**Karyological study on Bighead gobie (Neogobius kessleri) in Mahmoudabad Area (South Caspian Sea)**

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**Abstract**

Karyological characters of *Neogobius kessleri*, in Caspian sea were studied by examining metaphase chromosome spreads taken from the kidney tissues. The examination of 30 metaphase spread prepared from 10 specimens indicated that the chromosome numbers of this species was found 2n=46 and the arm number was determined as NF=46. The prepared karyotype of this species was consisted of 23 pairs acrocentric (at) chromosomes. The chromosome formula can be stated as 2n=46(at). Karyological parameters showed that relative length and length variation range of chromosomes were between 2.34-7.04, 1.67-5.01 respectively and total length is 71.16µ. In this study, it was found that the best chromosomal spread quality were obtained from 40 µg/gr Colchicine injection, height dropping of 120 cm, cooled slide with flame and %1 Tri-sodium Citrat as a hypotonic solution in 4ºC.

**Keywords:** Chromosome, Karyology, Bighead gobie, *Neogobius kessleri*, South Caspian Sea.

**Introduction**

Gobies are the most abundant fish in freshwater in oceanic islands. The smallest fishes (and vertebrates) in the world belong to this family which mostly live in shallow coastal waters and around coral reefs. Some species have symbiotic relationships with invertebrates. Neogobius is found in the Black and Caspian seas in where there are about 11 species, some large enough to be the object of commercial fisheries. The general Farsi name for fishes in this genus is gav mahi. This species is separated from other Caspian gobies in Iran by having a completely scaled nape. Systematically *Neogobius kessleri* belongs to teleostei class, Perciformes order, Gobidae family and Neogobius genus. Which found in the inshore Caspian Sea and tributary rivers. In Iran, it is reported from a wide range of rivers from Astara to the Gorgan and probably Atrak, the Aras River, the Anzali Mordab and Gorgan Bay, the southeast Caspian Sea, southwest Caspian Sea and south-central Caspian Sea (Holčík and Oláh, 1992). That this fish is endemic in Caspian sea.

Since 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, pants of rapid production of inbred lines, taxonomy and evolutionary studies (Hosseini 2003). Karyological studies have provided basic information about 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 source for breeding programs in aquaculture and for fishery management (Philips & Rab, 2001). However, as happened in the Iranian Cyprinids, such as Rutilus frisii kutum (Nowruzfashkhami and Khosroshahi, 1995), Abramis brama (Nahavandi et al., 2001), Ctenopharyngodon idella (Nowruzfashkhami et al., 2002) and Hypophthalmichthys molitrix (Varasteh et al., 2002), cytogenetic studies in fish have not been comprehensive when compared to other vertebrate grows.

The aim of this study was to investigate the chromosomes and karyotype of Neogobius kessleri in Iran

Materials and methods
Ten, Neogobius kessleri weight 100-150 gr, were caught in Mahmood abad shores (Caspian sea) in north of Iran. The fishes were transported live to the laboratory, and kept in a well-aerated aquarium at 15 - 20°C before analysis.

2.1. Mitotic inhibitors
The stock solution of colchicine was made by dissolving 10 mg colchicine and 100 mg NaCl in 20 ml distilled water. The colchicine was administrated at dose of 25 and 40 µg/gr body weight (BW) and slowly injected into the intraperitoneal muscle. Then, fishes left in aquaria at 15-20°C for 5-10 hours before sacrificing, then, the fish were killed and their anterior kidneys removed, suspended and placed in hypotonic treatment (0.075M KCl and 1 % Sodium citrate solution) at two different temperature 4°C and 25°C. Lasting time for hypotonisation treatment was 45-50 min.

2.2. Fixation
The swollen cell suspensions were fixed in 3:1 cooled Carnoy’s fluid (3 parts methanol and 1 part glacial acetic acid) for 30 min, then, the old fixative was replaced with the fresh Carnoy’s. Lasting time for fixation treatment was 60 min.

2.3. Spreading
The slides, previously 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) in different heights (60, 90 and 120 cm). Immediately thereafter the fixative was burned off, using the technique developed by Mellman (1965), for obtaining better cell spread. The slides were stained in series of concentrations of Gimsa Merck solution in distilled water (5, 10 and 15%) and buffered by phosphate (40 mol Na2HPO4 and 26.6 mol K H2PO4) at PH 6.8 and were assessed at 7, 8, 9 and 10 min exposure times to determine optimum staining conditions. Slides were dipped into distilled water to wash out extra Giemsa solution and then were allowed to air dry at 25°C for 2–3 h.

