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Karyological Analysis of Two Endemic Tooth-Carps, *Aphanius persicus* and *Aphanius sophiae* (Pisces: Cyprinodontidae), from Southwest Iran

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Abstract: The karyotypes of 2 endemic tooth-carps of Iran, *Aphanius persicus* (Jenkis, 1910) and *Aphanius sophiae* (Heckel, 1849), were investigated by examining metaphase chromosomes spreads obtained from gill epithelial and kidney cells. The diploid chromosome numbers of both species were 2n = 48. The karyotypes consisted of 11 pairs of submetacentric and 13 pairs of subtelocentric chromosomes in *A. persicus* and 14 submetacentric and 10 subtelocentric chromosomes in *A. sophiae*. The arm numbers in *A. persicus* and *A. sophiae* were NF = 70 and NF = 76, respectively. Sex chromosomes were cytologically indistinguishable in both tooth-carps.

Key Words: *Aphanius persicus*, *Aphanius sophiae*, karyotype, chromosome, idiogram

Introduction

The Cyprinodontidae are represented in Iran by 6 species (Coad, 1988, 1995, 1996; Scheel, 1990): *Aphanius ginaonis* (Holly, 1929); *A. mento* (Heckel, 1843); *A. dispar* (Rüppell, 1828); *A. vladykovi* Coad, 1988; *A. sophiae* (Heckel, 1849); and *A. persicus* (Jenkins, 1910). They are very colorful fish and can be kept in aquaria; hence, they may become part of the aquarium trade. Three species of those tooth-carps are found in Fars province, Southwest Iran. They are *A. dispar*, *A. sophiae* and *A. persicus*. *A. dispar* is a euryhaline fish that apparently prefers brackish waters in Fars province. *A. persicus* or Persian tooth-carp is an endemic species found in the Lake Maharlu basin. This lake is located in a basin in Shiraz valley at an altitude of about 1460 m. This is a chloride lake and fishless. The fish is found in its incoming streams and springs. *A. sophiae* is found in the endorheic Kor River basin of this province. Tooth-carps of Iran have been studied mainly based on their morphology but their morphology is conservative and, due to morphological similarities, their distinction on this basis is not always possible (Esmaeili and Shiva, 2006). Their biology is also unknown and there is little information on these species. 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. Application of this type of study has received considerable attention in recent years (Ozouf-Costaz and Foresti, 1992; Galetti et al., 2000). 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 karyotype of Iranian fish encouraged us to perform this first cytogenetical analysis (i.e. diploid chromosome numbers, description of karyotypes, and idiograms) of 2 endemic tooth-carps, *A. persicus* and *A. sophiae*, from Southwest Iran.

Materials and methods

A. persicus specimens were collected from the Barm-e-Shoor spring stream system (29°27′ N-52°42′ E, alt. 1465 m) in the Lake Maharlu basin, and those of *A. sophiae* were collected from Ghadamgah spring stream system in Kor basin (30°15′N-52°25′ E, alt. 1660 m) in Fars province, Southwest Iran, using a dip net. The fishes were transported live to the laboratory, and kept in a

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well-aerated aquarium at 20-25 °C before analysis. For karyological studies the modified method of Uwa (1986) was used. Colchicine solution was prepared with 0.005 g in 20 ml of physiological serum. The fish were injected intraperitoneally with 0.02 ml of colchicine per gram of body weight using an insulin syringe, and then were put back in the aquarium for 4-5 h. The gill filaments and kidneys of those specimens were then removed and placed in hypotonic 0.36% KCl 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 10% Giemsa for 20 min. Chromosomes were observed, selected and photographed by Olympus 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 idiograms were prepared in Excel 2003 software (Microsoft).

Results

In 82.5% of the 57 observed metaphase images obtained from the kidney and gill epithelial cells of 5 *A. persicus* specimens, the diploid chromosome number was 2n = 48. The typical karyotype consisted of 11 pairs of submetacentric and 13 pairs of subtelocentric chromosomes (Figure 1). Different chromosome numbers in a total of 17.5% of the metaphase images were recorded ranging from 29 to 46 (Table 1). There were no additions in these numbers (>48). These variations or deviations from values were probably caused by losses during preparation or other artifacts. Relatively small chromosomes were observed in *A. persicus*. The size (total length) of chromosomes varied from 3.29 to 5.95 µm based on the mean values of the measurements of best mitotic metaphases. The results are presented in Table 2.

In the case of *A. sophiae*, the chromosome number was 2n = 48, comprising 14 submetacentric and 10 subtelocentric chromosomes (Figure 2 and Table 1). Different chromosome numbers in a total of 12.5% of the metaphase images were recorded ranging from 47 to 50 (Table 1). The total length of chromosomes varied from 4.21 to 7.15 µm, being longer than *A. persicus* chromosomes. The measurements used to classify chromosomes and idiograms are given in Table 2 and Figure 2.

