www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 10: 477-481 (2010) DOI: 10.4194/trjfas.2010.0406

Karyotype Analysis of the King Nase Fish, *Chondrostoma regium* (Heckel, 1843) (Actinopterygii: Cyprinidae) from Iran

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Received 26 June 2009
Accepted 26 August 2010

Abstract

The karyotypic and cytological characteristics of a native cyprinid fish of Iran *Chondrostoma regium* (Heckel, 1843) have been investigated by examining metaphase chromosomes spreads obtained from gill epithelial and kidney. The diploid chromosome number of this species was 2n=52. The karyotype consisted of 21 pairs of submetacentric and 5 pairs of subtelocentric chromosomes (12Sm, 5St). The arm number (NF) was 58. No heteromorphic sex chromosomes were cytologically detected. Chromosome number, karyotype formula and arm number of *C. regium* differentiate it from other closely related species. The results may be used for population studies, management and conservation programs.

Keywords: *Chondrostoma regium*, karyotype, chromosome, idiogram, Middle East.

Kababurun Balığının, *Chondrostoma regium (*Heckel, 1843) (Actinopterygii: Cyprinidae), Karyotip Analizi, İran

Özet

İran'ın yerli sazangil türü olan *Chondrostoma regium* (Heckel, 1843)'un karyotipik ve sitolojik özellikleri, solungaç epitelyumundan ve böbrekten elde edilen metafaz kromozom yayılımlarının incelenmesiyle araştırılmıştır. Bu türün diploid kromozom sayısı 2n=52 olarak tespit edilmiştir. Karyotip, 21 submetasentrik ve 5 subtelosentrik kromozom çiftinden oluşmaktadır (12Sm, 5St). Kol sayısı (NF) 58 olarak tespit edilmiştir. Sitolojik olarak hiçbir heteromorfik cinsiyet kromozomu bulunmamıştır. *C. regium*'un kromozom sayısı, karyotip formülü ve kol sayısı, bu türü birbirine yakın olan diğer türlerden ayırmaktadır. Sonuçlar; popülasyon çalışmaları, yönetim ve koruma programları için kullanılabilir.

Anahtar Kelimeler: *Chondrostoma regium*, karyotip, kromozom, idiogram, Ortadoğu

Introduction

Cypriniformes or carps are a group of freshwater fishes with 6 families, 321 genera and about 3,268 species found throughout the world except Australia and South America (Nelson, 2006). The Cyprinidae or minnows are found in North America, Africa and Eurasia. This family with 220 genera and about 2,420 species is the largest family of freshwater fishes and with possible exception of Gobiidae, the largest family of vertebrates (Nelson, 2006). They are mostly small (<5 cm) although some are very large (3 m). About 73 species of cyprinid fish are recognized from Iran. The genus *Chondrostoma* is one of this diverse group. There are about 26 species of which 2 are known for Iran (Elvira, 1997) including *Chondrostoma regium* (Heckel, 1843) and *C. cyri* (Kessler, 1877).

The king nase, *Chondrostoma regium* is found throughout the Tigris-Euphrates basin and the Mediterranean basins of south-eastern Turkey and the northern Levant (Coad, 2009). In Iran, it is found in the Tigris River and Kor River basins. Additional localities are Kermanshah, the Marun River, the Hawr al Azim marsh (Wossughi, 1978; Abdoli, 2000; Coad, 2009). Ghorbani Chafi (2000) lists the Bazoft and Kuhrang Rivers in the upper Karun River basin and also the Zayandeh River of the Esfahan basin of Iran (Coad, 2009). In Iran, it is known as jokhorak, nazok, nazi; heif-e nan (=waste of bread, i.e. valueless) in Khuzestan; siah deem and siah dom, (meaning blacktail) and kapur puzeh dar) (Coad, 2009).

