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Karyotype Analysis in *Schizothorax zarudnyi* from Hamoon Lake, Iran

Mohammad Reza Kalbassi[1,*], Seyed Vali Hosseini[1], Reza Tahergorabi[2]
[1] Tarbiat Modares University, Faculty of Marine Sciences, P.O.Box: 46414-356. Nour, Iran.
[2] Islamic Azad University, Department of Fishery, Tehran-North Branch, P.O. Box: 19737-33583, Iran.

* Corresponding Author: Tel.: +98.122 6253101; Fax: +98.122 6253499;
E-mail: kalbassi_m@modares.ac.ir

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Abstract

The karyotype and chromosomal characteristics of (anjak, hamoon mahi, shir mahi) *Schizothorax zarudnyi* (Nikolskii, 1897) in Hamoon Lake of Iran were investigated. The examination of 85 metaphases spread prepared from 20 fingerling specimens indicated that the chromosome numbers of this species was found 2n=96 and the arm number was determined as NF=142. The prepared karyotype of this species was consisted of 9 pairs of metacentric (M), 14 pairs of submetacentric (SM) and 25 pairs of subtelocentric (ST) chromosomes. The chromosome formula can be stated as 2n = 9m + 14sm + 25st.

Karyological parameters shown that centromeric index, arm ratio, relative length and so length variation range of chromosomes of this fish are between 25.00 – 49.24, 1.03 – 3.55, 0.88 – 4.13 and 14.57 – 67.70 respectively and total length of chromosome is 1639.16 µm. The largest chromosome in this species is a pair of the submetacentric chromosome. The sex chromosomes were cytologically indistinguishable. With respect to the number of *S. zarudnyi* chromosomes and resistance of this fish to the environmental conditions, it seems to be tetraploid origin fish. This study may provide the first knowledge on chromosome analysis in *S. zarudnyi* and add basic useful information for its chromosomal manipulations.

Key words: Karyotype, Chromosome, *Schizothorax zarudnyi,* Hamoon lake-Iran.

Introduction

The family Cyprinidae is the richest and most important family of fish, and its members are distributed throughout the world (Al-Sabti, 1991). The vast majority of boned fish belongs to this family in Iran, and these are distributed widely in freshwater resources (Abdoli, 1999). Although this family is represented by approximately 1500 species worldwide (Gül *et al*., 2004), there are only 38 Geni and over 80 species in Iran (Firouz, 2000).

Systematically, *S. zarudnyi* belongs to teleostei class, cypriniformes order, cyprinidae family and schizothorax genus (Mostajeer and Vossoughi, 1994). The world distribution of *S. zarudnyi* is in semi-temporal freshwater of western Asia (Bianco and Banarescu, 1982). This fish is endemic in Iran and found in Sistan region mainly (Abdoli, 1999).

Since 1960s, karyological studies in teleost fish have made noteworthy contributions to increasing knowledge in the fields of genetics, taxonomy and environmental toxicology (Cucchi and Baruffaldi, 1990). The progress in increasing such knowledge has been closely related to the evolution of application methodologies (Rivlin *et al*., 1985). 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 (Gold *et al*., 1990).

The study of fish chromosome has become an active area of research in recent years (Thorgaard and Disney, 1983). Chromosomal analysis is important for fish breeding from the viewpoint of genetic control, the rapid production of inbred lines, cytotaxonomy and evolutionary studies (Kirpichnikov, 1981). Karyological studies have provided basic information on the number, size and morphology of chromosomes (Tan *et al*., 2004) that is important to undertake chromosome manipulations in fish (Khan *et al*., 2000). In addition to karyological studies, it may be useful for addressing a variety of evolutionary and genetic question about animals (McGregor, 1993) and may permit detection of changes that modified an ancestral karyotype as it evolved into new lines (Winkler *et al*., 2004). Genetic divergences of populations and their local adaptation are a potential resource for breeding programs in aquaculture and for fishery management (Philips and Rab, 2001). Cytogenetic studies in fish have not been comprehensive when compared to other vertebrate groups in Iran. In this respect, the most important karyological studies of Cyprinids fish in Iran consist of *Rutilus frisii kutum* **(**Nowruzfashkhami and Khosroshahi, 1995), *Abramis brama* (Nahavandi *et al*., 2001), *Ctenopharyngodon idella* (Nowruzfashkhami *et al*., 2002) and *Hypophthalmichthys molitrix* (Varasteh *et al*., 2002).

