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Original Article

Karyosystematics of Kol tooth-carp, *Aphanius darabensis* (Teleostei: Cyprinodontidae)

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Abstract: The karyological and cytological characteristics of an endemic cyprinodont fish of Iran, *Aphanius darabensis* Esmaeili, Teimori, Gholami & Reichenbacher, 2014 have been investigated for the first time by examining metaphase chromosomes spreads obtained from gill epithelial and kidney cells. The diploid chromosome number of *A. darabensis* is 48. The karyotype consisted of five submetacentric and 19 subtelocentric pairs of chromosomes (5sm+19st). The fundamental number (FN) is 58. Sex chromosomes were cytologically indistinguishable in this tooth-carp. According to this study and previous karyological reports from other cyprinodont species, it can be suggested that the diploid number (2n=48) is common amongst cyprinodont fishes. These results can be used as basic informations in population studies and management and conservation programs.

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Introduction

Fishes represent more than half of all extant vertebrates with more than 33,984 recognized species (Eschmeyer and Fong, 2016). Cyprinidontiformes is a small fish order comprising about 1323 of mostly small species in 10 families (Eschmeyer and Fong, 2016). They live in fresh or brackish waters and some extreme environments, such as saline or very warm waters, or isolated water bodies where no other types of fishes occur (Gholami et al., 2014; Esmaeili et al., 2016). The Cyprinodontidae with 135 species worldwide (Eschmeyer and Fong, 2016) 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 alive species have been reported from Iranian drainages including *A. darabensis* or Kapour-e-dandandar-e-darab (Farsi); Kol tooth-carp (English) and Darab Zahnkärpfling (German) is an endemic species found in the uppermost reaches of the Kol River tributary which drains to the Persian Gulf (Esmaeili et al., 2014). *Aphanius darabensis* is closely related to *A. shirini* from which it is distinguished by higher number of flank bars in males (9–18 in *A. darabensis* vs. 7-10 in *A. shirini*), small irregular vertical patches of brown color on the flank of females (vs. prominent dark brown blotches of round or irregular shape), and symmetrically shaped triangular to trapezoid otoliths with a rostrum clearly longer than the antirostrum (vs. quadrangular to trapezoid otoliths with short and equally sized rostrum and antirostrum). It is distinguished from the other *Aphanius* species by the combination of four characters in both sexes: longer anal fin (15.5% SL in males, 12.1% SL in females), larger pelvic fin (8.1-12.5% SL in males, 7.04-10.3% SL in females), greater scale width (4.1-6.0% SL), and otolith characters. In addition, males can be distinguished by greater scale length (3.0-4.8% SL) and small caudal peduncle (0.9-1.5% minimum body depth); and females can be separated additionally by a short caudal fin length (12.7-19.2% SL) (Esmaeili et al., 2014).

Tooth-carps of Iran have been studied mainly

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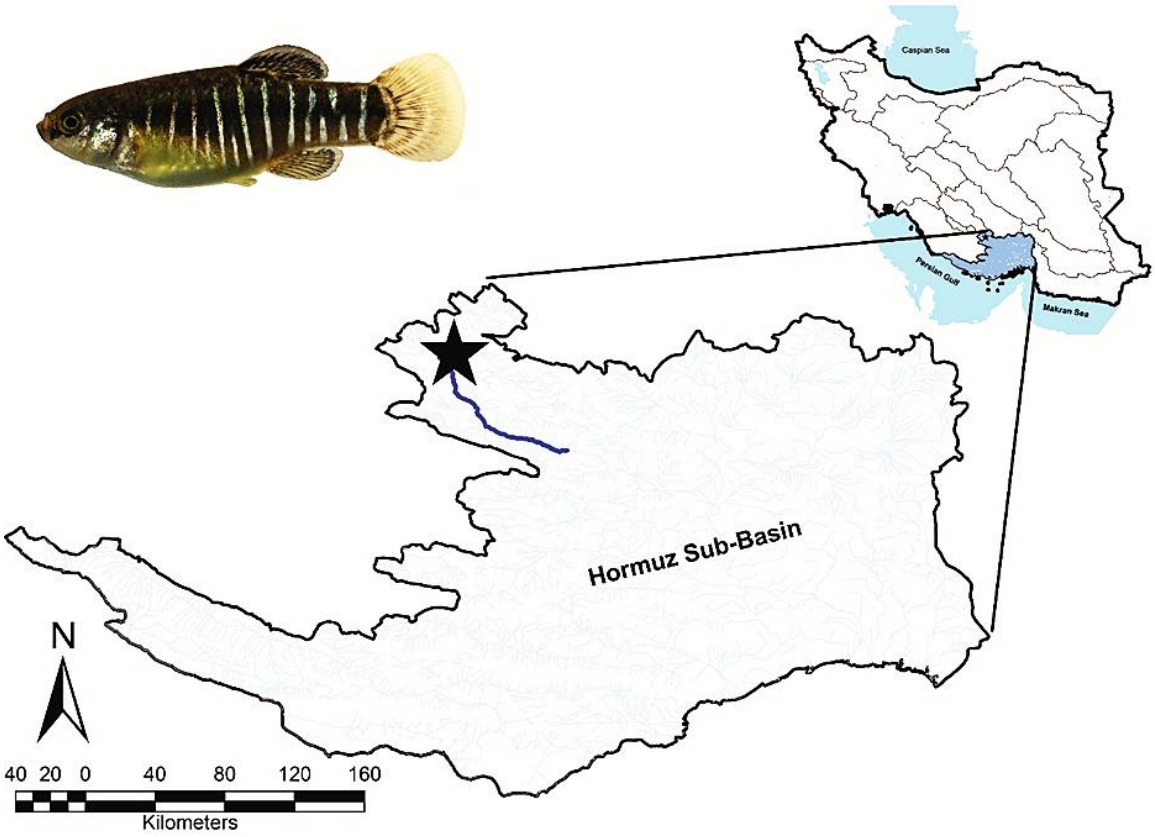

