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Karyological study on bighead goby (*Neogobius kessleri*) from southern part of the Caspian Sea

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Abstract: Karyological characteristics of bighead goby (*Neogobius kessleri*) in the Caspian Sea were studied by examining 30 metaphase chromosome spreads from the kidney tissue of 10 specimens. The chromosome number of this species was found 2n=46 and the arm number as NF=46. The prepared karyotype of this species consisted of 23 pairs acro-telocentric (a-t) chromosomes. The chromosomal formula can be stated as 2n=46 (a-t). Karyological parameters showed that relative length was between 2.34-7.04 and length variation range of chromosomes was between 1.67-5.01 and total length was 71.16µm. It was found that the best chromosomal spread quality were obtained from intraperitonial injection of 40µg/g colchicine for 5 hours, hypotonization of samples in %1 sodium tri-citrate solution in 4°C and preparation of spreads on cooled slide with flame technique.

Keywords: Chromosome, Karyology, Bighead goby, *Neogobius kessleri*, Caspian Sea, Iran

Introduction

Being among the world's smallest fishes and vertebrates, gobies are the most abundant fish in freshwater and oceanic islands. Mostly are marine fish and found in shallow coastal waters or around coral reefs. Some species have symbiotic relationships with invertebrates. *Neogobius* is found in the Black and Caspian Seas

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where there are about 11 species, some large enough to be the object of commercial fisheries. The general Persian name for this genus is gavmahi or sagmahi (Abdoli, 1999).

Systematically, *Neogobius kessleri* belongs to Actinopterygii class, Perciformes order, Gobiidae family. This endemic fish of the Caspian Sea has been reported from a wide range of rivers in Iran, from Astara to the Gorgan and probably the Atrak, Haraz River, Anzali Lagoon and Gorgan Bay as well as the southeast, southwest and south-central part of the Caspian Sea (Holčík & Oláh, 1992).

Since the 1960s, karyological studies in teleost fishes have made noteworthy contributions to increasing knowledge in the fields of genetics, taxonomy and environmental toxicology (Cucchi & Baruffaldi, 1990). The progress in increasing such knowledge has been closely related to the evolution of application methodologies (Rivlin *et al.*, 1985). Studies of the chromosomes of fishes have not been as successful or widespread as in other vertebrate groups. Standard karyotypes are reported for less than 10% of more than 20000 extant species of fishes (Gold *et al.*, 1990). The study of fish chromosome has become an active area of research in recent years (Thorgaard, 1983). Chromosomal analysis is important for fish breeding from the viewpoint of genetic control, the rapid production of inbred lines, taxonomy and evolutionary studies (Hosseini & Kalbassi, 2003). Karyological studies have provided basic information on the number, size and morphology of chromosomes that is important to undertake chromosome manipulations in fish (Khan *et al.*, 2000). Genetic divergences of populations and their local adaptation are a potential resource for breeding programs in aquaculture and for fishery management (Philips & Rab, 2001).

Although morphological and anatomical characteristics of this fish have been studied (Abdoli, 1999), application of non-morphological methods, such as cytogenetics studies, may provide a framework for the correct species identification of this fish. On the other hand, due to the lack of information on Iranian fish karyotypes (Kalbassi & Keyvanshokoh, 2004; Esmaili & Piravar, 2006), the results of this study could provide some chromosomes data and karyotype analysis of *N. kessleri* in the Caspian Sea shoreline of Iran.

Materials and methods

Specimens of *N. kessleri* (n=10, weight=100-150g) were caught in Mahmoud-abad shores of the southern Caspian Sea. The fishes were transported live to our laboratory, and kept in a well-aerated aquarium at 15-20°C before analysis.

The stock solution of colchicine was made by dissolving 10mg colchicine and 100mg NaCl in 20ml distilled water. The colchicine was administered intraperitoneally at dose of 25 and 40µg/gr body weight. Then, fishes left in aquaria at 15-20°C for 5-10 hours before sacrificing. The anterior kidneys were removed after killing the fish and then the well homogenized cell suspensions were transferred in a hypotonic solution (0.075M KCl or 1% sodium tri-citrate) at two different temperatures (4°C and 25°C) for about 45-50 min.

