

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

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Received: May 2012

Accepted: December 2014

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 ± 5 g 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; Esmaeiliet *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 *et 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

In November 2007, seven individuals of bigmouth Lotak (mean weight 30 ± 5 g and mean length 12 ± 3 cm) were caught in Godarkhosh River ($45^{\circ}54'3''\text{E}$ and $33^{\circ}30'16''\text{N}$) 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 aeration at 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) ($4 \mu\text{g}\cdot\text{g}^{-1}$ 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 $50 \mu\text{g}\cdot\text{g}^{-1}$ 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.

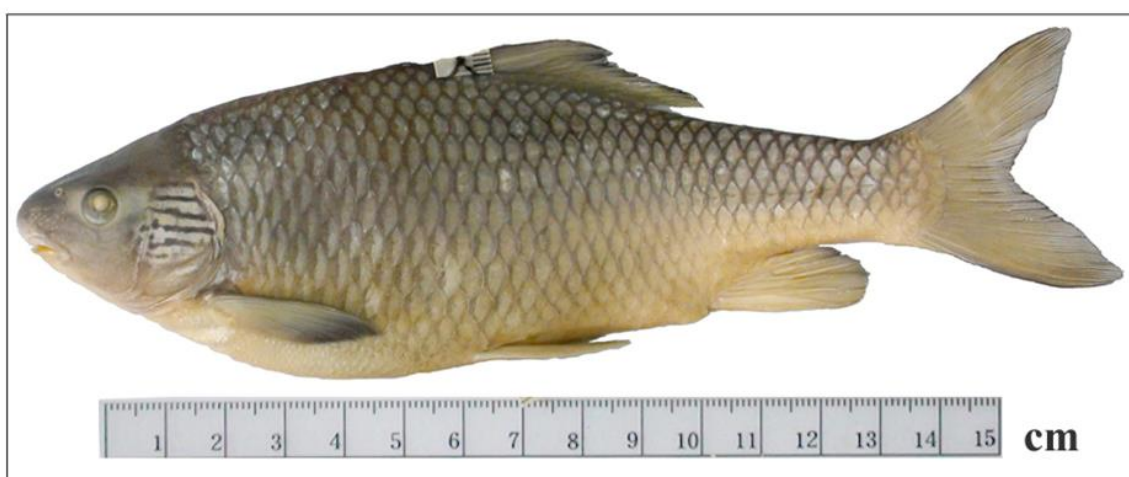


Figure 1: *Cyprinion macrostomum* from Godarkhosh River (Karkheh River basin).

