First record of karyotype analysis in Anjak, *Schizocypris altidorsalis* (Bianco and Banarescu, 1982) from Hamoun Lake, Iran

Hedari Salkhordeh M. R.[1]; Gharaei A.*[1,2]; Mirdar Harijani J.[1]

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Abstract

The chromosomal spread and karyotype of Anjak (*Schizocypris altidorsalis*) from Hamoun Lake were determined using tissue squashing techniques with an injection of 1 mL/100 g body weight of 0.01% colchicines solution. Kidney and gill epithelia tissues were removed and used for karyotype analysis. The analysis of 145 chromosome spreads revealed the diploid chromosome number of this fish, 2n=48 and a fundamental arm number (FN) =88. The diploid complements comprised 12 metacentric pairs, 8 submetacentric pairs, 1 subtelocentric pair and 3 telocentric pairs (12m+8Sm+1St+3t). Total length of the haploid complement equaled 44μm with a range in the length of the shortest and longest chromosome between 0.76-2.78μm. The arm ratio and the centromeric index ranged between 1.00-∞ and 0-50 respectively. This is the first report on the chromosome number and karyotype of *S. altidorsalis* from the Hamoun Lake in Iran.

Keywords: *Schizocypris altidorsalis,* Chromosome, Karyotype, Hamoun Lake, Iran.

1- Department of Fisheries, Faculty of Natural Resources, University of Zabol, Iran.
2- Department of Fisheries, Hamoun International Wetland Research Institute, University of Zabol, Iran.
* Corresponding author's Email: agharaei551@gmail.com

Introduction

As an endemic species at the Sistan region of Iran, *Schizocypris altidorsalis* Bianco and Banarescu, 1982 (Cyprinidae: Schizothoracinae) resides only in the Hamoun Lake, Helmand River and its tributaries and presumably adjacent Afghanistan (Coad, 2013).

The Sistan area is located in the southeastern of Iran. This inland basin is fed by Helmand River and other smaller rivers originating in the central highlands of Afghanistan. The basin includes a complex and unique wetlands named Hamouns which are, from an environmental perspective, the most important parts of the Sistan region (Vekerdy *et al.*, 2006). The south ends of Hamoun-e-Puzak and the contiguous Hamoun-e-Sabari (or Lake Hamoun) are recorded in Ramsar Sites (World Conservation Monitoring Centre, 1990). In the Ramsar Site, Hamoun Lake listed in the threatened of National Parks (Anonymous, 1988).

The Hamoun Wetland provides a habitat for diverse and globally significant fauna and flora such as snow trouts including Schizothoracinae fish. The genus *Schizocypris* of medium-sized snow trouts contains only 2 species that found in Pakistan, Afghanistan and Iran. The *S. altidorsalis* is a benthopelagic species that has been reported from pools in dry river beds and still, reedy channels. The fish enter the Hamoun Lake from the upstream parts of the rivers, and return to more permanent rivers when water levels fall (Coad, 2013).

Basic information on the number, size and morphology of chromosome could be obtained by karyotype studies (Khan *et al.*, 2000). Chromosome analysis is a valuable tool for systematic and evaluation, biodiversity conservation, stock assessment and aquaculture e.g. interspecific hybridization and polyploidy studies (Dorafshan and Kalbassi, 2006; Pisano *et al.*, 2007). Despite the importance of fish cytogenetic, when available data sets on fish karyotype are analysed, it is clear that they are still very incomplete, only 10-15% of all taxonomically known species were karyotyped (Gromicho and Collares-Pereira, 2007). The most important karyological studies have been conducted on several Cyprinids spieces in Iran such as *Rutilus frisii kutum* (Nowruzfashkhami and Khosroshahi, 1995), *Abramis brama* (Nahavandi *et al.*, 2001), *Schizothorax zarudnyi* (Hosseini and Kalbassi, 2002), *Hypophthalmichthys molitrix* (Varasteh *et al.*, 2002), *Barbus capito* and *Copoeta copoeta gracilis* (Pourali Darestani *et al.*, 2006). The aim of this study was to investigate the karyotype of *S. altidorsalis* for basic information on evaluation, conservation and/or aquaculture purposes. To best of our knowledge, this paper is the first report to provide detailed information on the chromosome number and karyotype of *S. altidorsalis* from Sistan Basin.