Cytogenetic studies in fishes have not been comprehensive when compared to other vertebrate

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in cooperation with Japan International Cooperation Agency (JICA), Japan

groups in Iran (Kalbassi *et al*., 2008). In this respect, the most karyological studies in Iran consist of *Abramis brama* (Nahavandi *et al*., 2001), *Ctenopharyngodon idella* (Nowruzfakhshami *et al.,* 2002), *Petroleuciscus persidis* and *Cyprinion tenuiradius* (Esmaeili and Piravar, 2006a, 2006b), *Iranocichla hormuzensis and Mastacembelus mastacembelus* (Esmaeili *et al*., 2006a, 2006b), *Schizothorax zarudnyi* (Kalbassi *et al*., 2008), *Aphanius* spp. (Esmaeili *et al*., 2008) and *Garra persica* (Esmaeili *et al*., 2009). However *Chondrostoma regium* has been described and compared morphologically, but it's karyotype has not been investigated yet.

As study of fish chromosomes has received considerable attention in recent years because of its usefulness in addressing problems of classification, evolution and heredity, this first report, could provide the detailed karyological features of this endemic carp from Iran.

Material and Methods

Chondrostoma regium specimens (Figure 1) were collected from Fahlian River (Figure 2), Nur Abad (Fars province, south, Iran) (30°11'06.2" N., 51°31'27.9" E., alt. 919 m) using electro fishing device and dip net. The other fish species including *Copeta trutta*, *Copoeta aculeata, Barbus lutes, Barbus sublimus, Cyprinion macrostomum, Alburnus mossulensis* and *Barilius mesopotamicus* Berg, 1932 (Cyprinidae) and *Nemacheilus* sp. (Balitoridae) and *Mastacembelus mastacembelus* (Mastacembelidae) were also collected from this river. The fishes 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 (1986) was used. Colchicine solution was prepared with 0.005 g in 20 ml physiological serum. The 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 these 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 5 ml fresh and cold fixative was added to 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

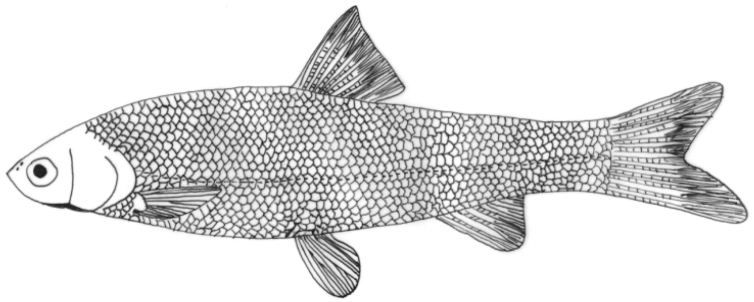

Figure 1. *Chondrostoma. regium* from Iran

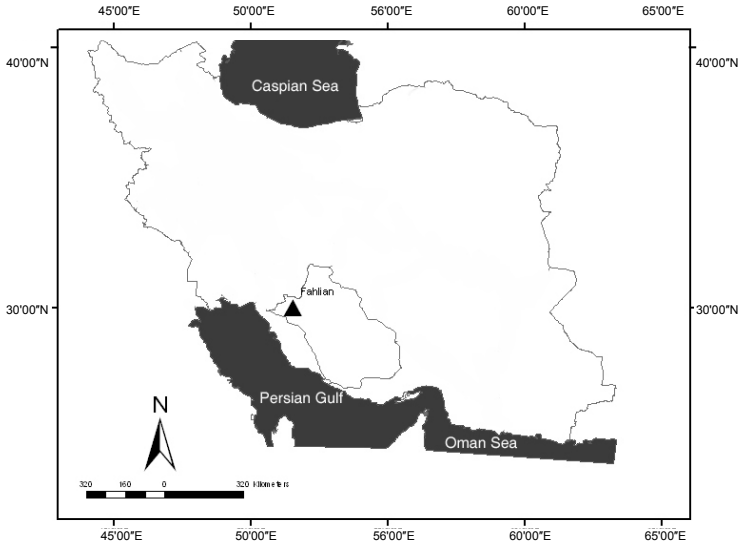

Figure 2. Collection site of *C. regium*, Fahlian River, Nur Abad, Fars province, Iran.

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*. (1964). Fundamental number (NF) expressed as of twice the number of atelocentric plus the number of telocentric chromosomes. The idiogram was prepared in Harvard Graphics 2.0 software.

