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"Research Note"

FIRST KARYOLOGICAL ANALYSIS OF AN ENDEMIC FISH, ZAGROS TOOTH-CARP, *APHANIUS VLADYKOVI* COAD, 1988 (ACTINOPTERYGII: CYPRINODONTIDAE) FROM IRAN*

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Abstract – The karyotype of Zagros tooth-carp, *Aphanius vladykovi* (Coad) has been investigated by examining metaphase chromosomes spreads obtained from gill epithelial and kidney cells. The diploid chromosome number of this species was 2n=48. The karyotype consisted of 8 submetacentric and 40 subtelocentric chromosomes (8Sm+40 St). The arm number (NF) was 28. Sex chromosomes were cytologically indistinguishable in this tooth-carp.

Keywords – Cyprinodontiformes, chromosome, cytogenetical analysis, idiogram

1. INTRODUCTION

The genus *Aphanius* is the only genus of Cyprinodontidae available in Iran which is represented by at least eight named species [1]: *Aphanius ginaonis* (Holly, 1929), *A. mento* (Heckel, 1843), *A. dispar* (Rüppell, 1828), *A. vladykovi* Coad, 1988, *A. sophiae* (Heckel, 1849), *A. persicus* (Jenkins, 1910) and *A. isfahanensis* Hrbek, Keivany and Coad, 2006 and A. *mesopotamicus* Coad, 2009 (Fig. 1). *Aphanius vladykovi* or Zagros tooth-carp is an endemic species found in a restricted area of the central Zagros Mountains [2-3].

Little work has been done on the Iranian populations of *Aphanius* [1]; however, basic data on the reproduction, alimentation and habitat of *A. vladykovi* [3], reproductive biology of *A. persicus* [4] and karyotypes of *A. sophiae* and *A. persicus* [5] are available. Tooth-carps of Iran have been studied mainly based on their morphology, but genetic diversity studies using different markers including karyotype morphological markers are essential for recognition and understanding of these tooth-carps. The application of such non-morphological methods may provide a complementary data source for more accurate and precise identification of these fish. Application of this type of study has received considerable attention in recent years [6-8]. Fish chromosome data have a great importance in studies concerning evolutionary systematics, aquaculture, mutagenesis, genetic control and the rapid production of inbred lines [9]. The increasing importance of chromosomal studies on fish and the lack of data on the karyotypy of Zagros tooth-carp encouraged us to do this first cytogenetical analysis (i.e., diploid chromosome number, description of karyotype, idiogram) of the this endemic tooth-carp of Iran .

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2. MATERIALS AND METHODS

Aphanius vladykovi specimens were collected from Chagha Khur wetland 31° 55.307' N, 50° 56.325" E. alt. 2278m in the province of Chahar-Mahall-va-Bakhtiari, Iran (Fig. 1) using a dip net. The specimens 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 was used [10]. Colchicine solution was prepared with 0.005 g in 20 ml distilled water. Five fish were injected intraperitonally with 0.02 ml of colchicine per gram of body weight using an insulin syringe, and then were replaced in the aquarium for 4-5 hours. 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 fixative (1:3, Acetic acid: Methanol) before centrifugation. The supernatants were then discarded and 5ml fresh and cold fixative was added to the sediments, mixed thoroughly and then were left for 1 hour. The fixation and centrifugation stages were repeated 2 times. The suspensions now were trickled to cold slides. These slides were stained with 10% Giemsa for 20 min. Chromosomes were observed, selected and photographed by Olympus light microscope mounted by a camera. 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 of Levan et al. [11]. Fundamental number (NF) was expressed as twice the number of atelocentric plus the number of telocentric chromosomes. The idiogram was prepared in Harvard Graphics 2.0 software.