Materials and methods

Live fish were obtained (8 females and 11 males) from local fishermen in the rivers around Zabol and live transported to the Fisheries Laboratory in University of Zabol, and maintained in a well-aerated aquarium at 20-24°C before analysis. Each specimen was injected intraperitoneally with 0.01% colchicine (1 mL/100 g fish weight) for stopping dividing cells at metaphase. The fish were maintained in a well aerated aquarium and after 4 h they were sacrificed. The gill filaments and cephalic kidneys of these specimens were then removed and placed in hypotonic treatment (0.075 M KCl) for 45 minutes at room temperature (25°C) and chopped by scalpel. After 45 min in the hypotonic solution, the cellular suspension was centrifuged at 1,000 rpm for 10 minutes. The swollen cell suspensions were fixed in a fresh and cold Carnoy solution (3: 1 methanol /glacial acetic acid) and with three changes of fixative at 15 min intervals. The suspension was ready for slide preparation. At first, the slides were washed with alcohol, and then 3-4 drops of the suspension fell onto the microscope slides at 50-60 cm height by Pasteur pipette. The chromosome slides were stained with 10% Giemsa in phosphate buffer of pH 6.8 for 10 min and gently washed with distilled water, and air dried. Mitotic metaphases were observed under a microscope (Olympus, Tokyo, Japan) with an oil immersion lens at 1000 magnifications. The chromosomes at the meta phase stage of somatic cells were photographed with a digital camera. The length of each arm was measured, centromeric index was calculated by dividing the length of the shorter of two chromosome arm by total length of chromosome and expressing it as percentage and the arm ratio was calculated by dividing the length of larger arm of chromosome by the length of its shorter arm, and data were transferred to the Excel 2007 (Microsoft) for analysis (Tan *et al.*, 2004).

Results

One hundred and forty five metaphase plates from 19 individuals of *S. altidorsalis* were available for the karyotype characteristics. The counts of chromosome ranged from 46 to 50 per metaphases with a mode at 48, representing 67.58% of the metaphases (Fig. 1). In 98 metaphases from the anterior kidney cells of this specimen, the diploid chromosome number was 2n=48 (Fig. 2). The karyotype consisted of 12 pairs of metacentric (m), 8 pairs of submetacentric (Sm), 1 pair of subtelocentric (St) and 3 pairs of telocentric chromosome. The karyotype formula considers as 2n=l2m+8sm+1st+3t (Fig. 3). The number of fundamental chromosome arms was determined as NF=88. Total length of the haploid complement equaled 44µm with a range in the length of the shortest and longest chromosome between 0.76 - 2.78µm.

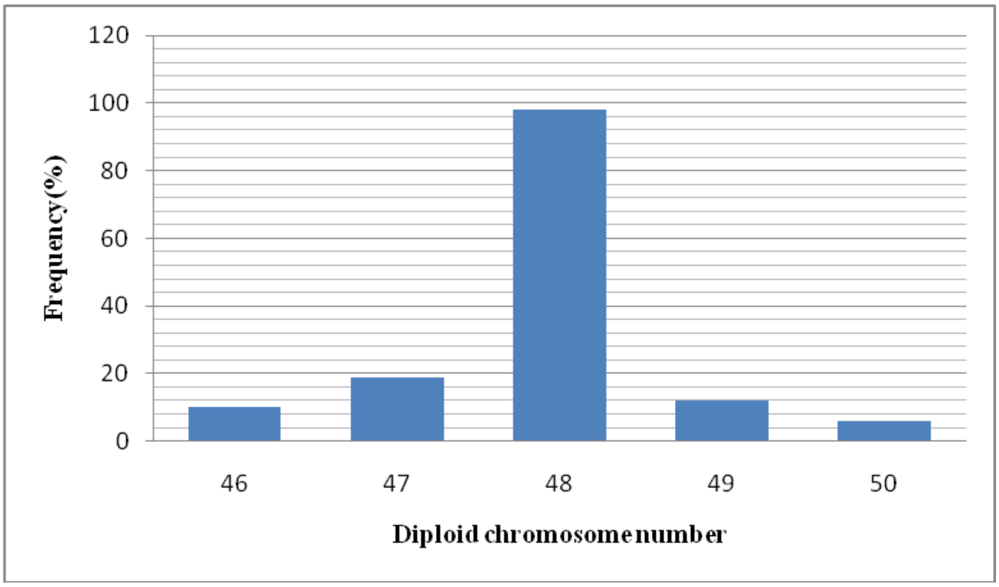

Figure 1: Frequency of diploid chromosome number recorded in 145 metaphases of *Schizocypris altidorsalis* from Hamoun Lake, Iran. Analysis of 145 metaphase plates shows the frequency of diploid chromosome number ranging from 46 to 50 with a modal diploid number 2n=48 which is valid over 67.58% .