Results

Metaphase spread of this species is given in Figure 3a. The diploid chromosome number in was 2n=52 (Figure 3b). The quantitative data of the different measurements used to classify chromosomes and idiogram are given in Table 1 and Figure 4. The karyotype consisted of 21 submetacentric and 5 subtelocentric (21Sm, 5St), and the arm number was 58. No heteromorphic elements indication sex chromosomes were detected in this native carp.

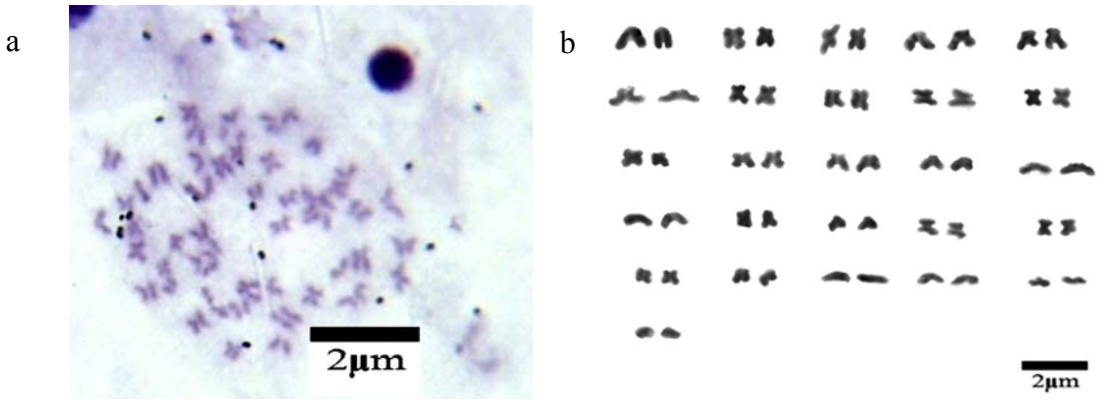

Figure 3. Giemsa stained chromosome spread (a) and karyotype (b) of *C. regium* from Iran.

Table 1. Chromosome measurements (in µm) and classification of *C. regium* chromosomes

Chromosome number	Chromosome type	Arm value	Short arm length (µm)	Long arm length (µm)
1	St	3.20	0.17±0.14	0.54±0.02
2	Sm	1.76	0.25±0.05	0.45±0.08
3	Sm	1.70	0.25±0.07	0.42±
4	Sm	1.80	0.23±0.05	0.42±0.10
5	Sm	1.95	0.21±0.01	0.40±0.05
6	Sm	2.29	0.17±0.08	0.40±0.06
7	Sm	2.31	0.18±0.04	0.42±0.06
8	Sm	1.93	0.20±0.05	0.39±0.02
9	Sm	2.95	0.14±0.07	0.41±0.05
10	Sm	Sm	0.17±0.05	0.40±0.1
11	Sm	Sm	0.18±0.02	0.38±0.01
12	Sm	1.84	0.21±0.01	0.39±0.01
13	Sm	Sm	0.20±0.02	0.35±0.04
14	Sm	Sm	0.20±0.01	0.35±0.02
15	Sm	Sm	0.20±0.02	0.34±0.01
16	Sm	Sm	0.14±0.08	0.39±0.1
17	Sm	Sm	0.21±0.03	0.32±0.03
18	Sm	Sm	0.17±0.1	0.34±0.09
19	Sm	1.55	0.21±0.03	0.32±0.03
20	St	5.00	0.08±0.01	0.40±0.05
21	Sm	2.04	0.15±0.03	0.31±0.03
22	Sm	2.07	0.14±0.06	0.29±0.03
23	St	3.46	0.09±0.02	0.32±0.06
24	St	6.44	0.05±0.04	0.34±0.02
25	Sm	2.89	0.09±0.05	0.26±0.05
26	St	3.79	0.06±0.02	0.24±0.02

m: Metacentric; Sm: Submetacentric; St: Subtelocentric; t: Telocentric

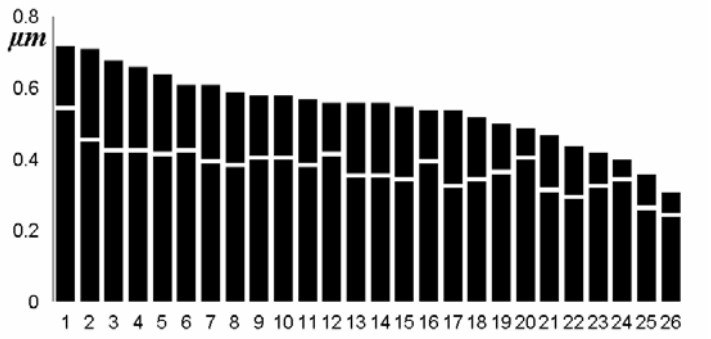

Figure 4. Haploid idiogram of *C. regium* from Iran.