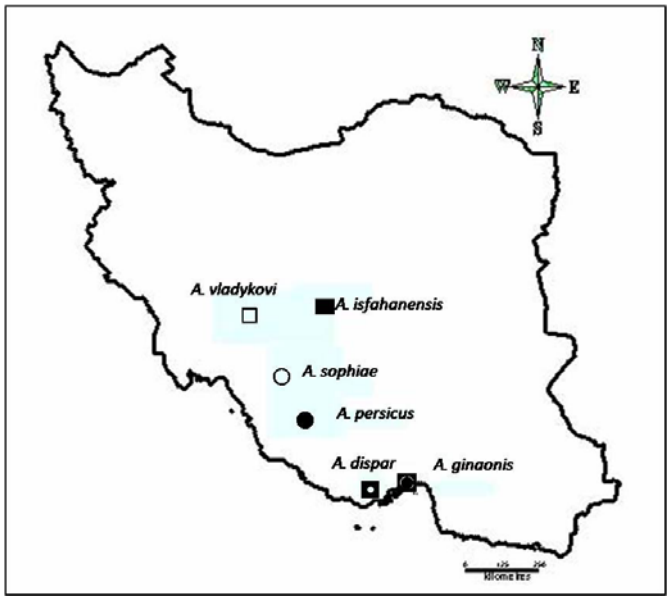

Fig. 1. Map of Iran showing distribution of some *Aphanius* species

3. RESULTS

Metaphase spread of this species is given in Fig. 2. The diploid chromosome number was 2n=48 (Fig. 3). The quantitative data of the different measurements used to classify chromosomes and the idiogram are given in Table 1 and Fig. 4. The karyotype consisted of 8 submetacentric and 40 subtelocentric chromosomes (8Sm+40St), and the arm number was 28. Sex chromosomes were cytologically indistinguishable in this endemic tooth- carp.