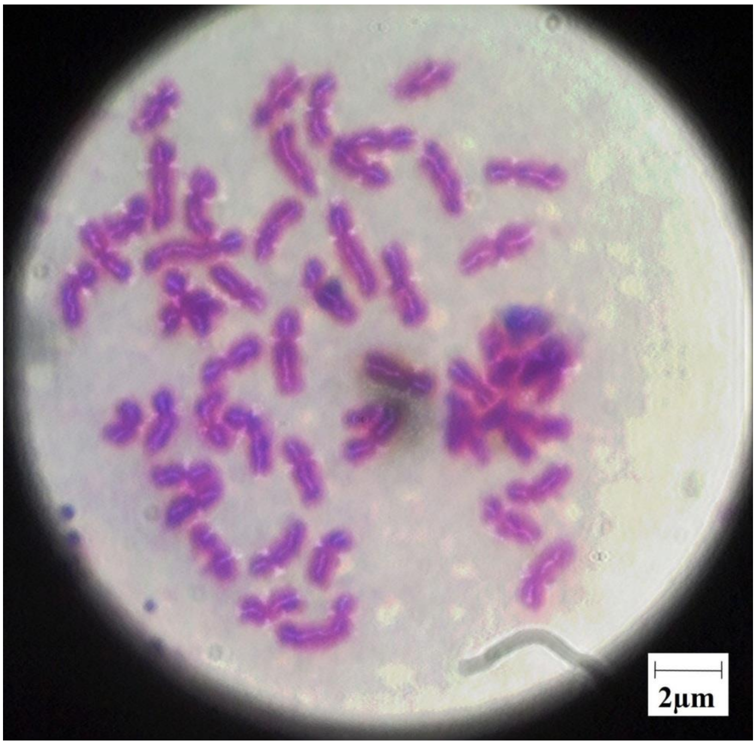

Figure 2: Metaphors spread of snow trout (*Schizocypris altidorsalis*).

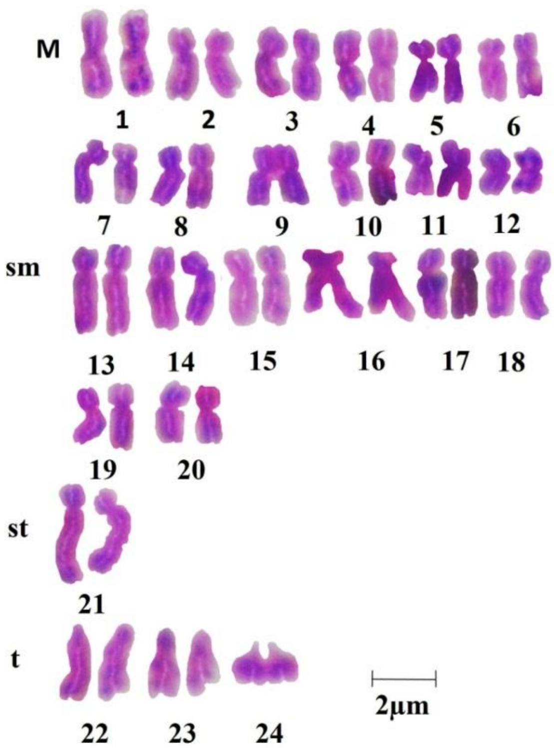

Figure 3: Karyotype of *Schizocypris altidorsalis* .