2.4. Chromosome examinations and morphometric measurements
Metaphases were examined under a microscope (Leca SER. NO. 990398, Equipted with a green filter and digital camera) with an oil immersion lens at 1000 magnification. The chromosomes at the metaphase were photographed with a digital camera (Sony SSC-DC 58 AP) onto Kodak colour films (ASA 25). In the course of the microscopic examinations the chromosome 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.

2.5. Chromosome pairing
To increase differences 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 Colour 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 were definitely attributed following this classification and the decreasing length order within each class. Finally, the karyotype was constructed by first dividing arranging the homologous pairs in the decreasing length order within each group.

Results
Relatively small and high numbers of chromosomes were observed in Neogobius kessleri. The counts of chromosome was 46 in per metaphases. In 30 metaphases from the anterior kidney cells of 10 N.kessleri specimens, the diploid chromosome number was 2n=46 (Fig.1). All chromosomes in the karyotype have a homologous pair. Homologous pairs of chromosomes were arranged in decreasing size. The investigation of metaphases showed notable difference in size of chromosomes in N.kessleri but in type there is no difference between chromosomes. In addition, the sex chromosomes could not be distinguished in this species.

The representative karyotype for N.kessleri is shown in Fig2. The karyotype of N.kessleri (Fig.2) has 23 pairs acro-telocentric chromosomes. The number of chromosome arms were determined NF=46 and chromosome formula can be expressed as 2n = 46(a-t). The morphological and numerical data are summarized in Table 1. Other data are represented in Table 2. According to this table, relative length and length variation range of chromosomes are between 2.34-7.04 and 1.67-5.01 respectively. Total length of chromosomes was 71.16µ. The ideogram of the N.kessleri was made on the basis of the karyotype(Fig.3).

In this study, the optimum colchicine concentration for N.kessleri was determined to be 40 µg/gr BW of colchicine solution for five hours. This concentration have effectively arrested dividing cells in metaphase. In addition the best chromosomal spread quality(well-spread metaphase) were obtained from treatment of cells with 1% Sodium citrate solution at 4°C for 45-50min and height dropping 120 cm ,cooled slide with flame.

The other hypotonic solution tested, 0.075M KCl, did not result in many scorable metaphases.
Figure 1- metaphase chromosomes of bighead goby \((Neogobius kessleri)\)

Table 1- centromeric index of bighead goby \((Neogobius kessleri)\)

| Pair | arm(\(\mu\text{m})\) | chromosome total length \((\mu\text{m})\) | centromer index arms ratio relative length Chromosome type |
|------|-----------------|---------------------------------|-----------------|-----------------|-----------------|
| 1    | 5.01            | 5.01                            | 0               | \(\infty\)       | 7.04            | A*              |
| 2    | 4.34            | 4.34                            | 0               | \(\infty\)       | 6.09            | A               |
| 3    | 4.01            | 4.01                            | 0               | \(\infty\)       | 5.63            | A               |
| 4    | 3.84            | 3.84                            | 0               | \(\infty\)       | 5.39            | A               |
| 5    | 3.84            | 3.84                            | 0               | \(\infty\)       | 5.39            | A               |
| 6    | 3.51            | 3.51                            | 0               | \(\infty\)       | 4.93            | A               |
| 7    | 3.34            | 3.34                            | 0               | \(\infty\)       | 4.69            | A               |
| 8    | 3.34            | 3.34                            | 0               | \(\infty\)       | 4.69            | A               |
| 9    | 3.17            | 3.17                            | 0               | \(\infty\)       | 4.45            | A               |
| 10   | 3.01            | 3.01                            | 0               | \(\infty\)       | 4.22            | A               |
| 11   | 3.01            | 3.01                            | 0               | \(\infty\)       | 4.22            | A               |
| 12   | 3.01            | 3.01                            | 0               | \(\infty\)       | 4.22            | A               |
| 13   | 3.01            | 3.01                            | 0               | \(\infty\)       | 4.22            | A               |
| 14   | 2.84            | 2.84                            | 0               | \(\infty\)       | 3.99            | A               |
| 15   | 2.84            | 2.84                            | 0               | \(\infty\)       | 3.99            | A               |
| 16   | 2.67            | 2.67                            | 0               | \(\infty\)       | 3.75            | A               |
| 17   | 2.67            | 2.67                            | 0               | \(\infty\)       | 3.75            | A               |
| 18   | 2.67            | 2.67                            | 0               | \(\infty\)       | 3.75            | A               |
| 19   | 2.67            | 2.67                            | 0               | \(\infty\)       | 3.75            | A               |
| 20   | 2.51            | 2.51                            | 0               | \(\infty\)       | 3.52            | A               |
| 21   | 2.51            | 2.51                            | 0               | \(\infty\)       | 3.52            | A               |
| 22   | 1.67            | 1.67                            | 0               | \(\infty\)       | 2.34            | A               |
| 23   | 1.67            | 1.67                            | 0               | \(\infty\)       | 2.34            | A               |