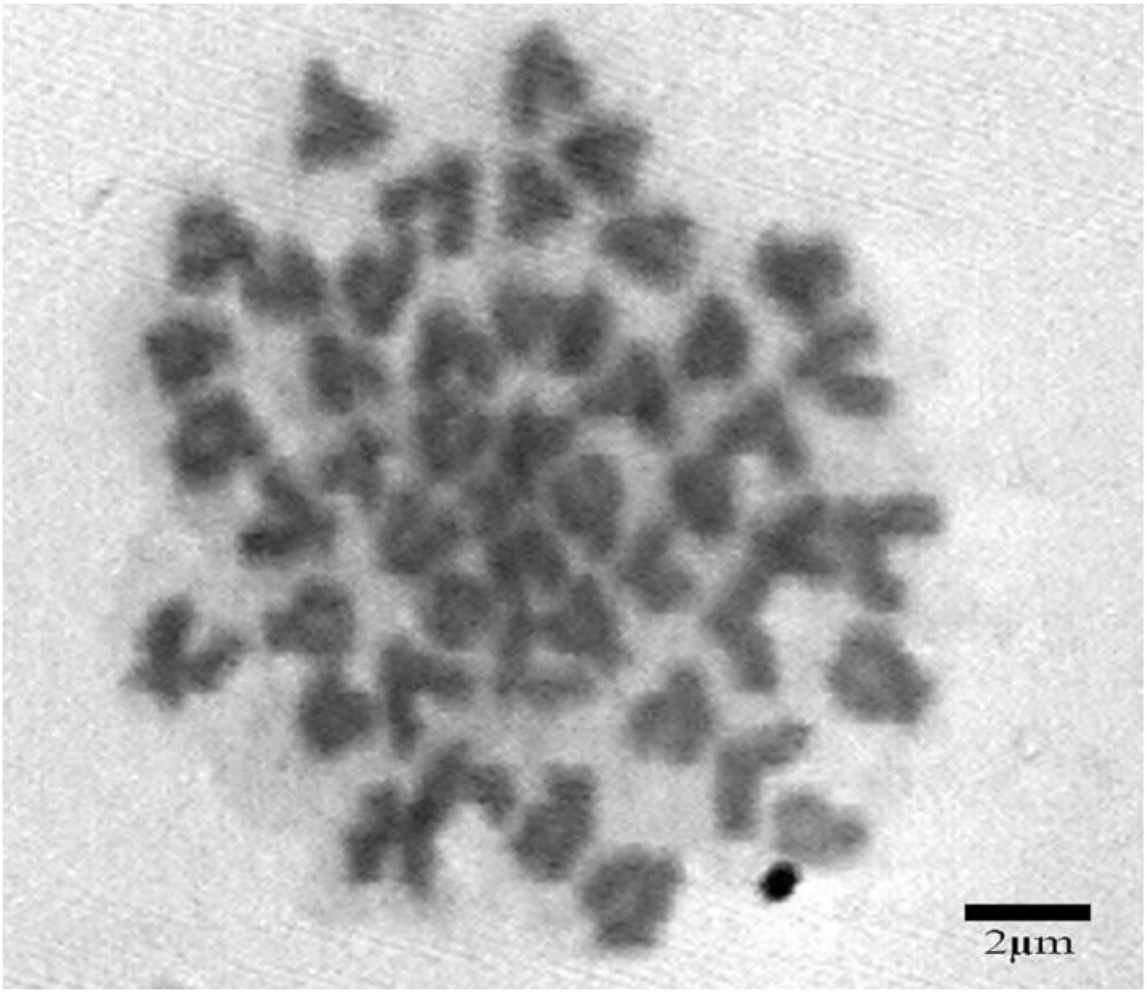

Fig. 2. Giemsa stained chromosome spread of Zagros tooth-carp from Iran

Fig. 3. Giemsa stained karyotype of *Aphanius vladykovi* from Iran

Table 1. Mean relative length, arm ratio and chromosome type of Zagros tooth-carp. St, subtelocentric; Sm, submetacentric

A. vladykovi			
No.	5.41± 0.21	**Arm ratio**	**Type**
1	5.08± 0.10	3.80± 0.19	St
2	4.98± 0.10	3.70± 0.57	St
3	4.82± 0.15	3.41± 0.77	St
4	4.72± 0.11	3.49± 0.51	St
5	4.61± 0.07	3.39± 0.20	St
6	4.50± 0.08	4.72± 2.60	St
7	4.38± 0.06	3.198± 2.37	St
8	4.35± 0.04	3.91± 0.32	St
9	4.31± 0.05	4.02± 1.13	St
10	4.28± 0.06	3.05± 0.40	St
11	4.21± 0.06	3.01± 0.63	St
12	4.17± 0.08	3.12± 0.52	St
13	4.09± 0.09	3.24± 0.48	St
14	4.06± 0.07	3.47± 0.48	St
15	3.98± 0.13	3.52± 0.25	St
16	3.90± 0.10	3.45± 0.52	St
17	3.86± 0.10	3.10± 0.77	St
18	3.75± 0.08	3.70± 2.25	St
19	3.72± 0.08	3.42± 0.72	St
20	3.55± 0.11	2.92± 0.32	Sm
21	3.35± 0.14	2.71± 0.35	Sm
22	3.15± 0.35	3.16± 0.60	St
23	2.78± 0.35	2.71± 0.48	Sm
24	5.41± 0.21	2.37± 0.65	Sm

