Karyological analysis of *Cyprinion macrostomum* Heckel, 1843, from Godarkhosh River, Ilam Province, Iran

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Abstract

In this study, for the first time in Iran, the karyotype of bigmouth Lotak, *Cyprinion macrostomum* Heckel, 1843, was investigated through examining metaphase chromosomes of seven fish with mean weight 30±5g caught by electrofishing from Godarkhosh River in Ilam Province. To stimulate cell divisions, fish were injected intraperitoneally two times by phytohemagglutinin (PHA). The cell divisions were arrested in metaphase stage by intraperitoneal injection of colchicine. Well-separated cells were obtained from kidney and gill filament and chromosome spreads were prepared and stained with giemsa. Karyotype was obtained as 2n=50. The karyotype consisted of 5 metacentric, 12 submetacentric and 8 telocentric chromosome pairs. Centromeric index, arm ratio and Fundamental Number (FN) were determined as 0-50, 1-∞, and 84, respectively.

Keywords: Bigmouth lotak, *Cyprinion macrostomum*, Godarkhosh River; Iran, Karyotype.

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Introduction

The genus *Cyprinion* (Cyprinidae) comprises nine species, among which five are reported from Iran and three from Tigris-Euphrates basin (*C. kais*, *C. macrostomum*and *C. tenuiradius*). The first two species are well distributed in inland waters of Iran, Iraq, Turkey, and Syria (Coad, 1995, 1996, 2015; Epler *et al*., 2001; Eschmeyer and Fricke, 2014; Froese and Pauly, 2015; Keivany *et al*. 2015). In Iran, *C. macrostomum* is named Lotak-e Dahan Bozorg (Big mouth Lotak) (Figure 1). Bigmouth Lotak is edible and fished by natives of the region and considered a valuable species for sport fishing (Abdoli, 2000).

There are some uncertainties about the taxonomy and phylogenetic status of *Cyprinion* species and several authors considered the systematic status of Cyprininae species and genera with their phylogenetic links still doubtful (Howes, 1982). Some researchers considered *C. kais* and *C. macrostomum* as synonyms (Berg, 1949), but Bianco and Banarescu (1982) denoted that they were wrongly considered as synonymous.

Karyology is a useful tool to study the taxonomy and phylogenetic relationships among fishes. The study of fish chromosomes is a routine activity in studying fish biology and taxonomy nowadays (Kalbassi *et al*., 2006; Esmaeili*et al*., 2010; Nasri *et al*., 2010; Okonkwo and Obiakor, 2010; Nezamoleslami *et al*., 2013; Singh *et al*., 2013). By karyological studies, we can obtain basic information including number and morphology of chromosomes to study systematic and evolutionary states of the animals (Macgregor and Varley, 1983). In addition, we can pursuit ancestral karyological changes and fixation in various new species (Winkler *et al*., 2004). Karyological study of fishes has several usages in aquaculture (e.g., to identify chromosome-manipulated fish, fish breeding and the rapid production of inbreed lines) (Chingjiang *et al*., 1986; Gül *et al*., 2004). Due to their smaller and more contracted chromosomes, the main difficulty in working with fish chromosomes is to obtain high quality metaphase spreads (Gül *et al*., 2004).

Howes (1982) reviewed the genus and Durand *et al*. (2002)conducted some phylogenetic and biogeographical studies on *C. macrostomum* and *C. kais* in the Middle East. Patimar and Nasri (2007) studied the age structure and growth of *C. macrostomum* in Ilam Province, Iran. Nasri (2008) studied the taxonomy and Nasri *el al*. (2013) investigated the osteology of *C. macrostomum* and *C. kais* in Karkheh River basin. Karyological analyses of *C. macrostomum* by Gaffaroğlu and Yüksel (2004), Yilmaz *et al*. (2005) and Yüksel and Gaffaroğlu (2008) were conducted in Turkey, but karyological study on this genus in Iran was restricted to *C. tenuiradius* (Esmaeili and Piravar, 2006) and *C. kais* (Nasri *et al*., 2010).

This study is the first karyological analysis of *C. macrostomum* in Iran. The result of this study would shed light on the systematics and taxonomy of the genus and could be used to differentiate between similar species which are morphologically hard to recognize.