Figure 1. Collection site of *Aphanius darabensis* in the upper reaches of Kol River drainage.

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 fishes. Fish karyosystematics is a branch of systematics that links systematics, cytology and genetics to find out structure and evolution of karyotypes and to reconstruct phylogenetic relationship of fish taxa (Yu et al., 1987). A considerable attention has been paid to this type of studies in recent years (Galetti Jr et al., 2000; Esmaeili and Shiva, 2006; 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 study of chromosomes in fishes has been expanding significantly due to the development of refined techniques of cell and tissue culture originally developed for mammals, but later adapted to the fish physiology (Clem et al., 1961; Booke, 1968; Wolf and Quimby, 1969; Denton, 1973) and the development of less expensive *in vivo* direct methods (Ozouf-Costaz and Foresti, 1992). Due to the particular phylogenetic position of the ray-finned fishes among vertebrates, studies on their chromosomes have provided valuable information for understanding mechanisms of sex determination, evolution of sex chromosomes, distribution of the nucleolus organizers regions (NOR), existence of supernumerary chromosomes and the role of polyploidy in evolution (Pisano et al., 2007; Nirchio et al., 2014).

In this study we examined cytogenetical characteristics (i.e., diploid chromosome numbers, description of karyotypes, ideograms) of Kol tooth-carp, *A. darabensis* from the Persian Gulf basin in order to help future taxonomical and genetic studies.

Materials and Methods

Twelve's adult specimens of *A. darabensis* specimens were collected from the Golabi spring

Table 1. Chromosome measurements (in µm) and classification of *Aphanius darabensis* 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	2.99	0.91	3.91	3.25	St
2	2.98	0.82	3.81	3.62	St
3	2.96	0.72	3.69	4.08	St
4	2.98	0.64	3.63	4.60	St
5	2.83	0.72	3.55	3.89	St
6	2.76	0.75	3.52	3.67	St
7	2.78	0.69	3.48	4.01	St
8	2.67	0.77	3.45	3.42	St
9	2.61	0.74	3.35	3.53	St
10	2.66	0.67	3.34	3.93	St
11	2.51	0.80	3.32	3.13	St
12	2.62	0.65	3.28	3.98	St
13	2.58	0.64	3.23	3.97	St
14	2.45	0.73	3.18	3.33	St
15	2.47	0.68	3.16	3.58	St
16	2.29	0.81	3.10	2.82	Sm
17	2.28	0.79	3.07	2.87	Sm
18	2.33	0.71	3.04	3.28	St
19	2.28	0.69	2.97	3.29	St
20	2.27	0.57	2.85	3.95	St
21	2.18	0.62	2.80	3.46	St
22	1.96	0.71	2.67	2.76	Sm
23	1.85	0.65	2.51	2.83	Sm
24	1.65	0.80	2.45	2.05	Sm

located in the uppermost reaches of Kol River tributary, Darab City, Fars, Iran, 28°47′15″ N 54°22′19″ E, (Fig. 1) using a dip net. The fishes were transported alive 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 hours. 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. 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 hour. The fixation and centrifugation 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 and shape. 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 (FN) was expressed as twice the number of atelocentric chromosomes plus the number of telocentric chromosomes. The ideogram 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 (Fig. 3). The quantitative data of the different measurements used to classify chromosomes and the

