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Research Article

Karyosystematics of an Endemic tooth-carp, *Aphanius shirini* (Teleostei: cyprinodontidae) from Iran

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Abstract

The karyological and cytological characteristics of an endemic cyprinodont fish of Iran, *Aphanius shirini* have been investigated for the first time by examining metaphase chromosomes spreads obtained from gill epithelial and kidney cells. The diploid chromosome number of this species is 48. The karyotype consisted of one submetacentric and 23 subtelocentric pairs of chromosomes (2Sm + 46St). The chromosome arm number (NF) is 50. Sex chromosomes were cytologically indistinguishable in this tooth-carp. Based on the present and previous reported diploid chromosome number for other cyprinodont species, it can be suggested that the diploid chromosome number of 2n = 48 is the modal number of the cyprinodont fish.

***Keywords*:** Cyprinodontiformes, Chromosome, Cytogenetical analysis, Idiogram

Introduction

Cyprinodontiformes order comprises 10 families and about 1326 species (Eschmeyer and Fong, 1998) of mostly small, fresh and brackish water fish inhabiting harsh environments, such as saline or very warm waters, water of poor quality, or isolated situations where no other types of fish live (Esmaeili et al., 2016; Gholami et al., 2014). The Cyprinodontidae are represented in Iran by only one genus *Aphanius* Nardo, 1827. From a total of 32 *Aphanius* species which have been described around the world, one fossil record, *Aphanius persicus* and 14 species have been reported from Iranian drainages: *A. arakensis* Teimori, Esmaeili, Gholami, Zarei and Reichenbacher, 2012 ; *A. darabensis* Esmaeili, Teimori, Gholami and Reichenbacher, 2014; *A. dispar* Rüppell, 1829; *A. farsicus* Teimori, Esmaeili and Reichenbacher, 2011; *A. furcatus* Teimori, Esmaeili, Erpenbeck and Reichenbacher, 2014; *A. ginaonis* Holly, 1929; *A. isfahanensis* Hrbek, Keivany and Coad, 2006; *A. kavirensis* Esmaeili, Teimori, Gholami and Reichenbacher, 2014; *A. mento* Heckel, 1843; *A. mesopotamicus* Coad, 2009; *A. pluristriatus* Jenkins, 1910; *A. shirini* Gholami, Esmaeili, Erpenbeck and Reichenbacher, 2013; *A. sophiae* Heckel, 1847 and *Aphanius vladykovi* Coad, 1988, (Jouladeh-Roudbar et al., 2015). Till now, the karyological studies of seven *Aphanius* species (out of 14 described species) have been reported from Iran consisted of *A. dispar* and *A. ginaonis* (Esmaeili et al., 2008a), *A. farsicus* and *A. sophiae* (Esmaeili et al., 2007), *A. isfahanensis* (Esmaeili et al., 2008b), *A. mento* (Arai, 2011) and *Aphanius vladykovi* (Esmaeili et al., 2009). *Aphanius shirini*, *Gholami*, Esmaeili, Erpenbeck and Reichenbacher, 2014 or Kapour-e-dandandar-e-Khosroshirin (Farsi); Shirin or Khosroshirin tooth-carp (English) and Khosroshirin Zahnkärpflinge (German) is an endemic species found in the uppermost reaches of the Kor River Basin. Khosroshirin tooth-carp is distinguished from other Iranian species of *Aphanius* by having the lowest number of flank bars among the Iranian inland *Aphanius* species, molecular characters of mitochondrial cytochrome b, DNA sequence data and multivariable morphometric and meristic traits. Thus, the Khosroshirin population clearly represents a new species based on both molecular and morphological evidence (Gholami et al., 2014).