*A: acrocentric

Table 2- Karyotype characters of bighead goby \((Neogobius kessleri)\)

<table>
<thead>
<tr>
<th>Number of chromosome</th>
<th>Chromosome number ((2n))</th>
<th>Total length chromosomes</th>
<th>Haploid total chromosome</th>
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Discussion

Rapidly growing tissues are required to obtain a large number of chromosome spreads in metaphase for karyotypical studies (Tan et al., 2004). Several techniques have been developed to examine chromosomes in tissues of adult fish. These include squashed (Ojima et al., 1963; Roberts, 1967; Al-Sabti et al., 1983), blood leucocyte culture (Barker, 1972; Al-Sabti, 1985; Hartley & Hornne, 1985) and cell suspensions from tissues such as gill, kidney, intestine (McPhail & Jones, 1966; Gold, 1974; Klingerman & Bloom, 1977), cornea (Drewery, 1964) and scales (Denton & Howell, 1969). Each of these procedures was optimized to obtain large numbers of well-spread metaphases and was used regularly for karyotype analysis that in present lecture we utilized anterior kidney. Karyological study of teleost fishes presents

<table>
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<th>arms(NF)</th>
<th>(µm)</th>
<th>length (µm)</th>
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<tbody>
<tr>
<td>46</td>
<td>46</td>
<td>71.16</td>
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Figure 2- Karyogram of bighead goby (Neogobius kessleri)

Figure 3- Idiogram of bighead goby (Neogobius kessleri)
technical difficulties which are not encountered in the study of other vertebrates, and these difficulties are due to the small size and high number of chromosomes (Cucchi & Baruffaldi, 1990). Different techniques are presently being used to perform such studies: direct, in vivo; and indirect, in vitro. With those forms employing direct techniques, the preparation of slides for optical microscopy is quite easy. Furthermore, these techniques are rather inexpensive and results are obtained relatively quickly. Such techniques are based on the use of Colchicine to block quickly-proliferating cell populations at the metaphase.

Karyological study has in different steps. Each of the steps involves in the preparation of tissues and slides for cytogenetic analysis is which important in attaining large number of well-spread metaphases. The first step in the procedure is the treatment of the cells with Colchicine, which arrests cell division at metaphase (Baski & Means, 1988). High concentration and long period of Colchicine treatment affect chromosome, causes to aggregate and shrink of chromosome and their arms, so it is difficult to identify short arm of acrocentric chromosomes and other chromosomes. This study suggests that colchicines concentrations of 50 µg/gr BW can effectively arrest dividing cells in metaphase in kidney tissues. But the maintenance periods may vary according to species. Type of hypotonic treatment and the length of exposure affect on the degree of chromosome spreading. In this study, 0.075M KCl hypotonic treatment was 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 been used fish standard karyotypes to examine taxonomic or systematic problems (Bolla, 1987). The major difficulty encountered is the current morphological variation even between homologous chromosomes in the same nucleus (Al-Sabti, 1991 & Levan et al., 1964). Sometimes it could happen that some chromosomes get more contracted than others, so chromosome measurements are very small 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 cannot have a standard karyotype for fish not only because there are differences between species, but also 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) where differences in the number of chromosome arms were seen, aril usually on the result of a difference in the scoring of subtelocentric chromosomes by different authors (Philips & Rab, 2001). The majority of Gobidae species have 2n = 46 chromosomes while Neogobius fluviatilis and Neogobius melanostomum have 2n= 42-46 (Klinkhardt, M,1995) and fishes which have normal chromosome series (2n = 50) are called diploids fish. Until now, karyotype of some member of Neogobius genus were determined such as Neogobius melanostomus affinis (2n=46, NF=46 2n=46a-t) (Klinkhardt, M, 1995). The karyotype analysis is a key step toward the stock improvement by polyploidy manipulation, hybridisation and related genetic engineering (Tan et al., 2004). Therefore like to other animal species, comprehensive genetic researches are required this fish too.

References:


Anonym., 1982.


Magdeburg: Westarp Wissenschaften