Materials and methods

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In November 2007, seven individuals of bigmouth Lotak (mean weight 30±5 g and mean length 12±3 cm) were caught in Godarkhosh River (45°54'3"E and 33°30'16"N) in Ilam Province. through electrofishing. Fish were transferred alive to the Ichthyology Laboratory at Isfahan University of Technology and stored in a 50-liter aquarium with continuous aerationat water temperatures of 15°C for adaptation to laboratory conditions.

To study karyotype, the air-dried chromosome preparation method as described by Thorgaard and Disney (1990) was used with some modifications. To stimulate mitotic divisions, the fish were injected intraperitoneally with Phytohemagglutinin (PHA) (4 $\mu g.g^{-1}$ b.w) in two steps with a 20-hour interval at 20°C. Eight hours after the second PHA injection, fish were divided into two groups (four and three fish) and colchicine was injected intraperitoneally (25 and 50 $\mu g.g^{-1}$ b.w, in the first and second group, respectively) to depress the mitotic division at metaphase stage and left for 7 hours before sacrificing. Kidney and gill filament cells were removed, homogenized and hypotonized simultaneously by tri–sodium citrate 1% for 45 minutes at room temperature. Because of their tiny tissues, the obtained tissues from each group were mixed. Then, samples were centrifuged at 1300 rpm for 10 minutes and supernatant was removed and cold fresh carnoy (3:1 methanol and glacial acetic acid) was added to fix the cells. Samples were stored at 4°C for 30 minutes then centrifuged. This process was repeated three times and carnoy was replaced in 30-minute intervals. After the last centrifugation, cold and fresh carnoy was added and samples were stored at 4°C. Smears were prepared using splash method (cold lamella) and air dried for 24 hours, then, stained with giemsa 10%. Metaphasic chromosomes were analyzed and photographed using a Nikon microscope model Fujix Digital Camera, HC–300zi by 100x magnification lens, immersion oil, and blue photo filter.

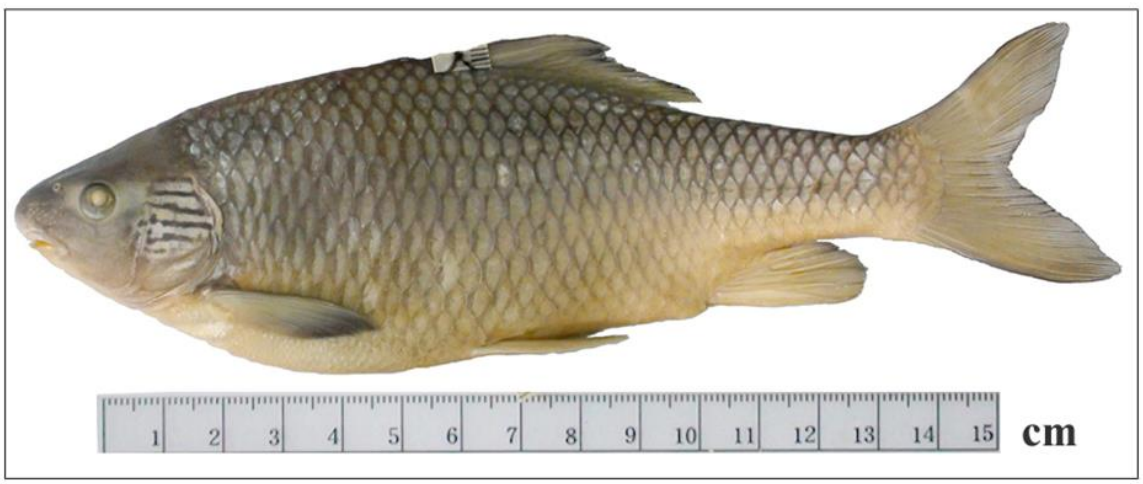

Figure 1: *Cyprinion macrostomum* from Godarkhosh River (Karkheh River basin).