Discussion

The diploid chromosome number of fishes varies from 2n= 22-26, in some species of Nototheniidae an Antarctic fish group, to 2n=240-260, in some anadromous Acipenseridae which show several microchromosomes (Fontana *et al*., 1997)

According to our observations, the diploid chromosome number of *Chondrostoma regium* was 2n=52 and it is not in conformation with the chromosome number of its closely related species. Karyotypes of some species of this genus including *C. genei, C. knerii, C. nasus, C, phoxinus, C. soetta and C. toxostoma* have been investigated (Arkhipchuk, 1999; Klinkhardt *et al*., 1995). The diploid chromosome number in all of them was 2n=50. It shows that members of the genus *Chondrostoma*, are not conserved from the cytogenetic point of view and it is an interesting feature in this taxon. Large variation in diploid number of the species of the other fish genera also have been reported (Alves *et al*., 2006). According to them, the species of the genus *Hypostomus* display a large variation in diploid numbers, with values ranging from 2n=52 to 2n=80 chromosomes and it may be due to high number of uniarmed chromosomes, suggesting the occurrence of a large number of centric fissions in the karyotype evolution of *Hypostomus*.

The majority of the cyprinid species have 2n=50 chromosomes (Al-Sabti, 1991; Gul *et al*., 2004) and it may be considered as modal number in cyprinid fishes. However some carps such as *Cyprinus carpio* has 2n=98-100 and the polyploid *Barbus* species from southern Africa has 2n=148 or 150 chromosomes (AL-Sabti 1991; Gul *et al*., 2004; Oellerman *et al*., 1990). According to Tsigenopoulos *et al.* (2002) three ploidy levels have been recognized in African species of cyprinine cyprinids: diploid (about 50 chromosomes), tetraploid (about 100 chromosomes), and hexaploid (about 150 chromosomes). Occurrence of polyploidy in fishes has been discussed and reviewed by Tsigenopoulos *et al.* (2002), Leggatt and Iwama (2003) and Steven *et al.* (2004). According to Leggatt and Iwama (2003) polyploidy has occurred extensively, independently, and is often repeated in many groups of fish, from the sharks to the higher teleosts and is more common in the lower teleosts than the higher teleosts, possibly due to an advantage gained through decreased specialization in the lower teleosts, a decreased viability of polyploidy in the higher fish, or both.

In the present study, no cytological evidence was found for sex chromosome dimorphism in *Chondrostoma regium* which agrees with reports on many fish species (Yu *et al*., 1987; Esmaeili *et al*., 2008). In marine fishes also, despite of the large number of living species, the occurrence of cytologically differentiated sex chromosomes appears to be rare although it has been described in platyfishes and in some catfishes (Galetti *et al*., 2000; Alves *et al*., 2006).

The arm number of *Chondrostoma regium* was 58. Chromosome number, karyotype formula and arm number of *Chondrostoma regium* may differentiate this species from other species in this genus. Karyotype of other related native species of this genus, *Chondrostoma cyri*, has not been investigated yet, so no comparison is possible. Study of the karyotype of these taxa and assessment of chromosomal evolution may aid our understanding the evolutionary study of these groups.

Further researches are needed on molecular, cytological, anatomical, morphological and biological aspects for better recognition and understanding of the cyprinid fish. It is also important to study karyotype of specimens of *Chondrostoma* from other latitudes using modern techniques of chromosome banding to see if there are intraspecific variations.

Acknowledgments

The authors are grateful to N. Nazari, F. Shahryari, R. Choobineh and S. Baghbani for helping in fish collection and Shiraz University for financial support.

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