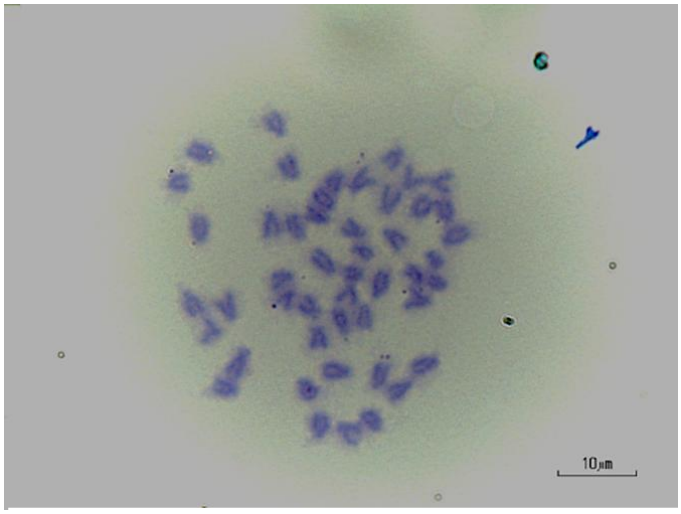

Figure 2. Giemsa stained chromosome spread of *Aphanius darabensis*.

Figure 3. Giemsa stained karyotype of *Aphanius darabensis*.

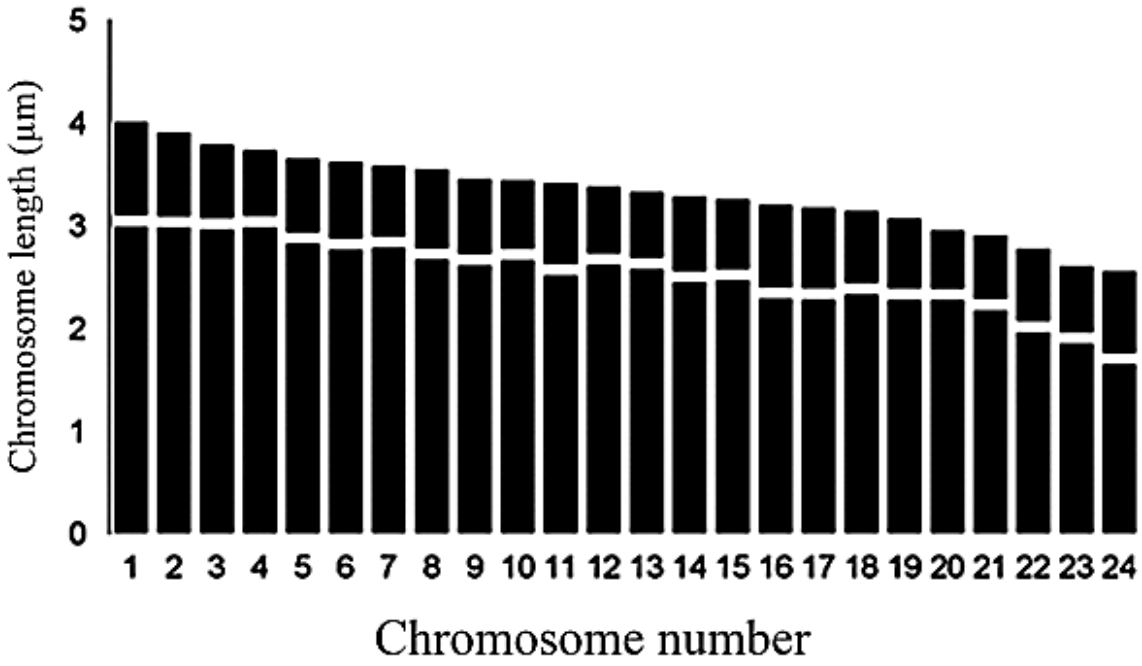

Figure 4. Haploid ideogram of *Aphanius darabensis*.

ideogram are given in Table 1 and Figure 4, respectively. The karyotype consisted of five submetacentric and 19 subtelocentric pairs of chromosomes (5sm+19st). The chromosome arm number (FN) was 58.