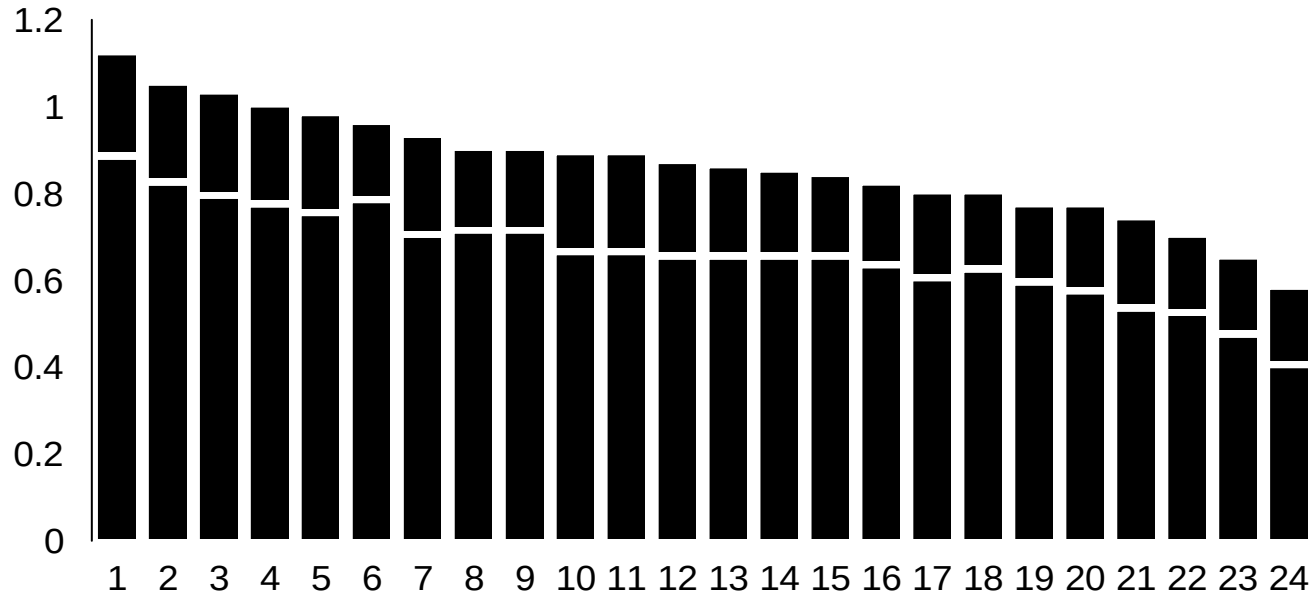

Fig. 4. Haploid idiogram of *A. vladykovi* from Iran

DISCUSSION

According to our observations, the diploid chromosome number of *Aphanius valdykovi* species was 2n= 48 and is in conformation with the chromosome number of other species of this genus. The chromosome number of *A. sophiae* has been reported to be 2n= 48 and that of *A. mento* 2n= 48 [12-14]. The chromosome numbers of *A. dispar, A. asquamatus, A. iberus, A. fasciatus* and *A. persicus* were also 2n= 48 [4-5, 12-13, 15-16]. It can be concluded that the chromosome number in this genus is conservative. The number of chromosomes in this tooth-carp is also similar to that of other species of Cyprinodontidae such as *Orestias agassizii*, *Cyprinodon bovines* and *Cyprinodon macularius* [12-13]. In the order Cyrinodontiformes, the most common fish species which have so far been cytologically investigated, such as *Gambusia holbrooki*, *G. affinis*, *G. hurtadoi*, *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, X. variata* (Goodeidae), have the diploid chromosome number 2n = 48 [12-13,17]. Yet in a few species of Cyprinodontiformes such as *Aphyosemion viride*, *Fundulopanchax sjostedti* (Aplocheilidae); *Allodontichthys hubbsi* and *Ameca splendens* (Goodeidae) the diploid chromosome number is reported to vary from 2n=26 to 2n=42 [12-13]. It could be suggested that the diploid chromosome number of 2n=48 is the modal number in cyprinodont fish.

The karyotype formula of this tooth-carp was 8Sm + 40St and the chromosome arm number was 28. In the present study, no cytological evidence was found for sex chromosome dimorphism in any of the Zagros tooth-carp, which agrees with reports on many fish species [5, 18-19]. In marine fishes also, despite the large number of living species, the occurrence of cytologically differentiated sex chromosomes appears to be rare [6].

Further molecular, cytological, anatomical, morphological and biological investigations towards better recognition and understanding of the genus *Aphanius* in Iran need to be made.

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