As this species is a good candidate for other genetic investigations such as hybridization and chromosomal manipulation and so due to lack of their chromosomal data, this first report could provide the detailed information on the chromosome number and karyotype of this species in the world.

Materials and Methods

Twenty *S. zarudnyi,* weight 10±1 g, were caught in Hamoon Lake (Lat 31º02' to 30º54', Long 30º54' to 31º02') in East of Iran. The fish were transported live to the laboratory, and kept in a well-aerated aquarium at 20-24ºC before analysis.

Mitotic Inhibitors

The stock solution of colchicine was made by dissolving 10 mg colchicine and 100 mg NaCl in 20 ml distilled water. The colchicine was administrated at dose of 25 and 50 µg/g body weight (BW) and slowly injected into the intraperitoneal muscle. Then, fish were left in aquaria at 20-24°C for 5-10 hours before sacrificing. And then the fish were killed and their anterior kidneys removed, suspensioned and placed in hypotonic treatment (0.075M KCl and 1% Sodium citrate solution) at two different temperature, 4ºC and 25°C. Lasting time for hypotonisation treatment was 45-60 min (with interval of 5 min).

Fixation

The swollen cell suspensions were fixed in 3:1 cooled Carnoy's fluid (3 parts methanol and 1 part glacial acetic acid) for 30 min, then the old fixative was replaced with the fresh Carnoy's. Lasting time for fixation treatment was 60 min.

Spreading

The slides, previously washed in alcohol and ether and kept at -1°C, were prepared by letting two drops of the fixing solution containing the cell suspension fall onto the slide in different heights (60, 90 and 120 cm). Immediately thereafter the fixative was burned off, using the technique developed by Mellman (1965), for obtaining better cell spread. The slides were stained in series of concentrations of Gimsa Merck solution in distilled water (5, 10 and 15%) and buffered by phosphate (40 mol Na_2HPO_4 and 26.6 mol K H_2PO_4) at pH 6.8 and were assessed at 7, 8, 9 and 10 min exposure times to determine optimum staining conditions. Slides were dipped into distilled water to wash off extra Gimsa solution and then were allowed to air dry at 25ºC for 2–3 h.

Chromosome Examinations and Morphometric Measurements

Mitotic metaphases were examined under a microscope (Leica SER. NO. 990398, Equipped with a green filter and digital camera) with an oil immersion lens at 1000 magnification. The chromosomes at the metaphase were photographed with a digital camera (Sony SSC-DC 58 AP) onto Kodak colour films (ASA 25). In the course of the microscopic examinations, the chromosome sets of 80 cells were counted and 10 of the best metaphases were used to measure karyotypes. The morphometric measurements of chromosome pictures were conducted with photographic software Photoshop 6.0 (Adobe Systems). Each chromosome was tagged with a reference number, and then the chromosome was divided into four arms at the centromere. The length of each arm was measured, and the measurements were standardized by using a micrometer scale. The data were transferred to the Excel 2000 (Microsoft) for analysis (Tan *et al*., 2004)

Chromosome Pairing

For each chromosome, the average lengths of the short and long arms, and the centromeric index (CI, expressed as the ratio of the short arm length to the total length of chromosomes), and the arm ratio (the ratio of the long arm length to the short arm length) were calculated. The relative length of each pair was expressed by the percentage of the absolute length of each chromosome pair divided by the sum of the absolute length of total chromosomes (i.e., 48). The Excel application paired up all the chromosomes using criteria of maximum resemblance based on the total length and the centromere position (Tan *et al*., 2004). The homologous chromosome pairs were classified according to increasing differences between the homologous chromosomes. The total length of chromosome was computed by summing up the average chromatid lengths of each diploid complement. The length recorded in pixels by the Colour Image Analysis System Video Pro 32 (Leading Edge) was converted into micrometers after the scale factor was calibrated with a stage micrometer.

