.95	4.93	2.80	26.32	A
22	0.60 ± 0.05	0.31 ± 0.07	0.91	4.72	1.94	34.07	A
23	0.62 ± 0.04	0.23 ± 0.06	0.85	4.41	2.70	27.06	A
24	0.64 ± 0.03	0.20 ± 0.04	0.84	4.36	3.20	23.81	SM
25	0.54 ± 0.08	0.14 ± 0.06	0.73	3.79	3.86	19.18	SM
26	0.45 ± 0.02	0.18 ± 0.05	0.63	3.27	2.50	28.57	A
27	0.50 ± 0.02	0.10 ± 0.01	0.60	3.12	5.00	16.67	A

[a] M = metacentric; SM = submetacentric; A = acrocentric

somes, causing them to aggregate and shrink which leads to difficulties in classification. The present study suggests that a colchicine concentration of 0.3% per gram body weight for 2 h of treatment in this species can effectively arrest dividing cells in metaphase in the gill tissue. In slide preparation, sometimes several incomplete metaphases were encountered and these probably resulted from hypotonic over-treatment[12].

Bagridae show a great diversity in the organization of the number and shape of the chromosome in each nucleus. For example, Bagridae species of *Coreobagris brevicorpus* has only 2n=44 chromosomes[13], while *Mystus macropterus* has 2n=60 chromosomes[3]. The number of chromosomes varies between species in genus *Mystus* at various geological regions of India (Table 2). However in the present work, the karyotype of the representative species of the Bagridae of *M. vittatus* has a diploid number of 2n=54. Furthermore, the karyotypic configuration comprised 3 pairs of metacentric, 9 pairs of submetacentric, and 15 pairs of acrocentric chromosomes (Table 1). This cytological count for *M. vittatus* from freshwater system of Chidambaram shows that this species has more acrocentric chromosomes than in the same species studied at various geographical regions reported earlier[6].

Most authors classify the uni-armed bi-armed chromosomes according to the guidelines of Macgregor[9], where differences in the number of chromosomal arms were seen. This was usually the result of differences in the scoring of telocentric or acrocentric chromosome in different species of *Mystus* from India[5,15]. In the present study, we also find differences in the counting of chromosomal arms. A few studies have used fish standard karyotypes to examine taxonomic or systematics of fish species[16]. The major difficulty encountered is the morphological variation existing even between homologous chromosomes in

Table 2 Chromosome complements of Bagridae fishes in different regions of India.

Species	Region	2n	an	m	sm	t	st	a	Reference
M. cavasius	Berhampur Orisa	58	98	18	22	18	-	-	Tripathy and Das [14]
M. gulio	Kalyani Maharashtra	58	120	30	12	14	2	-	Das and Khuda-Bukhsh [7]
M. vittatus	Culcutta West Bengal	58	104	16	10	12	20	-	Manna and Prasad [6]
M. vittatus	Chidambaram Tamil Nadu	54	78	6	18	-	-	30	Present study

an = arm number; m = metacentric; sm = submetacentric; t = telocentric; st = subtelocentric; a = acrocentric

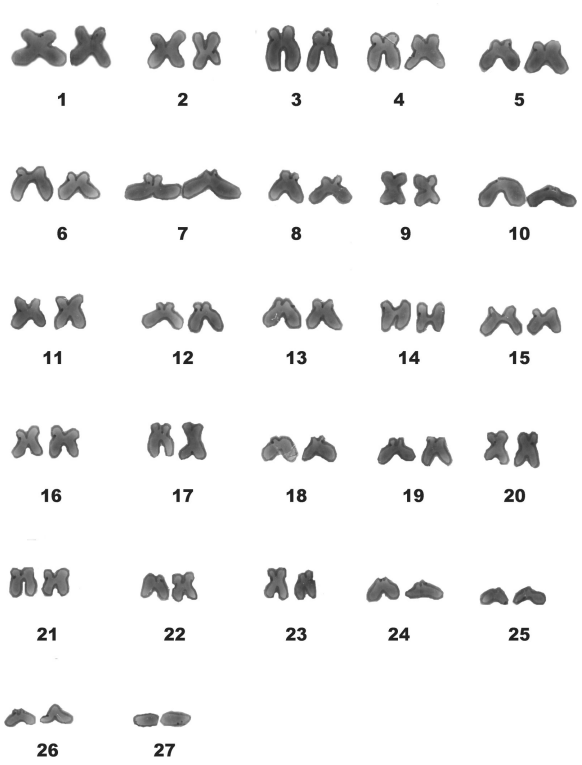

Fig. 3 Ideogram showing karyotypic arrangement (2n=54) of *M. vittatus*, according to morphometric measurement.

the same nucleus [17]. Another problem is that fish karyotypes are not identical, as in humans or other animal species, so we do not have a standard karyotype for fish because not only are there differences between species, but polymorphism often occurs within the same species [17].

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