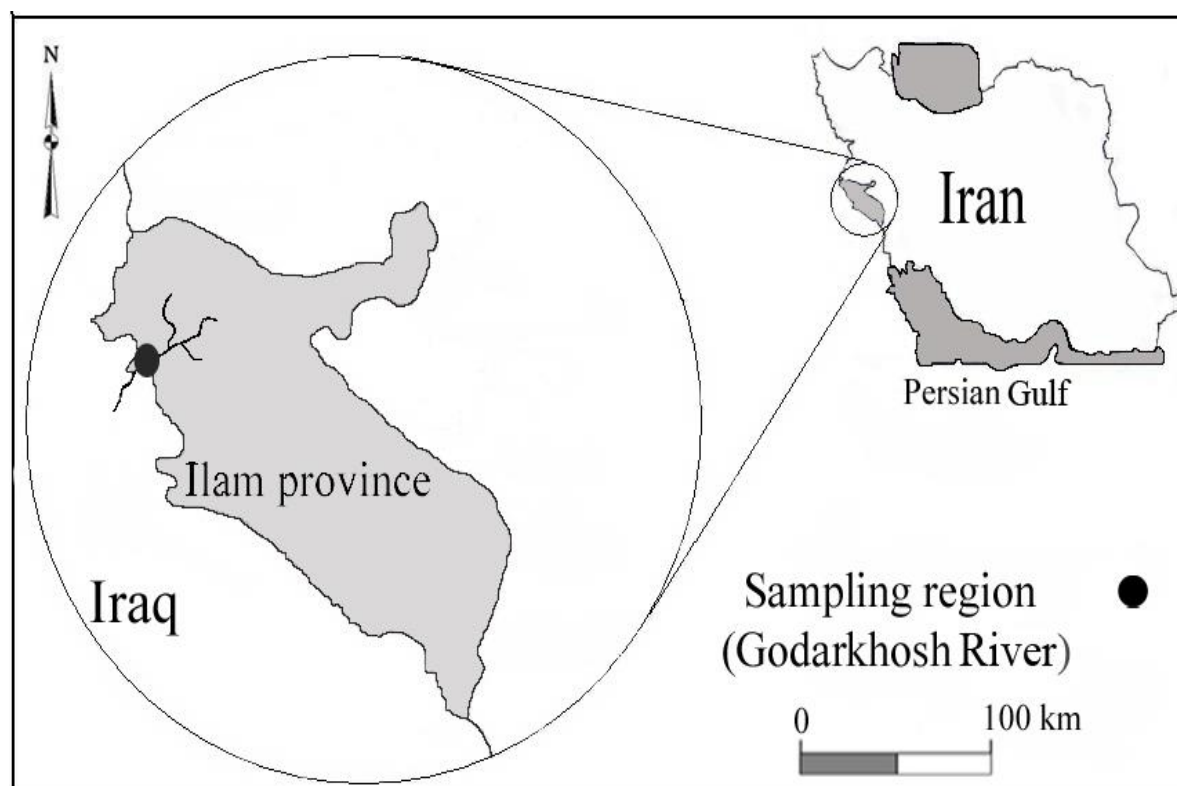


Figure 2: Map of the study area showing the Godarkhosh River (sampling region) and its position in Ilam Province in Western Iran.

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 ($R=100 \times C/L$), (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