The swollen cell suspensions were centrifuged at 800 G for 10 min and then fixed in fresh and cold Carnoy's fixative solution (3 parts methanol and 1 part glacial acetic acid) for 30 min; then, the old fixative was replaced with the fresh Carnoy's. Duration of exposure for fixation treatment was 60 min.

The slides, already washed in alcohol and ether and kept at -1°C, were prepared by letting two drops of the fixing solution containing the cell suspension fall onto the cooled slide with flame and warm slide (40°C) at different height (60, 90 and 120cm). Thereafter, the fixative was burned off immediately, using the technique developed by Mellman (1965), for obtaining better cell spread. The slides were stained in series of concentrations (5, 10 and 15%) of Gimsa Merck solution in distilled water and buffered by phosphate (40 mol Na_2HPO_4 and 26.6 mol KH_2PO_4) at pH 6.8 and were assessed at 7, 8, 9 and 10 min exposure times to determine optimum staining conditions.

Metaphases were examined under a photomicroscope (Leica SER. No. 990398, Equipped with a green filter and digital camera). The chromosomes at the metaphase were photographed with a digital camera (Sony SSC-DC 58 AP) onto Kodak color films (ASA 25). In the course of the microscopic examinations, the chromosomal sets of 30 cells were counted and 10 of the best mitotic metaphases were used to measure karyotypes. The morphometric measurements of chromosome pictures were conducted with photographic software Photoshop 6.0 (Adobe Systems). Each chromosome was

tagged with a reference number. The data were transferred to the Excel 2000 (Microsoft) for analysis.

To increasing distinguishability between the homologous chromosomes, the total length of chromosome was computed by summing up the average chromatid lengths of each diploid complement. The length recorded in pixels by the Color Image Analysis System Video Pro 32 (Leading Edge) was converted into micrometers after the scale factor was calibrated with a stage micrometer.

The chromosome pairs were classified following the recommendations of Macgregor (1993). The pair numbers and the decreasing length order within each class were definitely attributed following this classification. Finally, the karyotype was constructed by first dividing arranging order of the homologous pairs in the decreasing length order within each group.

Results

Results showed that the number of diploid chromosome in 30 metaphases from the anterior kidney cells of ten *N. kessleri* specimens was 2n=46 (Fig. 1). All chromosomes in the karyotype had a homologous pair, which were arranged in decreasing size. The investigation of metaphases showed notable difference in size of chromosomes, but no difference between chromosomal type was evident. In addition, the sex chromosomes could not be distinguished without banding techniques in this species.

The representative karyotype for *N. kessleri* is shown in Fig. 2. It has 23 pairs of acro-telocentric chromosomes. The number of chromosomal arms was determined as NF=46 and chromosome formula would be expressed as 2n=46 (a-t). The morphological and numerical data summarized in Tables 1 and 2 show that relative length and length variation range of chromosomes are between 2.34-7.04 and 1.67-5.01 respectively. Total length of chromosomes were 71.16μm. The idiogram of the *N. kessleri* was made based on the haploid set of chromosomes (Fig. 3).

In this study, the optimum colchicine concentration for *N. kessleri* was determined to be 40μg/gr BW of colchicine solution for five hours. This concentration effectively arrested dividing cells in metaphase stage. In addition, the best chromosomal spread quality (well-spread metaphase) were obtained from treatment of cells with 1%

sodium tricitrate solution at 4°C for 45-50min, while 0.075M KCl did not result in considerable metaphases.