The arm numbers in *A. persicus* and *A. sophiae* were NF = 70 and NF = 76, respectively. Sex chromosomes were cytologically indistinguishable in both tooth-carps.

Discussion

According to our observations, the diploid chromosome numbers of both *Aphanius persicus* and *A. sophiae* were 2n = 48 and this is in agreement with the chromosome number of other species of this genus. Klinkhardt et al. (1995) and Arkhipchuk (1999) reported the chromosome number of *A. sophiae* to be 2n = 48 and that of *A. mento* to be 2n = 48 (Vasil'ev, 1980; Klinkhardt et al., 1995; Arkhipchuk, 1999). The chromosome numbers of *A. dispar*, *A. asquamatus, A. iberus* and *A. fasciatus* were also 2n = 48 (Karbe, 1961; Gyldenholm and Scheel, 1974; Arkhipchuk, 1999). It can be concluded that the chromosome number in this genus is conserved. The number of chromosomes in these tooth-carps is also similar to that of other species of Cyprinodontidae such as *Orestias agassizii, Cyprinodon bovines* and *Cyprinodon macularius* (Klinkhardt et al., 1995; Arkhipchuk, 1999). In the order Cyrinodontiformes, the most common fish species that have so far been cytologically investigated, such as *Gambusia holbrooki, G. affinis, G. hurtadoi* and *Girardinus metallicus; Poecilia latipinna* (Poecillidae); *Fundulus majalis* (Fundulidae); *Allotoca diazi, Ameca splendens, Goodea atripinnis, Goodea gracilis, Hubbsina turneri, Hyodon furcidenes, Skiffia bilineata, Xenotaenia resolanae, Xenotoca eiseni, X. melanosoma* and *X. variata* (Goodeidae), have the diploid chromosome number 2n = 48 (Sola et al., 1990; Klinkhardt et al., 1995; Arkhipchuk, 1999). Yet in a few species of

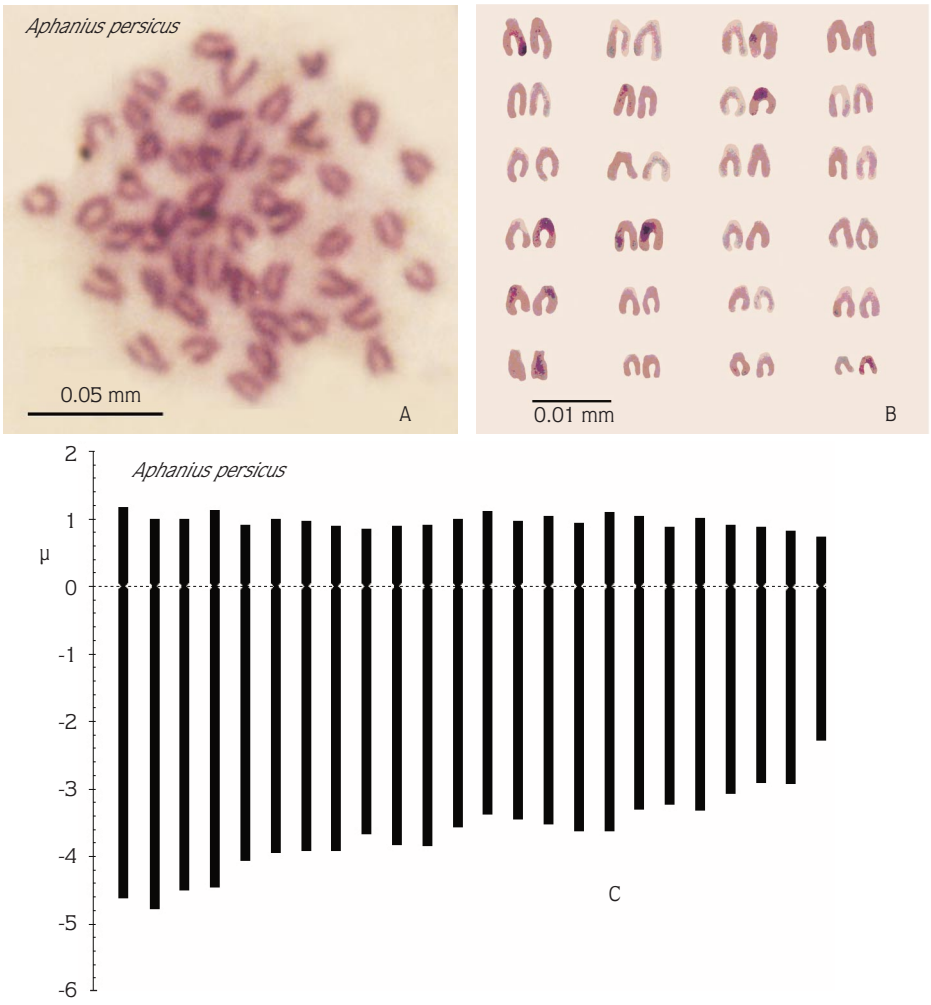

Figure 1. Metaphase spread from gill epithelial cell (A), giemsa stained karyotypes (B) and haploid idiogram (C) of *A. persicus*.