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

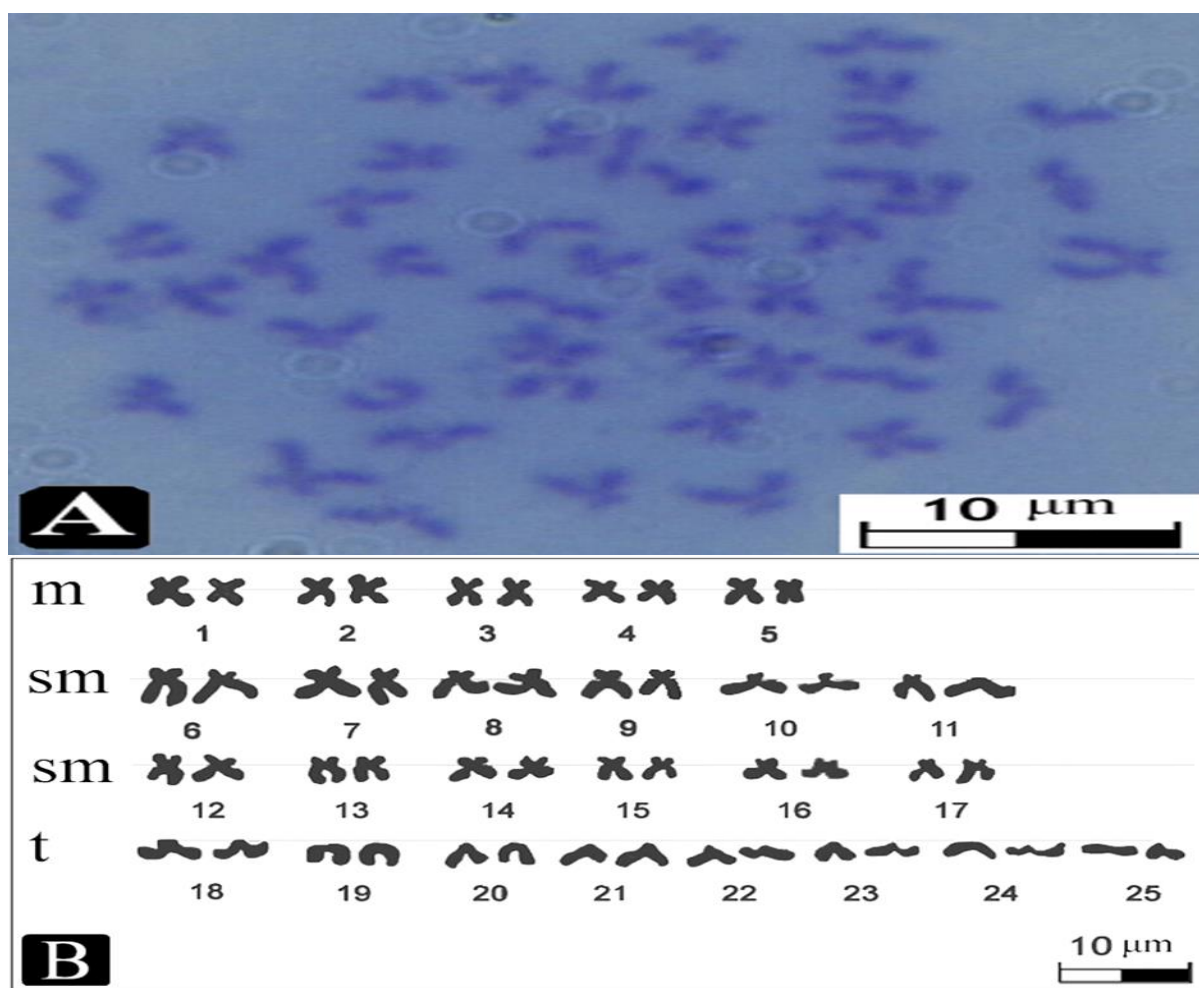


Figure 3: Chromosomal spread (A) and karyogram (B) of *Cyprinion macrostomum*.

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

Species	2n	Chromosome formula				NF	Region	Author
		m	sm	st	t			
<i>C. macrostomum</i>	48	2	13	9	-	-	Turkey	(Colak <i>et al.</i> , 1985)
	48	-	-	-	-	-	Turkey	(Ünlü <i>et al.</i> , 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 <i>et al.</i> , 2005)
<i>C. macrostomum</i>	50	3	12	6	4	92	Turkey	(Yılmaz <i>et al.</i> , 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
<i>C. tenuiradius</i>	50	13	5	-	7	86	Iran	(Esmaeili and Piravar, 2006)
<i>C. kais</i>	50	8	7	3	7	86	Iran	(Nasri <i>et al.</i> , 2010)