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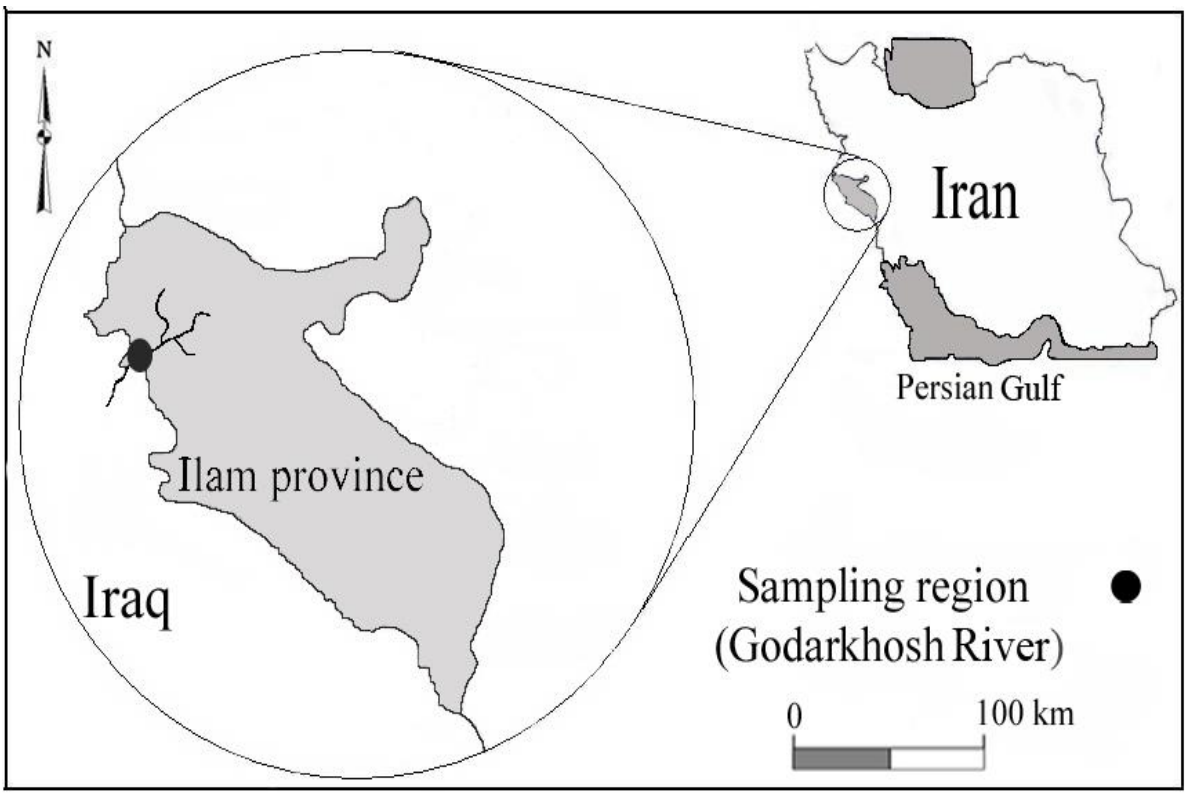

Figure 2: Map of the study area showing the Godarkhosh River (sampling region) and its position in Ilam Province in Western Iran.

About 120 metaphasic plates were counted and a proper plate was selected to obtain karyotype formulae and karyogram. Measurements were performed by Adobe Photoshop CS5 professional software. Calculation of data and drawing the ideogram were performed in Microsoft Office Excel 2010 software.

For each chromosome, centromeric index (I=100 S/C), (*S*: short arm length &*C*: total length of chromosome), arm ratio (R = L/S), (L: long arm) and relative chromosomes length (R=100×C/L), (L: summation of all chromosomes length) were calculated as described by Levan *et al*. (1964) and the Fundamental Number (FN) was calculated. Preparation and ranking of chromosomes were performed using Levan *et al*. (1964) method, with some modifications, and metacentric, submetacentric and telocentric chromosomes were denoted.

Results

One hundred and twenty metaphase plates of the seven specimens of *C. macrostomum* were counted. The diploid number per each metaphase plate ranged between 35 and 57. Diploid number of 2n=50 constituted 60% and 2n=48 constituted 18.33% of the metaphase plates (Table 1). Using a proper metaphase plate (Figure 3A) and based on chromosomal indicators (Table 2), chromosomal formulae was obtained as 5 metacentric, 12 submetacentric and 8 telocentric. Centromeric index, arm ratio and Fundamental Number (FN) were determined as 0-50, 1-∞, and 84, respectively. The largest chromosome was a submetacentric (5.62 μm) and the smallest

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was a telocentric one (2.23 μm) (Figure 3). Based on the chromosomal indicators (Figure 3 and Table 2), a karyogram (Figure 3B) was drawn and an ideogram was depicted. The diploid numbers, rather than 2n=50 (Table 1), are usually the result of losses or additions from nearby cells during preparation or other artifacts as reported in other studies (Gül *et al.*, 2004; Esmaeili and Piravar, 2006).