Table 1. Chromosome complements of *A. persicus* and *A. sophiae*.

Species	No. of metaphase plates	No. of chromosomes	Frequency	Percentage
A. persicus	57	48	47	82.5
		46	5	9
		31	3	5
		29	2	3.5
A. sophiae	40	48	35	87.5
		47	3	7.5
		19	1	2.5
		50	1	2.5

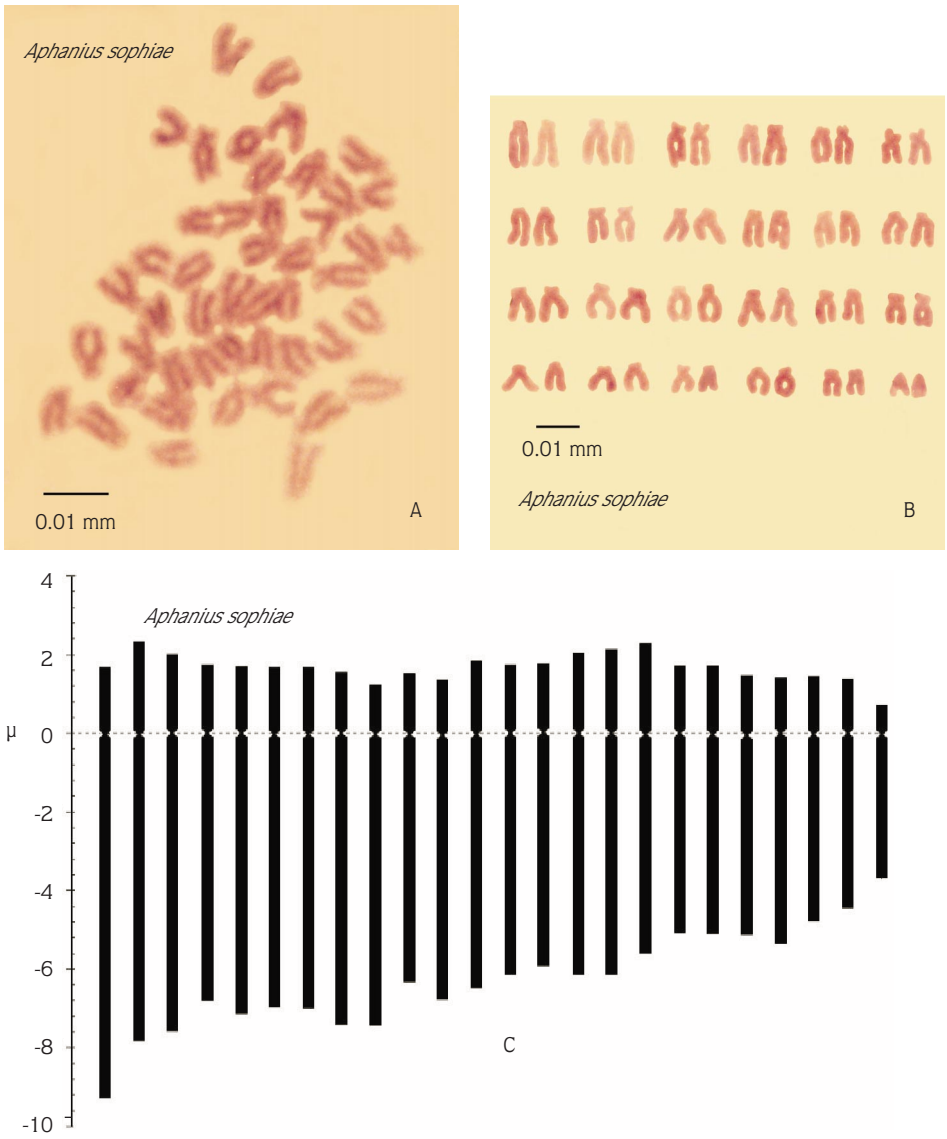

Figure 2. Metaphase spread from gill epithelial cell (A), giemsa stained karyotypes (B) and haploid idiogram (C) of *A. sophiae*.