Tooth-carps of Iran have been studied mainly based on their morphology but species identification on this basis is not always possible. The application of non-morphological methods such as cytogenetic studies may provide a complementary data source for more accurate and precise identification of these fishes. Fish karyosystematics is a branch of systematics that links systematics, cytology, and

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genetics to find out structure and evolution of karyotypes and to reconstruct phylogenetic relationship of fish taxa (Yu et al., 1987). Application of this type of studies has received considerable attention in recent years (Esmaeili and Shiva, 2006; Galetti Jr et al., 2000; Harrison et al., 2007). Fish chromosome data have great importance in studies concerning evolutionary systematics, aquaculture, mutagenesis, genetic control and the rapid production of inbred lines (Al-Sabti, 1991). The increasing importance of chromosomal studies on fish and lack of data on karyotyping of Khosroshirin tooth-carp encouraged us to do first cytogenetical analysis (i.e., diploid chromosome number, description of karyotype, idiogram) of this endemic tooth-carp of Iran.

Materials and Methods

Aphanius shirini specimens were collected from the Paselari spring of the Khosroshirin spring-stream system, uppermost reaches of Kor River basin, Khosroshirin Village, Abadeh City, Fars, Iran, 30° 53′29.5″ N 52° 00′36.8″ E, Alt. 2327 m (Figure 1) using a dip net. The fishes were transported live to the laboratory, and kept in a well-aerated aquarium at 20 – 25°C before analysis. For karyological studies, the modified method of Uwa (1986) was used. Vinblastine solution was prepared with 0.005 g in 20 ml of physiological serum. The fish were injected intraperitoneally with 0.02 ml of vinblastine per gram of body weight using an insulin syringe and then were put back in the aquarium for 3 - 4 h.

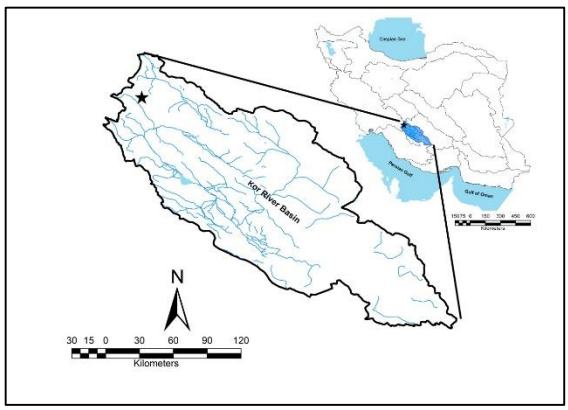

Figure 1. Location of *Aphanius shirini* population analyzed in this study.

The gill filaments and kidneys of those specimens were then removed and placed in hypotonic 0.36% KCI solution for 45 min at room temperature (25°C). Thereafter, the solutions were centrifuged for 10 min at 1000 rpm, adding 2 - 3 drops of fresh and cold Carnoy's fixative (1 : 3, acetic acid: methanol) before centrifugation. The supernatants were then discarded and 5 ml of fresh and cold fixative was added to the sediments, which were mixed thoroughly and then left for 1 h. The fixation and centrifugation stages were repeated twice. The suspensions were then trickled onto cold slides. These slides were stained with 20% Giemsa for 20 min. Chromosomes were observed, selected and photographed by Nikon light microscope with a camera mounted on it. Karyotypes were prepared by arranging chromosomes in pairs by size. For each chromosome, the average lengths of the short and long arms and arm ratio (the ratio of the long arm length to the short arm length of chromosomes) were calculated and then the chromosomes were classified according to the criteria given by Levan et al. (1964). Fundamental number (NF) was expressed as twice the number of atelocentric chromosomes plus the number of telocentric chromosomes. The idiogram was prepared in Harvard Graphics 2.0 software.

Results

Metaphase spread of this species is given in Figure 2. The diploid chromosome number was 2n = 48 (Figure 3).