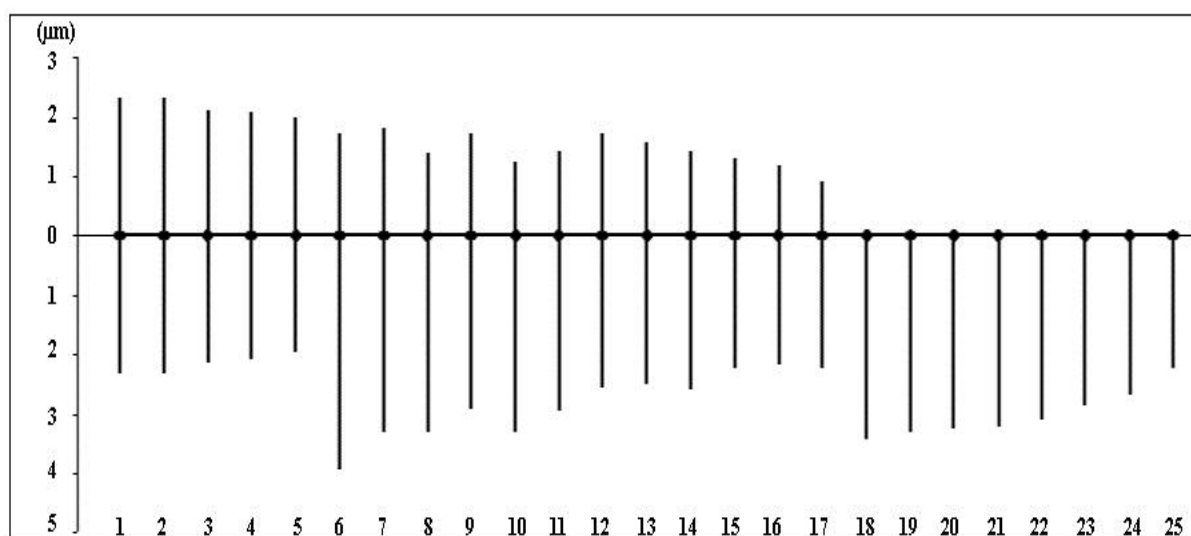


Figure 4: Ideogram of *Cyprinion macrostomum*. Chromosomes arranged according to their forms and grouped as metacentric (1-5), sub metacentric (6-17) and telocentric (18-25).

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* (Esmaili and Piravar, 2006; Nasri *et al.*, 2010) and *C. kais* in Iran (Esmaili 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

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).

Acknowledgements

We would like to thank Mr. G. Abdali, Environmental Protection, Ilam Headquarter, Iran, Dr. M.R. Sabzalian and Miss. S. Mahjoori, Department of Agronomy and Plant Breeding, Faculty of Agriculture, Isfahan University of Technology. This project was financially supported by Isfahan University of Technology.

References

- Abdoli, A., 2000.** The inland water fishes of Iran. Tehran: Naghsh Mana Publication. 378P.
- Al-Sabti, K., 1986.** Karyotypes of *Cyprinus carpio* and *Leuciscus cephalus orientalis*. *Cytobios*, 47, 19-25.
- Al-Sabti, K., 1987.** Cytogenetic studies on five species of Pisces from Yugoslavia. *Cytobios*, 49, 175-188.
- Al-Sabti, K., 1991.** Handbook of genotoxic effects and fish chromosomes. Ljubljana: Joseph Stephane Institute. 221P.
- Berg, L.S., 1949.** Freshwater fishes of Iran and adjacent countries). *Trudy Zoologicheskogo Instituta Akademii Nauk SSSR*, 8, 783-858.
- Bianco, P.G. and Banarescu, P., 1982.** A contribution to the knowledge of the Cyprinidae of Iran (Pisces, Cypriniformes). *Cybium*, 6, 75-96.
- Chingjiang, W., Yuzhen, Y. and Rongde, C., 1986.** Genome manipulation in carp (*Cyprinus carpio* L.). *Aquaculture*, 54(1-2), 57-61.
- Coad, B.W., 1995.** Freshwater fishes of Iran. *Acta Scientiarum Naturalium Academiae Scientiarum Bohemicae Brno*, 29(1), 1-64.
- Coad, B.W., 1996.** Zoogeography of the fishes of the Tigris-Euphrates basin. *Zoology in the Middle East*, 13, 51-70.
- Coad, B.W., 2015.** *Freshwater fishes of Iran* [online]. Ottawa. Available from: www.briancoad.com [Accessed 11 June 2014].
- Colak, A., Sezgin, I. and Süngü, Y.S., 1985.** Sazangiller familyası (Cyprinidae) Öna ait beni balýúýnda (*Cyprinion macrostomus* Heckel, 1843) kromozomal araštýrmalar. *Turkish Journal of Biology*, 9(2), 193-195.
- Durand, J.D., Tsigenopoulos, C.S., Ünlü, E. and Berrebi, P., 2002.** Phylogeny and biogeography of the family Cyprinidae in the middle East inferred from cytochrome b DNA—evolutionary significance of this region. *Molecular Phylogenetics and Evolution*, 22(1), 91-100.
- Epler, P., Bartel, R., Szczerbowski, J.A. and Szyputa, J., 2001.** The ichthyofauna of lakes Habbaniya,