The chromosome pairs were classified following the recommendations of Macgregor (1993) into metacentric (M), submetacentric (SM), subtelocentric (ST) and telocentric(T), with CI ranges of 46-49, 26-45, 15-26 and less than 15, respectively. The pair numbers were definitely attributed following this classification and the decreasing length order within each class. Finally, the karyotype was constructed by first dividing the chromosome pairs into classes on the basis of centromere position, and then arranging the homologous pairs in the decreasing length order within each group.

Results

Relatively small and high numbers of chromosomes were observed in *S. zarudnyi*. The scattering of the diploid chromosome number values are shown in Figure 1. The counts of chromosome ranged from 95 to 101 per metaphases with a mode at 96, representing 92.94% of the metaphases. In 85 metaphases from the anterior kidney cells of 20 *S. zarudnyi* specimens, the diploid chromosome number was 2n = 96 (Figure 2), which is valid over 90% of

metaphases cells. Cells lacking a normal number of chromosome values (2n = 95-101) were probably caused by losses during preparation or additions from nearby cells. All chromosomes in the karyotype have a homologous pair. Homologous pairs of chromosomes were arranged in decreasing size and centromeric indexes. The investigation of metaphases showed notable difference in size and type of chromosomes in *S. zarudnyi*. In addition, the sex chromosomes could not be distinguished in this species.

The representative karyotype for *S. zarudnyi* is shown in Figure 3. The karyotype of *S. zarudnyi* has 9 pairs metacentric, 14 pairs submetacentric and 25 pairs of acro-telocentric chromosomes. The number of chromosome arms was determined NF = 142 and chromosome formula can be expressed as 2n = 9 m + 14 sm + 25 a-t. The morphological and numerical data are summarized in Table 1. Other data are represented in Table 2. According to this table, centromeric index, arm ratio, relative length and length variation range of chromosomes are between 25.00-49.24, 1.03-3.55,

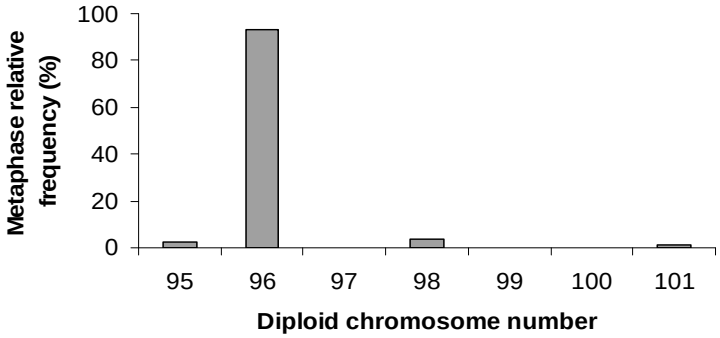

Figure 1. Distribution of chromosome number recorded in 85 diploid metaphases of *S. zarudnyi.*

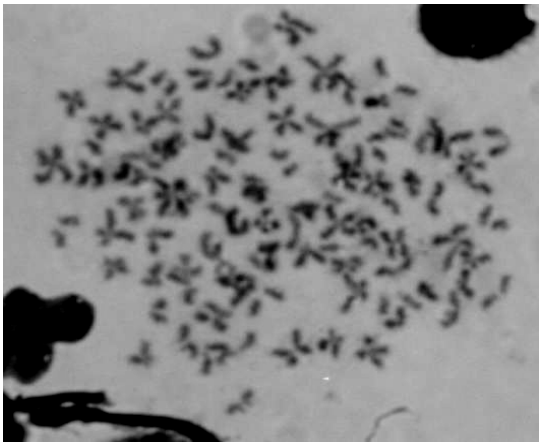

Figure 2. Metaphase spread from kidney tissue of *S. zarudnyi* from Hamoon Lake × 1000, 2n=96.