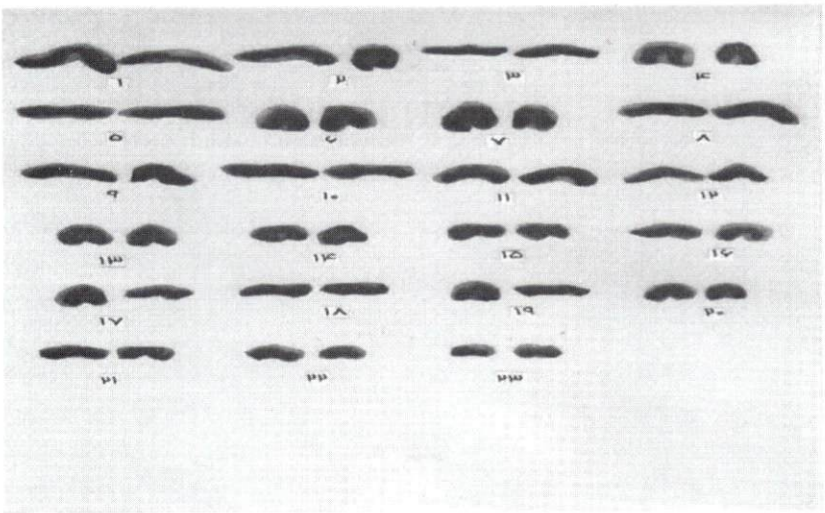

Figure 1: Karyogram of bighead goby (*N. kessleri*)

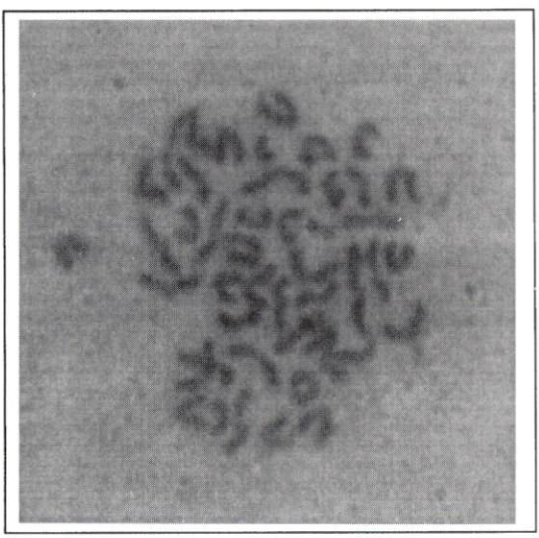

Figure 2: Metaphase chromosomes of bighead goby (*N. kessleri*) × 1000, 2n=46

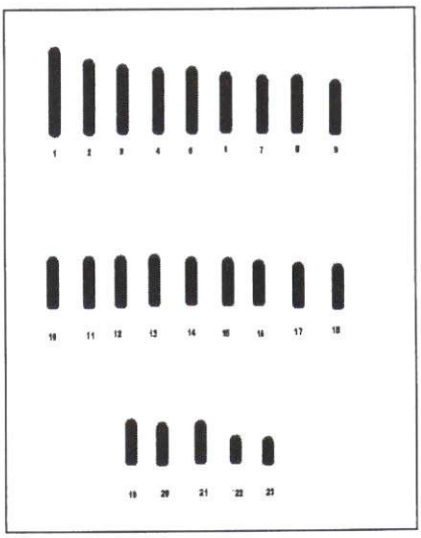

Figure 3: Idiogram of bighead goby (*N. kessleri*), n=23

Table 1: Centromeric index of bighead goby (*N. kessleri*)

Chromosome No.	chromosome total length (µm)	centromer index	arms ratio	relative length	Chromosomal type
1	5.01	0	∞	7.04	A*
2	4.34	0	∞	6.09	A
3	4.01	0	∞	5.63	A
4	3.84	0	∞	5.39	A
5	3.84	0	∞	5.39	A
6	3.51	0	∞	4.93	A
7	3.34	0	∞	4.69	A
8	3.34	0	∞	4.69	A
9	3.17	0	∞	4.45	A
10	3.01	0	∞	4.22	A
11	3.01	0	∞	4.22	A
12	3.01	0	∞	4.22	A
13	3.01	0	∞	4.22	A
14	2.84	0	∞	3.99	A
15	2.84	0	∞	3.99	A
16	2.67	0	∞	3.75	A
17	2.67	0	∞	3.75	A
18	2.67	0	∞	3.75	A
19	2.67	0	∞	3.75	A
20	2.51	0	∞	3.52	A
21	2.51	0	∞	3.52	A
22	1.67	0	∞	2.34	A
23	1.67	0	∞	2.34	A