- Tharthar and Razzazah. *Archives of Polish Fisheries*, 9, 171-184.
- Eschmeyer, W.N. and Fricke, R., 2014.** Catalog of fishes electronic version [online]. California Academy of Sciences. Available from: <http://research.calacademy.org/research/ichthyology/catalog/fishcatmain.asp> [Accessed 14 June 2014].
- Esmaeili, H.R. and Piravar, Z., 2006.** On the karyotype of *Cyprinion tenuiradius* Heckle, 1849 (Pisces: Cyprinidae) from the Southwest of Iran. *Zoology in the Middle East*, 39, 75-80.
- Esmaeili, H.R., Zareian, H., Gholamhosseini, A., Ebrahimi, M., Gholami, Z., Teimori, A. and Ansari, T.H., 2010.** Karyotype analysis of the king nase fish, *Chondrostoma regium* (Heckel, 1843) (Actinopterygii: Cyprinidae) from Iran. *Turkish Journal of Fisheries and Aquatic Sciences*, 10, 477-481.
- Eşref, Y. and Muhammet, G., 2008.** NOR phenotype of *Cyprinion macrostomus* (Osteichthyes, Cyprinidae). *Journal of Fisheries Science*, 2(2), 114-117.
- Froese, R. and Pauly, D., 2015.** *FishBase* [online]. Available from: www.fishbase.org [Accessed 14 June 2014].
- Gaffaroğlu, M. and Yüksel, E., 2004.** Karyotype analysis of *Cyprinion macrostomus* Heckel, 1843 (Pisces: Cyprinidae). *Gazi Üniversitesi Kirsehir Eğitim Fakültesi*, 5(2), 235-239.
- Gül, S., Çolak, A., Sezgin, İ. and Kaloğlu, B., 2004.** Karyotype analysis in *Alburnus heckeli* (Battalgil, 1943) from Lake Hazer. *Turkish Journal of Veterinary and Animal Sciences*, 28, 309-314.
- Howes, G.J., 1982.** Anatomy and evolution of the jaws in the semiplotine carps with a review of the genus *Cyprinion* Hechel, 1843 (Teleostei: Cyprinidae). *Bulletin of the British Museum (Natural History) Zoology*, 42(4), 299-335.
- Kalbassi, M.R., Dorafshan, S., Tavakolian, T., Khazab, M. and Abdolhay, H., 2006.** Karyological analysis of endangered Caspian salmon, *Salmo trutta caspius* (Kessler, 1877). *Aquaculture Research*, 37(13), 1341-1347.
- Keivany, Y., Nasri, M., Abbasi, K. and Abdoli, A., 2015.** Atlas of inland water fishes of Iran. Iran Department of Environment Press, Tehran. In press.
- Kılıç-Demirok, N., 2000.** Dicle su sisteminde yaşayan bazı Cyprinid tür ve alttürlerinin kromozomları üzerine çalışmalar. (Ph.D.). Dicle Üniversitesi.
- Kilic-Demirok, N. and Ünlü, E., 2001.** Karyotypes of Cyprinid fish *Capoeta trutta* and *Capoeta capoeta umbla* (Cyprinidae) From the Tigris River. *Turkish Journal of Biology*, 25, 389-393.
- Levan, A., Fredga, K. and Sandberg, A. A., 1964.** Nomenclature for centromeric position on chromosomes. *Hereditas*, 52, 201-220.
- Macgregor, H.C. and Varley, J.M., 1983.** Working with animal chromosomes. John Wiley and Sons Ltd. 266P.
- Muhammet, G. and Eşref, Y., 2004.** Karyotype analysis of *Cyprinion macrostomus*, (Heckel, 1843) (Pisces: Cyprinidae). *Gazi Üniversitesi*.