Table 1: Abundance of chromosomes in the counted plaques of *Cyprinion macrostomum*.

Number of Chromosomes in Each Plaque	35	45	47	48	49	50	51	52	54	57
Number of Metaphase Plates	2	3	5	22	2	72	6	5	2	1
Frequency %	1.66	2.5	4.16	18.33	1.66	60	5	4.16	1.66	0.83

Table 2: Centromeric index in *Cyprinion macrostomum* (m: metacentric; sm: sub metacentric; t: telocentric).

	Short arm	**Long arm**	**Chromosome length**	**Arm ratio**	**Centromeric index**	**Relative arm length %**	**Chromosome form**	**Arms Number**
1	2.31	2.31	4.62	1	50	4.79	m	4
2	2.3	2.3	4.6	1	50	4.47	m	4
3	2.11	2.11	4.22	1	50	4.38	m	4
4	2.07	2.07	4.14	1	50	4.3	m	4
5	1.96	1.96	3.92	1	50	4.07	m	4
6	1.7	3.92	5.62	2.31	30.25	5.84	sm	4
7	1.8	3.3	5.1	1.83	35.29	5.3	sm	4
8	1.38	3.3	4.68	2.39	29.49	4.86	sm	4
9	1.7	2.9	4.6	1.71	36.96	4.78	sm	4
10	1.23	3.3	4.53	2.68	27.15	4.71	sm	4
11	1.42	2.92	4.34	2.06	32.72	4.51	sm	4
12	1.7	2.53	4.23	1.49	40.19	4.4	sm	4
13	1.57	2.46	4.03	1.57	38.96	4.19	sm	4
14	1.42	2.58	4	1.82	35.5	4.16	sm	4
15	1.3	2.23	3.53	1.72	36.83	3.67	sm	4
16	1.19	2.15	3.34	1.81	35.63	3.47	sm	4
17	0.92	2.19	3.11	2.38	29.58	3.23	sm	4
18	0	3.42	3.42	∞	0	3.55	t	2
19	0	3.3	3.30	∞	0	3.43	t	2
20	0	3.23	3.23	∞	0	3.35	t	2
21	0	3.2	3.20	∞	0	3.32	t	2
22	0	3.07	3.07	∞	0	3.19	t	2
23	0	2.84	2.84	∞	0	2.30	t	2
24	0	2.65	2.65	∞	0	2.75	t	2
25	0	2.23	2.23	∞	0	2.31	t	2
total	27.24	69	96.24	-	-	100	-	84