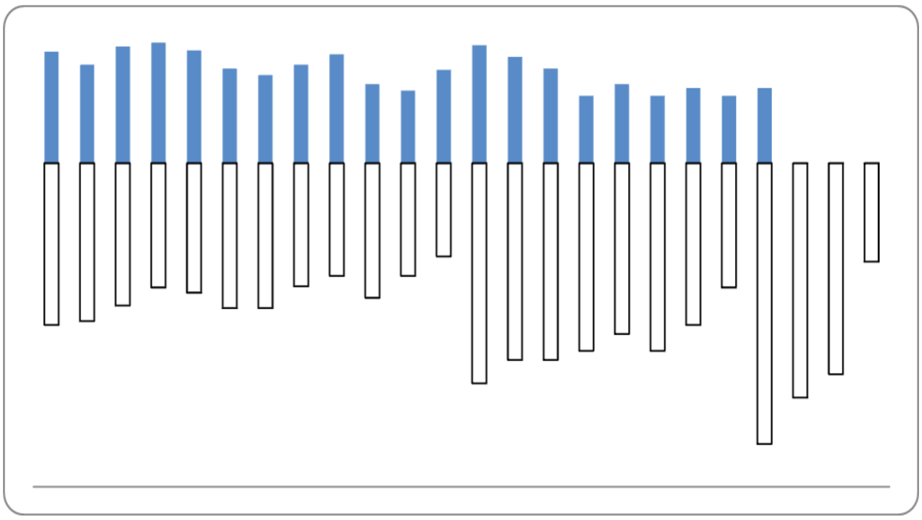

Figure 4: Haploid Karyogram of *Schizocypris altidorsalis* (n=24). The plus values represent the relative lengths of short arms of chromosome pairs, and the minus values those of long arms of chromosome pairs.

Table 1: Chromosome measurements and classification of *Schizocypris altidorsalis* chromosomes.

Chromosome pair no	Total length (μm)	Short arm (μm)	Long arm (μm)	Arm ratio	Centromeric index	Classification
1	2.10	0.85	1.25	1.47	40.47	Metacentric
	2.11	0.86	1.25	1.45	40.75	Metacentric
2	1.97	0.77	1.20	1.55	39.08	Metacentric
	1.98	0.76	1.22	1.60	38.38	Metacentric
3	1.90	0.90	1.00	1.11	47.36	Metacentric
	1.91	0.90	1.10	1.22	47.12	Metacentric
4	1.88	0.90	0.98	1.08	47.87	Metacentric
	1.89	0.93	0.96	1.03	49.20	Metacentric
5	1.87	0.87	1.00	1.14	46.52	Metacentric
	1.83	0.72	1.11	1.48	39.34	Metacentric
6	1.85	0.71	1.14	1.60	38.37	Metacentric
	1.85	0.73	1.12	1.53	39.45	Metacentric
7	1.80	0.67	1.13	1.68	37.22	Metacentric
	1.80	0.68	1.12	1.46	37.77	Metacentric
8	1.71	0.76	0.95	1.25	44.44	Metacentric
	1.75	0.76	0.99	1.30	43.42	Metacentric
9	1.72	0.86	0.86	1.00	50.00	Metacentric
	1.71	0.84	0.87	1.03	49.12	Metacentric
10	1.67	0.62	1.05	1.69	37.12	Metacentric
	1.65	0.61	1.04	1.50	36.86	Metacentric
11	1.43	0.57	0.86	1.50	39.86	Metacentric
	1.43	0.56	0.87	1.55	39.16	Metacentric
12	1.43	0.71	0.72	1.01	49.65	Metacentric
	1.42	0.72	0.72	1.00	50.00	Metacentric
13	2.61	0.91	1.70	1.86	31.27	Submetacentric
	2.64	0.88	1.76	2.00	33.33	Submetacentric
14	2.35	0.84	1.51	1.79	35.74	Submetacentric
	2.34	0.82	1.52	1.85	35.04	Submetacentric
15	2.28	0.77	1.51	1.96	33.77	Submetacentric
	2.25	0.73	1.52	2.14	31.81	Submetacentric
16	1.99	0.53	1.46	2.75	26.63	Submetacentric
	1.97	0.52	1.45	2.78	26.39	Submetacentric
17	1.93	0.62	1.31	2.11	32.12	Submetacentric
	1.93	0.61	1.32	2.16	31.60	Submetacentric
18	1.99	0.53	1.46	2.75	26.63	Submetacentric
	1.97	0.52	1.45	2.78	26.39	Submetacentric
19	1.84	0.58	1.26	2.17	31.52	Submetacentric
	1.83	0.58	1.25	2.15	31.69	Submetacentric
20	1.48	0.52	0.96	1.84	35.13	Submetacentric
	1.48	0.54	0.94	1.84	36.48	Submetacentric
21	2.78	0.64	2.14	3.34	23.02	subtelocentric
	2.75	0.58	2.17	3.74	21.09	subtelocentric
22	1.86	0	1.86	∞	0	telocentric
	1.81	0	1.81	∞	0	telocentric
23	1.63	0	1.63	∞	0	telocentric
	1.69	0	1.69	∞	0	telocentric
24	0.76	0	0.76	∞	0	telocentric
	0.76	0	0.76	∞	0	telocentric