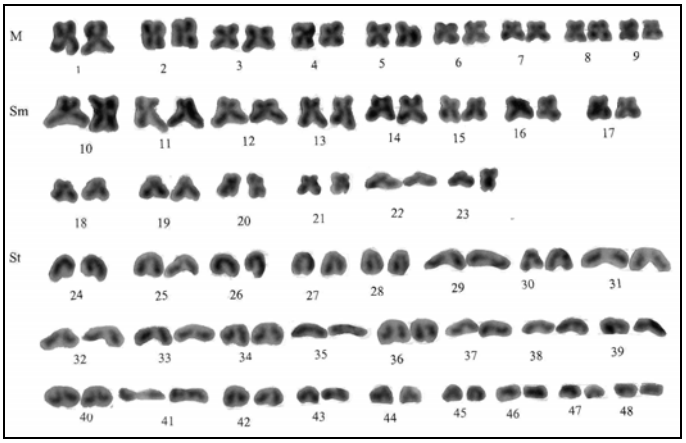

Figure 3. Karyotype of *S. zarudnyi* from Hamon Lake, 2n = 96.

Table 1. The morphological and numerical data are summarized in *S. zarudnyi*

Total length of haploid chromosome (µm)	Total length long arm (µm)	Total length short arm (µm)	2n	NF*
1638/58	1226/27	412/31	96	142

***** Arm number

Table 2. Numeral characteristics of the karyotype of *S. zarudnyi* showing the mean values of measurements from ten best mitotic metaphases

Chromosome pair no.	Total length (µm)	Long arm (µm)	Short arm (µm)	Relative length (%)	Centromeric index	Arm ratio	Classification
1	61/26	31/83	29/43	3/73	48/02	1/08	Metacentric
2	52/76	28/86	23/90	3/21	45/29	1/28	Metacentric
3	46/69	24/76	21/93	2/84	46/94	1/13	Metacentric
4	43/34	23/32	20/02	2/64	46/19	1/16	Metacentric
5	43/01	22/92	20/09	2/62	46/95	1/13	Metacentric
6	41/16	20/93	20/23	2/51	49/24	1/03	Metacentric
7	41/02	22/00	19/02	2/50	46/48	1/15	Metacentric
8	40/95	22/00	18/95	5/49	46/40	1/16	Metacentric
9	36/95	19/85	17/10	2/25	46/27	1/16	Metacentric
10	67/70	38/20	29/50	4/13	46/48	1/34	Submetacentric
11	57/16	34/24	22/92	3/48	40/01	1/49	Submetacentric
12	55/75	37/50	18/25	3/4	32/73	2/05	Submetacentric
13	53/48	32/26	21/22	3/26	39/68	1/52	Submetacentric
14	52/77	29/43	23/34	3/21	44/26	1/26	Submetacentric
15	41/73	23/34	18/39	2/54	43/83	1/03	Submetacentric
16	37/91	25/18	12/73	2/31	33/48	1/99	Submetacentric
17	37/37	26/88	10/49	2/27	28/68	3/55	Submetacentric
18	37/35	22/07	15/28	2/27	41/70	1/47	Submetacentric
19	37/18	26/99	10/19	2/26	27/43	2/64	Submetacentric
20	36/22	20/31	15/91	2/20	43/26	1/50	Submetacentric
21	35/64	26/17	9/47	2/17	26/56	2/49	Submetacentric
22	28/20	21/15	7/05	1/72	25/00	3/00	Submetacentric
23	27/20	20/30	6/90	1/65	25/36	2/94	Submetacentric
24	33/95	33/95	0/00	2/07	0/00	∞	Subtelocentric
25	32/00	32/00	0/00	1/95	0/00	∞	Subtelocentric
26	30/24	30/24	0/00	1/85	0/00	∞	Subtelocentric
27	28/69	28/69	0/00	1/75	0/00	∞	Subtelocentric
28	28/58	28/58	0/00	1/74	0/00	∞	Subtelocentric
29	28/30	28/30	0/00	1/72	0/00	∞	Subtelocentric
30	28/02	28/02	0/00	1/70	0/00	∞	Subtelocentric
31	28/01	28/01	0/00	1/70	0/00	∞	Subtelocentric
32	27/16	27/16	0/00	1/66	0/00	∞	Subtelocentric
33	26/60	26/60	0/00	1/62	0/00	∞	Subtelocentric
34	25/53	25/53	0/00	1/55	0/00	∞	Subtelocentric
35	25/48	25/48	0/00	1/55	0/00	∞	Subtelocentric
36	25/25	25/25	0/00	1/54	0/00	∞	Subtelocentric
37	24/81	24/81	0/00	1/51	0/00	∞	Subtelocentric
38	24/76	24/76	0/00	1/51	0/00	∞	Subtelocentric
39	24/37	24/37	0/00	1/48	0/00	∞	Subtelocentric
40	24/05	24/05	0/00	1/46	0/00	∞	Subtelocentric
41	23/75	23/75	0/00	1/44	0/00	∞	Subtelocentric
42	23/91	23/91	0/00	1/45	0/00	∞	Subtelocentric
43	22/64	22/64	0/00	1/38	0/00	∞	Subtelocentric
44	21/93	21/93	0/00	1/33	0/00	∞	Subtelocentric
45	21/01	21/01	0/00	1/28	0/00	∞	Subtelocentric
46	16/29	16/29	0/00	0/99	0/00	∞	Subtelocentric
47	15/70	15/70	0/00	0/99	0/00	∞	Subtelocentric
48	14/57	14/57	0/00	0/88	0/00	∞	Subtelocentric