*A: acrocentric

Table 2: Karyotype characteristics of bighead goby (*N. kessleri*)

Chromosome number (2n)	Number of chromosome arms (NF)	Total length of Chromosome (µm)	Haploid total chromosome length (µm)
46	46	71.16	35.58

Discussion

Several techniques have been developed to examine chromosomes in tissues of adult fish. These include squashed (Al-Sabti, 1983), blood leucocyte culture (Baker, 1972; Al-Sabti, 1985) and cell suspensions from tissues such as gill, kidney and intestine (Kligerman & Bloom, 1977; Gold *et al.*, 1990), and scales (Denton & Howell, 1969). Due to previous succesfull results on some species karyotyping in our lab (Hosseini & Kalbassi, 2003; Kalbassi & Keyvanshokoh, 2004; Kalbassi & Dorafshan, 2005; Kalbassi *et al.*, 2006) we utilized cell suspension from anterior kidney in the present study. This technique is rather inexpensive and results are obtained relatively fast. Such techniques are based on the use of colchicine to block quickly proliferating cell populations at the metaphase stage. Due to the small size and high number of chromosomes, karyological study of teleost fishes presents technical difficulties that are not encountered in the study of other vertebrates (Cucchi & Baruffaldi, 1990).

Karyological study has some different steps. The first step in the procedure is treatment of the cells with colchicine, which arrests cell division at metaphase (Baski & Means, 1988). High concentration and long period of colchicine treatment effect on chromosome, cause to aggregate and reduce the size of chromosome and their arms, so it is difficult to identify short arm of an acrocentric chromosome or other types of chromosomes. This study suggests that colchicines concentrations of 50μg/gr BW can effectively arrest dividing cells in metaphase in kidney tissues. But the maintenance periods may vary according to species. Also type of hypotonic solutions treatment as well as duration of exposure time, affect the amount of chromosome spreading. In this study, 0.075 M KCl hypotonic treatments were ineffective in obtaining well-spread metaphases. Although condensed chromosomes could be observed, they were often seen inside an intact cell or only slightly spread. Fixative treatment was not found to be as important as hypotonic treatment in obtaining well-spread metaphases.

The main difficulty in working with fish chromosomes is in obtaining high quality metaphase spreads. A few studies have used fish standard karyotypes to examine taxonomic or systematic problems (Bolla, 1987). The major difficulty encountered is the morphological variation existing even between homologous chromosomes in the same nucleus (Al-Sabti, 1991; Levan *et al.*, 1964). Sometimes it could happen that some chromosomes are more contracted than others, so chromosome measurements are very difficult in fishes which have small chromosomes compared to those of man and mammals. Another problem is that fish karyotypes are not identical as in human being or other animal species, so we can not have a standard karyotype for fish because not only are there differences between species, but polymorphism often occurs within the same fish species (Al-Sabti, 1991). Several incomplete metaphases were encountered in the preparation, and these probably resulted from hypotonic over treatment (Nanda *et al.*, 1995). The majority of authors classify uni-armed and bi-armed chromosomes according to the guidelines of Macgregor (1993). The majority of Gobiidae species have 2n= 46 chromosomes while *Neogobius fluviatilis* and *Neogobius melanostomum* have 2n=42-46 (Klinkhardt *et al.*, 1995). Until now, karyotype of some members of *Neogobius* genus as *Neogobius melanostomus affinis* has been determined (2n=46, NF=46 2n=46a-t) (Klinkhardt *et al.*, 1995). Additional data from this species and related taxa may provide beneficial insights into the value of conventional cytogenetic data for reconstructing Gobiidae family. However, the value of karyological data can be better utilized if combined with the highest possible taxonomic elements for the diagnosis of species. In addition, the karyotype analysis is a key step toward the stock improvement by polyploidy manipulation, hybridization and related genetic engineering (Tan *et al.*, 2004). Therefore, like other animals, comprehensive genetic researches is needed for this fish as well.

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