The arm ratio and the centromeric index ranged between 1.00-∞ and 0-50, respectively. The sex chromosomes were not detected in this species. The quantitative data of the different measurements used to classify chromosomes and diagrams are given in Table 1 and Fig.4.

Discussion

Karyotypes are prepared from good metaphase spread. The major difficulty encountered is the morphological variation existing even between homologous chromosomes in the same nucleus (Al Sabti, 1991; Vitturi *et al.*, 1993). Sometimes it could happen that some chromosomes are more contracted than the others, so chromosome measurements and classification are very difficult, and especially in fish, which have very small chromosomes compared to those of mammals. Another major problem is that fish karyotypes are not identical as in human or in other animal species. Therefore, for fish, we cannot have a standard karyotype because differences not only exist between species, but polymorphism often occurs within the species (Al Sabti, 1991).

Usually, the mitotic metaphase cell in blood and kidney tissues of fishes in vivo or in vitro can present clear chromosome spreads. The chromosome slides for optical microscopy in this study were prepared from the anterior portion of the kidney and gill filaments in vivo of *S. altidorsalis*. With this technique, the preparation of the chromosome slides was inexpensive and the result could be obtained very quickly.

The present study revealed that *S. altidorsalis* has a consistent diploid number of 48. The chromosome number of this species is similar to the most common karyotype (48-50). These positions demonstrate that *S. altidorsalis* do not corporate in duplication events in early vertebrate evolution.

Heteromorphic sex chromosomes have been identified in several salmonid fishes, there was an XY /XX system in *Oncorhynchus mykiss*, and an XYY system in *Coregonus sardinella*. The formation of heteromorphic sex chromosomes often involves heterochrornatin addition, as in other animals (Philips and Rab, 2001). Occurrence of cytologically differentiated sex chromosomes in a large number of living marine fish species appears to be rare (Galleti *et al*, 2000). However sex chromosomes were indistinguishable in several cyprinid fishes (Kilic-Demirok and Unlii, 2001; Kalbassi *et al*, 2008; Esmaeili *et al*, 2009; Ergene *et al*, 2010). There was no evidence of sexual dimorphism of the chromosomes in this species and similar results were also documented in most fish species.

Species with high numbers are considered to have result through polyploidy from ancestral 2n = 48 or 50 (Rishi *et al.*, 1998). Chromosome counts in nearly cyprinid polyploids occur in multiples or combinations of the most common karyotype (48-50) and tetraploids 96, 98 or 100) and hexaploids (148-150) have arisen through hybridization (Dowling and Secor, 1997).

Most of the cyprinids karyotypes are characterized by a relatively large number of bi-armed meta- and submetacentric) compared to uni-armed (subtelo and acrocentric) chromosomes, which is expected if NF is commonly above 80 in species with diploid chromosome numbers of 48 and 50 (Klinkhardt *et al.*, 1995). A pair of large acrocentric chromosomes has been proposed as a marker for the genus *Alburnus* (Gold and Avise, 1977) as well as for some other cyprinid genera (Cataudella *et al.*, 1977). The karyotypes of *S. altidorsalis* are composed mainly of biarmed elements and a pair of large acrocentric chromosomes, perhaps suggesting evolutionary position among the other cyprinidae.

While *S. altidorsalis* is as economic species, the study of its karyotype in order to planning to chromosome manipulation and inter or intera-specific hybridization in aquaculture is very important. Also knowledge about chromosome numbers of this species associate with behavior patterns, ecology, genetics and biology studies can be a useful tool in fish evolutionary and phylogeny studies.

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