0.88–4.13 and 14.57–67.70, respectively. Total length of chromosome was 1639.16 µm. The largest chromosome is a pair of submetacentric chromosome. The idiogram of the *S. zarudnyi* was made on the basis of the karyotype (Figure 4).

In this study, the optimum colchicine concentration for *S. zarudnyi* was determined to be 50 µg/g BW of colchicine solution for five hours. This concentration has effectively arrested dividing cells in metaphase. In addition, the best chromosomal spread quality (well-spread metaphase) was obtained from treatment of cells with 1% Sodium citrate solution at

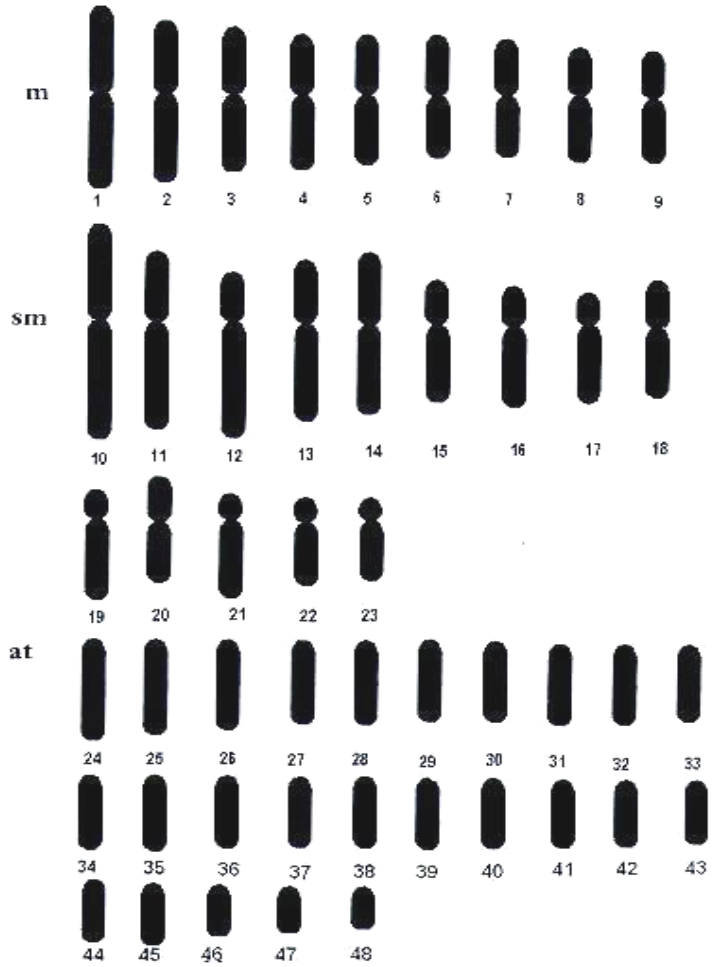

Figure 4. Idiogram of *S.zarudnyi* from Hamon lake, n = 48.

4°C for 45 min and height of dropping in 120 cm. The other hypotonic solution tested, 0.075 M KCl, did not result in many scorable metaphases.

Discussion

Rapidly growing tissues are required to obtain a large number of chromosome spreads in metaphase for karyotypical studies (Tan *et al*., 2004). Karyological study of teleost fish presents technical difficulties which are not encountered in the study of other vertebrates, and these difficulties are due to the small size and high number of chromosomes (Cucchi and Baruffaldi, 1990). Different techniques are presently being used to perform such studies: direct, *in vivo*; and indirect, *in vitro*. With those forms employing direct techniques, the preparation of slides for optical microscopy is quite easy. Furthermore, these techniques are rather inexpensive and results are obtained relatively quickly. Such techniques are based on the use of colchicine to block quickly-proliferating cell populations at the metaphase.

The main difficulty in working with fish chromosomes is in obtaining high quality metaphase spreads. A few studies have used fish standard karyotypes to examine taxonomic or systematic problems (Bolla, 1987). The major difficulty encountered is the morphological variation existing even between homologous chromosomes in the same nucleus (Al-Sabti, 1991; Levan *et al*., 1964). Sometimes, it could happen that some chromosomes are more contracted than others, so chromosome measurements are very small chromosomes compared to those of man and mammals. 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 because not only are there differences between species, but polymorphism often occurs within the same fish species (Al-Sabti, 1991).