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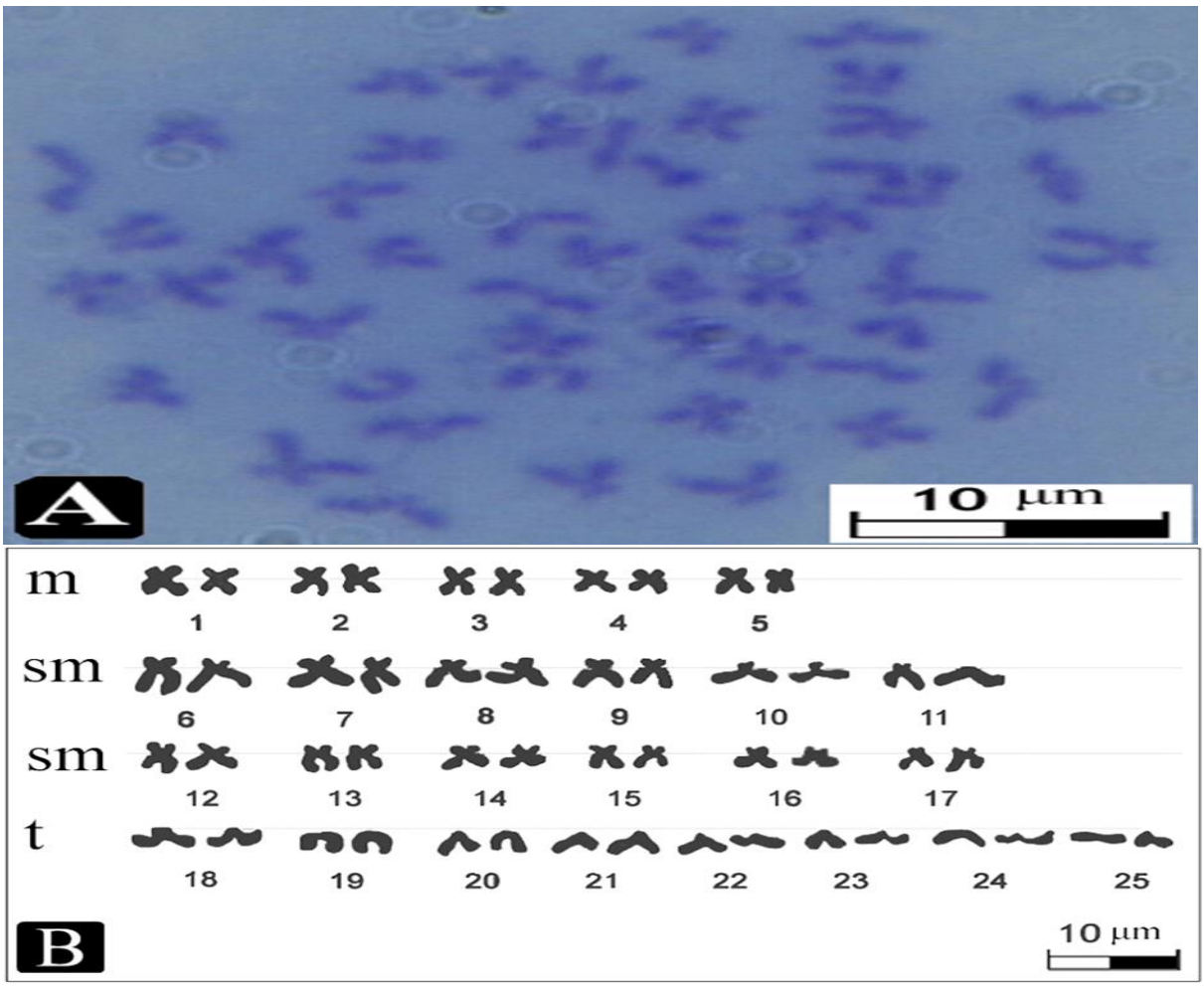

Figure 3: Chromosomal spread (A) and karyogram (B) of *Cyprinion macrostomum*.

Table 3: Chromosome formulae of *Cyprinion* species obtained by various authors.

Species	2n	Chromosome formula				NF	Region	Author
		m	sm	st	t			
C. macrostomum	48	2	13	9	-	-	Turkey	(Colak *et al*., 1985)
	48	-	-	-	-	-	Turkey	(Ünlü *et al*., 1997)
	50	3	13	9	-	82	Turkey	(Kılıç-Demirok, 2000)
	50	3	12	6	4	92	Turkey	(Gaffaroğlu and Yüksel, 2004)
	50	3	12	6	4	92	Turkey	(Muhammet and Eşref, 2004)
	50	3	12	6	4	92	Turkey	(Muhittin *et al.*, 2005)
C. macrostomum	50	3	12	6	4	92	Turkey	(Yilmaz *et al.*, 2005)
	50	3	12	6	4	92	Turkey	(Eşref and Muhammet, 2008)
	50	3	12	6	4	92	Turkey	(Yüksel and Gaffaroğlu, 2008)
	50	5	12	-	8	84	Iran	This study
C. tenuiradius	50	13	5	-	7	86	Iran	(Esmaeili and Piravar, 2006)
C. kais	50	8	7	3	7	86	Iran	(Nasri *et al*., 2010)