Cyprinodontiformes such as *Aphyosemion viride* and *Fundulopanchax sjostedti* (Aplocheilidae), and *Allodontichthys hubbsi* and *Ameca splendens* (Goodeidae) the diploid chromosome number was reported to vary from 2n = 26 to 2n = 42 (Klinkhardt et al., 1995; Arkhipchuk, 1999). It could be suggested that the diploid chromosome number 2n = 48 is the modal number in cyprinodont fishes. 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 (Kuda-Bukhsh et al., 1986) but the issue still seems unresolved. The discovery of 48 rather large acrocentric chromosomes in the Pacific hag fish, *Eptatretus stoutii*, belonging to the order Myxiniformes (Taylor, 1967; Vasil'ev, 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 chrodate might consist of 48 rods. Therefore, most of the subsequent researchers assumed the karyotypic

Table 2. Chromosome measurements (in µm) and classification of *A. persicus*e and *A. sophiae* chromosomes (Ch. No.: Chromosome number; LA: Long arm; SA: Short arm; TL: Total length; AR: Arm ratio; CT: Chromosome type; Sm: Submetacentric; St: Subtelocentric).

A. persicus						*A. sophiae*					
Ch. No.	LA	SA	TL	AR	CT	Ch. No.	LA	SA	TL	AR	CT
1	4.63	1.17	5.80	3.97	St	1	5.65	1.50	7.15	3.77	St
2	4.96	0.99	5.95	5.05	St	2	5.05	1.54	6.59	3.29	St
3	4.54	1.12	5.66	4.07	St	3	5	1.72	6.72	2.91	Sm
4	4.07	0.90	4.97	4.55	St	4	4.86	1.56	6.42	3.12	St
5	3.92	0.89	4.81	4.40	St	5	4.53	1.52	6.05	2.99	Sm
6	3.58	0.84	4.42	4.26	St	6	4.23	1.67	5.9	2.53	Sm
7	3.84	0.89	4.72	4.33	St	7	4.98	1.36	6.34	3.65	St
8	4.51	0.96	4.50	4.58	St	8	4.68	1.29	5.97	3.63	St
9	3.86	0.91	4.76	4.27	St	9	4.55	1.35	5.89	3.38	St
10	3.37	1.23	4.60	2.73	Sm	10	4.06	1.55	5.61	2.62	Sm
11	3.38	1.18	4.56	2.88	Sm	11	4.16	1.42	5.58	2.94	Sm
12	3.96	0.99	4.95	3.99	St	12	4.68	1.26	5.94	3.71	St
13	3.92	0.96	4.87	4.10	St	13	3.90	1.44	5.34	2.72	Sm
14	3.45	1.36	4.81	2.53	Sm	14	3.58	1.64	5.23	2.18	Sm
15	3.27	1.15	4.42	2.84	Sm	15	4.56	1.52	6.08	2.99	Sm
16	3.64	0.94	4.51	3.87	St	16	4.42	1.39	5.80	3.19	St
17	2.94	1.09	4.03	2.69	Sm	17	4.06	1.38	5.44	2.93	Sm
18	3.33	1.50	4.83	2.22	Sm	18	3.44	1.30	4.74	2.65	Sm
19	3.06	1.13	4.19	2.70	St	19	3.34	1.02	4.36	3.26	St
20	3.30	1.14	4.44	2.91	Sm	20	3.09	1.12	4.21	2.77	Sm
21	2.29	1.00	3.29	2.29	Sm	21	3.52	1.26	4.78	2.80	Sm
22	2.92	1.02	3.94	2.86	Sm	22	3.42	1.29	4.71	2.65	Sm
23	2.93	1.08	4.01	2.71	Sm	23	3.75	1.50	5.25	2.51	Sm
24	3.24	1.12	4.36	2.89	Sm	24	3.79	1.23	5.02	3.07	St

evolution in different groups of fishes based on this basic assumption of 48 rods as the primitive number (Kuda-Bukhsh et al., 1986). However, the discovery of 2n = 24 rods in 2 species of freshwater eels (Kitada and Tagawa, 1973; Rishi and Haobam, 1984), 2n = 36 rods in 2 species of *Myxine*, and low diploid numbers ranging between 14 and 42 in a large number of fish families showing NF less than 36 in some cases (Kuda-Bukhsh et al., 1986) would possibly call for a more cautious prediction on the primitive karyotype of fish.

The karyotypes of these 2 tooth-carps were found to be different. *A. persicus* had 11 pairs of submetacentric and 13 pairs of subtelocentric chromosomes, having smaller chromosomes, while *A. sophiae* had 14 pairs of submetacentric and 10 pairs of subtelocentric chromosomes, resulting in different idiograms. The chromosome arm number of *A. sophiae* was also higher than that of *A. persicus*. The geographical distribution of these species seems to be reflected in karyotype evolution and species differentiation of these tooth-carps. However, further molecular, cytological, anatomical, morphological and biological investigations towards better recognition and understanding of the genus *Aphanius* in Fars province need to be done. Obvious sex determination, beautiful coloration, small size, high tolerance, and easy adaptation to aquarium conditions make them a very suitable species for keeping in aquaria.

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