- Kirşehir Eğitim Fakültesi, Cilt*, 5(2), 235-239.
- Muhittin, Y., Yılmaz, Ç., Yusuf, T. and Muhammet, G., 2005.** A taxonomic study on *Orthrias insignis euphraticus* (Banarescu and Nalbant, 1964) and *Cyprinion macrostomus* (Heckel, 1843) by sarcoplasmic protein electrophoresis. *Gazi Üniversitesi Journal of Sciences*, 18(1), 61–68.
- Nasri, M., 2008.** Taxonomy of bigmouth lotak (*Cyprinion macrostomum* Heckel, 1843) and smallmouth lotak (*Cyprinion kais* Heckel, 1843) in Karkheh River basin and Godarkhosh River in Ilam Province. (MSc). Isfahan University of Technology.
- Nasri, M., Keivany, Y. and Dorafshan, S., 2010.** First karyological analysis of smallmouth lotak, *Cyprinion kais* Heckel, 1843, an endemic cyprinid fish from Tigris-Euphrates basin. *Italian Journal of Zoology*, 77(3), 272-276.
- Nasri, M., Keivany, Y. and Dorafshan, S., 2013.** Comparative osteology of lotaks, *Cyprinion kais* and *C. macrostomum* (Cypriniformes, Cyprinidae), from Godarkhosh River, Western Iran. *Journal of Ichthyology*, 53(6), 455–463.
- Nezameslami, A., Keivany, Y. and Dorafshan, S., 2015.** Karyology of *Garra rufa* from Semirom River, Isfahan Province (Actinopterygii: Cyprinidae). *Cell and Tissue*, 6: In press.
- Okonkwo, J.C. and Obiakor, M.O., 2010.** Karyological and Chromosomal Study of Catfish (Clariidae, Clarias gariepinus). *Pakistan Journal of Nutrition*, 9(2), 112-115.
- Patimar, R. and Nasri, M., 2007.** Investigation on age structure and growth of Lotak *Cyprinion macrostomum* Heckel, 1843, in Seimareh River, Ilam Province. *Journal of Agricultural Sciences and Natural Resources (Animal and Aquatic Sciences)*, 5(2), 12-22.
- Singh, S.S., Singh, C.B., and Waikhom, G., 2013.** Karyotype analysis of the new catfish *Mystus ngasep* (Siluriformes: Bagridae) from Manipur, India. *Turkish Journal of Fisheries and Aquatic Sciences*, 13, 179-185.
- Thorgaard, G.H. and Disney, J.E., 1990.** Chromosome preparation and analysis. In: Schreck, C.B. and Moyle, P.B. eds. *Methodes for fish biology*. Bethesda, Maryland: American Fisheries Society, pp. 171-190.
- Ünlü, E., Kilic-Demirok, N., Cengiz, E.I. and Karadede, H., 1997.** Karyology of *Garra rufa* (Cyprinidae) in River Tigris (Turkey). 9th International Congress of European Ichthyologists (CEI9) (Fish Biodiversity). Italy, 95P.
- Winkler, F.M., García-Melys, D. and Palma-Rojas, C., 2004.** Karyotypes of three South East Pacific flounder species of the family Paralichthyidae. *Aquaculture Research*, 35(13), 1295-1298.
- Yilmaz, M., Çiğremiş, Y., Türköz, Y. and Gaffaroğlu, M., 2005.** A taxonomic study on *Orthrias insignis euphraticus* (Banarescu and Nalbant, 1964) and *Cyprinion macrostomus* (Heckel, 1843) by sarcoplasmic protein electrophoresis. *Gazi University Journal of Science*, 18(1), 61-68.

Yüksel, E. and Gaffaroğlu, M., 2008.

NOR phenotype and ploidy level of *Cyprinion macrostomus* (Osteichthyes, Cyprinidae). *Journal of Fisheries Sciences*, 2(2), 114-117.