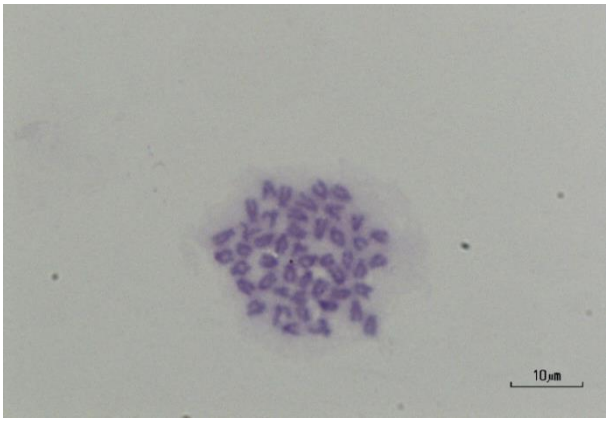

Figure 2. Giemsa stained metaphase chromosome spread of Aphanius shirini from Iran.

Figure 3. Giemsa stained karyogram of *Aphanius shirini* from Iran.

The quantitative data of the different measurements used to classify chromosomes and the idiogram are given in Table 1 and Figure 4 respectively. The karyotype consisted of one pair of submetacentric and 23 pairs of subtelocentric chromosomes (2Sm + 46St), and the arm number (NF) was 50. Sex chromosomes were cytologically indistinguishable in this endemic tooth-carp.

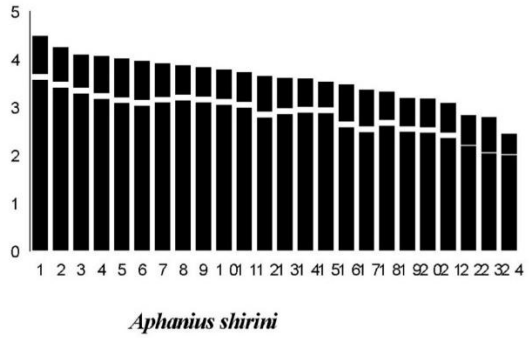

Figure 4. Haploid idiogram of *A. shirini* from Iran.

Table 1. Chromosome measurements (in µm) and classification of *Aphanius shirini* chromosomes (Ch. No.: Chromosome number; LA: Long arm; SA: Short arm; TL: Total length; AR: Arm ratio; CT: Chromosome type; Sm: Submetacentric; St: Subtelocentric).

Ch. No.	LA	SA	TL	AR	CT
1	3.58	0.81	4.39	4.37	St
2	3.42	0.74	4.17	4.58	St
3	3.30	0.71	4.01	4.63	St
4	3.18	0.79	3.98	4.00	St
5	3.10	0.83	3.93	3.70	St
6	3.04	0.83	3.88	3.63	St
7	3.11	0.72	3.83	4.29	St
8	3.15	0.63	3.79	4.97	St
9	3.11	0.63	3.75	4.89	St
10	3.06	0.63	3.69	4.83	St
11	2.99	0.65	3.64	4.61	St
12	2.79	0.77	3.56	3.63	St
13	2.87	0.65	3.52	4.39	St
14	2.90	0.61	3.51	4.74	St
15	2.89	0.54	3.43	5.30	St
16	2.59	0.79	3.39	3.26	St
17	2.49	0.78	3.27	3.17	St
18	2.62	0.61	3.24	4.28	St
19	2.49	0.60	3.10	4.14	St
20	2.47	0.61	3.09	4.00	St
21	2.36	0.63	3.00	3.71	St
22	2.20	0.63	2.84	3.45	St
23	2.05	0.75	2.80	2.70	Sm
24	2.01	0.44	2.46	4.48	St