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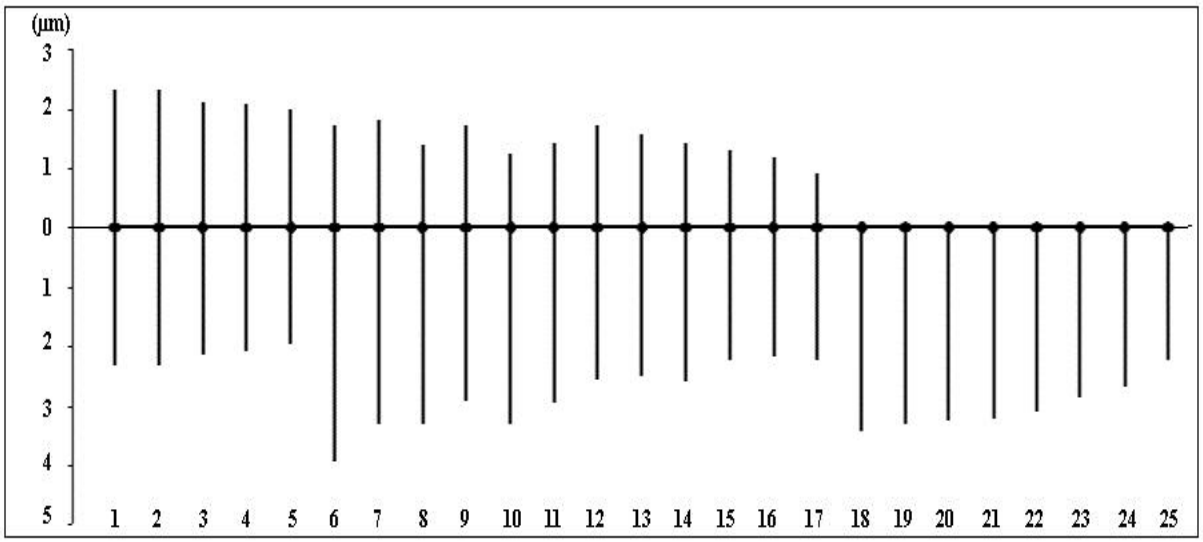

Figure 4: Ideogram of *Cyprinion macrostomum*. Chromosomes arranged according to their forms and grouped as metacentric (1-5), sub metacentric (6-17) and telocentric (18-25).

Discussion

Studying and measuring fish chromosomes is somehow difficult because of their smaller and more contracted structure than those of mammals (Gül *et al*., 2004). Another problem is that fish karyotypes are not identical as in other animal species, so we cannot have a standard karyotype for fish, because polymorphism are seen not only between species but also within the same fish species (Al-Sabti, 1991). According to studies performed by various methods on *C. macrostomum* in Turkey (Gaffaroğlu and Yüksel, 2004; Muhammet and Eşref, 2004; Muhittin *et al.*, 2005; Yilmaz *et al*., 2005; Eşref and Muhammet, 2008; Yüksel and Gaffaroğlu, 2008) on *C. tenuiradius* (Esmaeili and Piravar, 2006; Nasri *et al*., 2010) and *C. kais* in Iran (Esmaeili and Piravar, 2006; Nasri *et al*., 2010) and on *C. macrostomum* in the present study, it seems that 2n=50 in the genus *Cyprinion*, as in many other cyprinids, is a generality. Despite the similarity of diploid numbers in species of *Cyprinion*, there are some differences in their karyotype formula (**Error! Reference source not found.**). Colak *et al*. (1985) and Kılıç-Demirok (2000) did not recognize any teleocentric chromosomes in their populations. Gaffaroğlu and Yüksel (2004), Muhammet and Eşref (2004), Muhittin *et al.* (2005), Yilmaz *et al.* (2005), Eşref and Muhammet, 2008; and Yüksel and Gaffaroğlu (2008) recognized four teleocentric and six subteleocentric chromosomes in their populations in Turkey. We recognized eight teleocentric but no subteleocentric chromosomes in the population in Iran. The differences between *C. tenuiradius*, *C. kais* and *C. macrostomum* are normal, but the differences between *C. macrostomum* populations in Turkey and Iran, are thought to be chromosomal polymorphism. However, it could be also due to misinterpretation of the data. The other reasonable interpretation is that we might be dealing with two different species of *Cyprinion* in Iran and Turkey. The latter

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interpretation needs further examination of these populations in the two countries. Molecular analyses, especially Cyt-b sequencing could be fruitful. However, based on the present data and abundance of diploid number of 2n=50 with 60% and 2n=48 with 18.33%, we can assume dimorphism for the diploid number in this species. Such differences were observed in some other species, such as the grass carp (Al-Sabti, 1987), common carp, and *Squalius* (*Leuciscus*) *cephalus orientalis* (Al-Sabti, 1986) and *Gara rufa* (Nezameslami *et al*., 2015).

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