Discussion

According to our observations, the diploid chromosome number of *A. darabensis* (2n=48) is in confirmation with *A. sophiae*, *A. farsicus*, *A. asquamatus*, *A. dispar*, *A. fasciatus*, *A. iberus* and *A. mento*. 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*, *C. atrorus* and *C. beltrani* (Stevenson, 1981). 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*, *I. lennoni*, *Skiffia francesae*, *S. 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 the order such as *Aphyosemion bivittatum*, *A. bualanum*, *A. calliurum*, *Fundulopanchax sjostedti*, *F. 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 can be noted that the diploid number (2n=48) is modal in cyprinodont fish. In 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 FN 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. According to Nirchio et al. (2014) in freshwater fishes both the average number of chromosomes and the FN are higher than in marine fishes, and a general higher degree of cytogenetic diversification and karyotype variation is observed, compared to a more conserved cytogenetic pattern in marine fishes. Few decades ago the difference between karyotypes of freshwater and marine fishes was already observed and considered related to a more stable environment at sea as compared to inland waters, with some exceptions (Nikolsky, 1976; Nirchio et al., 2014).

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 consisted of 5 submetacentric and 19 subtelocentric pairs of chromosomes (5Sm+19st) and the chromosome arm number was 58. Chromosome formula of 16sm+32st was reported for *A. dispar* and *A. farsicus*; 14sm+34st for *A. ginaonis*; 12sm+34st in *A. isfahanensis* and 8sm+40st for *A. sophiae* and *A. vladykovi*. The arm number of FN=32 was reported for *A. dispar* and *A. farsicus* and FN=28 for *A. sophiae* and *A. vladykovi*. The arm number in *A. ginaonis* and *A. isfahanensis* were reported to be 31 and 30, respectively (Esmaeili et al., 2007; Esmaeili et al., 2008a ; Esmaeili et al., 2008b ; Esmaeili et al., 2009) (Table 2). Though chromosome numbers of *Aphanius* species are conserved despite of 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 changes in chromosome morphology without 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, leads to differences in chromosome classification. Based on Nirchio et al. (2002), species with high arm number would be more recently appeared in

Table 2. Karyotype characteristics of *Aphanius* species in Iran.

Species	Diploid number (2n)	Karyotype formula	FN	Reference
A. darabensis	48	10sm+38st	29	Present study
A. dispar	48	16sm+32st	32	Esmaeili et al. (2008a)
A. farsicus	48	16sm+32st	32	Esmaeili et al. (2007)
A. ginaonis	48	14sm+34st	31	Esmaeili et al. (2008a)
A. isfahanensis	48	12sm+36st	30	Esmaeili et al. (2008b)
A. sophiae	48	8sm+40st	28	Esmaeili et al. (2007)
A. vladykovi	48	8sm+40st	28	Esmaeili et al. (2009)
		12st+36t	30	Amini and Hemmatzadeh (2012)

evolutionary history of the lineage. In other word, low FN should be a plesiomorphy and high FN might be considered as apomorphy which suggested to be assessed for *Aphanius* species using molecular data set.

The data presented contributes with first knowledge on the karyotypes of *A. darabensis.* Camparing to pervoius reported diploid chromosome number for other species of the genus, it can be concluded that the chromosome number in this genus is conserved despite variation in fundamental arm numbers.

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چکیده فارسی

کاریوسیستماتیک گورماهی کل، *Aphanius darabensis* (ماهیان استخوانی عالی: کپورماهیان دندان‌دار)

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چکیده:

برای اولین بار ویژگی‌های کاریولوژیکی و سیتولوژیکی یک کپور دندان‌دار بومزاد ایران، *Aphanius darabensis* Esmaeili, Teimori, Gholami & Reichenbacher, 2014 به وسیله بررسی گسترش‌های کروموزومی متافازی به دست آمده از سلول‌های پوششی آبشش و کلیه مورد مطالعه قرار گرفت. عدد کروموزومی دیپلوئید این گونه ۲n=۴۸ و کاریوتایپ آن شامل پنج جفت کروموزوم ساب متاسنتریک و ۱۹ جفت کروموزوم ساب تلوسنتریک با فرمول کاریوتایپی (۵st+۱۹sm) می‌باشد. تعداد بازوی کروموزوم‌ها FN=۵۸ است. کروموزوم‌های جنسی از نظر سیتولوژیکی غیرقابل تشخیص است. بر اساس مطالعه کنونی و گزارش‌های کاریولوژیکی پیشین از دیگر گونه‌های کپور دندان‌دار می‌توان پیشنهاد کرد که ۲n=۴۸، عدد کروموزومی دیپلوئید معمول در بین کپورماهیان دندان‌دار می‌باشد. این نتایج می‌توانند به عنوان اطلاعات پایه‌ای در مطالعات جمعیتی، برنامه‌های مدیریت و حفاظت استفاده شوند.

کلمات کلیدی: کپورماهی شکلان دندان‌دار، کروموزوم، آنالیز سیتولوژیکی، ایدئوگرام.