Discussion and Conclusion

According to our observations, the diploid chromosome number of *Aphanius shirini* species was 2n = 48 and is in conformation with the chromosome number of other species of this genus. The chromosome numbers of studied *Aphanius* species including *A. sophiae*, *A. farsicus*, *A. asquamatus*, *A. dispar*, *A. fasciatus*, *A. iberus* and *A. mento* have been reported to be 2n = 48. Hence, it can be concluded that the chromosome number in this genus is conserved. The number of chromosomes in this tooth-carp is also similar to that of other species of Cyprinodontidae such as *Cyprinodon alvarezi*, *Cyprinodon atrorus* and *Cyprinodon beltrani*. In the order Cyrinodontiformes, the most common fish species which have so far been cytologically investigated, such as *Gambusia affinis*, *G. holbrooki*, *G. gaigei*, *G. nobilis*, *Girardinus metallicus*; *Poecilia vivipara* (Poecillidae); *Fundulus diaphanus* (Fundulidae); *Allotoca maculata*, *Goodea luitpoldi*, *G. atripinnis*, *G. gracilis*, *Hubbsina turneri*, *Ilyodon furcidens*, *Ilyodon lennoni*, *Skiffia francesae*, *Skiffia bilineata*, *Xenoophorus captivus*, *Xenotaenia resolanae*, *Xenotoca eiseni*, *X. melanosoma*, *X. variata* (Goodeidae), have the diploid chromosome number of 2n = 48 (Arai, 2011). Yet in a few species of Cyprinodontiformes such as *Aphyosemion bivittatum*, *A. bualanum*, *A. calliurum*, *Fundulopanchax sjostedti*, *Fundulopanchax mirabilis* (Aplocheilidae); *Allotoca dugesi* ,*Allodontichthys hubbsi* and *Ameca splendens* (Goodeidae) the diploid chromosome number is reported to vary from 2n = 26 to 2n = 42 (Arai, 2011). It could be suggested that the diploid chromosome number of 2n = 48 is the modal number of cyprinodont fish. In the interpretation of karyotypic evolution, it is often assumed that the primitive fish karyotype consists of 48 rods from which the karyotypes of all existing fish forms have been derived (Khuda-Bukhsh et al., 1986) but the issue seems yet to be resolved. The discovery of 48 rather large acrocentric chromosomes in the Pacific hagfish, *Eptatretus stoutii*, belonging to the order Myxiniformes (Taylor, 1967; Vasil'yev, 1980) and the occurrence of 48 rods in the majority of fishes studied prior to 1967 led to the idea that the primitive karyotype of ancestral vertebrate freshly evolved from chordate might consist of 48 rods (Khuda-Bukhsh et al., 1986). Therefore, most of the subsequent workers assumed the karyotypic evolution in different groups of fishes based on this basic assumption of 48 rods as the primitive number (Khuda-Bukhsh et al., 1986). But the discovery of 2n = 24 rods in two species of freshwater eels (Kitada and Tagawa, 1973; Rishi and Haobam, 1984), 2n = 36 rods in two species of *Myxine*, low diploid numbers ranging between 14 and 42 in a large

number of fish families showing NF less than 36 in some cases (Khuda-Bukhsh et al., 1986) would possibly call for a more cautious prediction on the primitive karyotype of fish.

In the present study, no cytological evidence was found for sex chromosome dimorphism which agrees with reports on many fish species such as Serranidae and Mugilidae (Aguilar 1997, Rossi *et al*. 1997).

The karyotype formula of this tooth-carp was 2Sm + 46St and the chromosome arm number was 50. Chromosome formula of 16Sm + 32St was reported for *A. dispar* and *A. farsicus*; 14Sm + 34St for *Aphanius ginaonis*; 12Sm + 34St in *A. isfahanensis* and 8Sm + 40St for *A. sophiae* and *Aphanius vladykovi*. The arm number of NF = 32 was reported for *A. dispar* and *A. farsicus* and NF = 28 for *Aphanius sophiae* and *A. vladykovi*. The arm number in *Aphanius ginaonis* and *A. isfahanensis* were reported to be 31 and 30 respectively (Esmaeili et al., 2008a; Esmaeili et al., 2008b, 2009; Esmaeili et al., 2007). Though chromosome numbers of *Aphanius* species are conserved despite different geographical locations, the fundamental arm numbers are different. These differences within *Aphanius* species of different geographical locations suggest that structural rearrangement in chromosome complements, as a consequence change in chromosome morphology without any change in chromosome number. This divergence may be attributed to differences in the karyotype macrostructure, reflecting a real geographical variation common to widespread species or may be the result of differences in the scoring of submetacentric or metacentric chromosomes as different degrees of chromosome condensation, leading to differences in chromosome classification.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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