Several incomplete metaphases were encountered in the preparation, and these probably resulted from hypotonic overtreatment (Nanda *et al*., 1995). The majority of authors classify uni-armed and bi-armed chromosomes according to the guidelines of Macgregor (1993). Where there are differences in the number of chromosome arms, this is usually the result of a difference in the scoring of subtelocentric chromosomes by different authors (Philips and Rab, 2001).

The majority of cyprinid species have 2n = 50 chromosome (Al-Sabti, 1985), while *Cyprinus carpio* has 2n = 98-100 (Demirok and Ünlü, 2001) and

polyploidy Barbus species from Southern Africa has 2n = 148 or 150 chromosome (Oellerman and Skelton, 1990), and fish which have several chromosome series (2n > 50) are called polyploids. The role of polyploidy in evaluation and survival of fish is very important because it provides from natural selection pressure (Oellerman and Skelton, 1990). Khuda and Nayak (1982) noticed that *S. nigar* caught in India is a polyploid fish. In other species of cyprinids such as *Tor putitora*, *Tor khudree* and *Tor tor*, polyploidy were reported too (Khuda, 1982). So with respect to the number of *S. zarudnyi* chromosomes and their resistance to the environmental conditions, it seems to be polyploidy fish.

Until now, karyotype of some members of Schizothorax genus were determined such as *S. richardsonii* (2n = 98, NF = 154, 2n = 16 m + 40 t + 42 a) and *S. kumaonensis* (2n = 98, NF = 126, 2n = 18 m + 10 t + 70 a) (Lakara *et al*., 1997); but there is not any report about *S. zarudnyi* and our study is the first report of the karyotype of *S. zarudnyi.*

The karyotype analysis is a key step towards the stock improvement by polyploidy manipulation, hybridization and related genetic engineering (Tan *et al*., 2004). Therefore, like for to other animals, comprehensive genetic researches will be needed for this fish. This paper is the first to provide the detailed information on the chromosome number and karyotype of *S. zarudnyi*.

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