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Karyotypic characterization of the pike, *Esox lucius* from the south Caspian Sea basin

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The karyotype of pike from Anzali lagoon in the south Caspian Sea basin have been investigated for the first time by conventional chromosome staining. The chromosomes spreads were stained with 7% Giemsa solution for 15 min and examined under a light microscope. Appropriate metaphase plates were photographed in order to prepare karyotype. All samples had a diploid number of 50 chromosomes (2n=50), with a karyotype consist of 12 metacentric, 24 submetacentric, 14 subtelocentric and fundamental number (NF) of 86. The largest chromosome in this species was a pair of metacentric chromosomes. Based on type of chromosomes, the karyotype of this species was nearly differed with what that found in previous studies, which could be attributed to the existence of different populations for this species. Despite the conserved diploid number, the data on the cytogenetic structure help characterize the karyotype of this species.

Key words: *Esox lucius*, Caspian Sea, Cytogenetic, Giemsa staining.

INTRODUCTION

The genus *Esox* belongs to the family Esocidae comprises five species distributed in the Holarctic region (Nelson, 1994) and there is only one species reported in Iran (Jolodar & Abdoli, 2004). The pike *Esox lucius* (Linnaeus, 1758) is distributed across northern Eurasia and northern North America. Iranian populations are found in the Caspian Sea basin, from the Anzali lagoon and the Sia Keshim Protected Region to Gorgan Bay and its tributaries such as the Karasu, described from the rivers including the Safid, Tajan, Babol and Haraz, and the Atrak River basin, and the Amirkelaye Lagoon near Lahijan (Nejatsanatee, 1994; Riazi, 1996; Abbasi et al., 1999; Kiabi et al., 1999; Jolodar & Abdoli, 2004). Pikes are important sport fishes and are found in lakes and rivers where the water is still or flowing slowly as well as marshes and ponds. They are found only in the lower reaches of rivers along the Iranian shore (Abbasi et al., 1999).

The study of karyotype, which is the chromosome number and morphology, is a relevant source of information on taxonomical and phylogenetic relationships among fishes (King, 1993). In the recent years, cytogenetic and molecular methods mentioned novel taxonomical and phylogenetic relationships among different aquatic species (Rab & Mayr, 1987; Pourkazemi et al., 2010; Nazari et al., 2011). Even though Euteleostei fishes belong to a cytogenetically fairly studied fish group, the family Esocidae has received far less attention, in particular in Iran. Although the chromosome

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number of the genus *Esox* have described, the karyotype of *Esox lucius* have not been studied in Iranian water (Arkhipchuk, 1999). The karyotypes of the family Esocidae seem to be close to the ancestral esocoid (Crossman & Rab, 1996). Studies of northern pike (*Esox lucius*) chromosomes concerned general cytogenetic characterization and its karyotype has been investigated from some European (Nygren, 1968; Rab & Mayr, 1987; Rab & Crossman, 1994; Jankun et al., 1998) and china populations (Zou & Li, 2006). Jankun et al. (1998) also analyzed heterochromatin and the location of nucleolar organizer regions (NOR) in the chromosomes of northern pike (*Esox lucius*) in Poland. Since karotypic polymorphism characterizes Esocae fishes (Jankun et al., 1998), it is significant to survey the chromosomal complements of the pike throughout its distribution range. In this paper we present the karyotype of *Esox lucius* from Caspian Sea basin and compare our results with the available data for this species.

MATERIALS AND METHODS

Eight individuals of *Esox lucius*, were collected from Anzali lagoon in north part of Iran (37º28'N, 49º27'E) between June and August of 2011 and kept in the laboratory in aerated fresh water until studied. The preparation of chromosomes was performed according to Collares- Pereira et al. (1998) with some modifications (Nazari et al., 2009). This technique is particularly convenient for the preparation of chromosomes from fishes. Briefly, The specimens were injected intraperitoneally with a 0.01% distill water solution of colchicine at 0.5 ml per 100 gram of body weight, allowed to remain in aerated fresh water at 19-21 °C for a period of 2-2.5 h, and then sacrificed. The gill filaments and the head kidney tissues of specimens were squashed and placed in a hypotonic solution of KCl (0.075 M) for 50 min in room temperature (22-23 °C), then drained and fixed in freshly prepared Carnoy fixative (3:1, methanol: acetic acid) at 4 °C. The supernatants were then discarded and 5 ml fresh and cold fixative was added to sediments, mixed thoroughly and three changes of fixative of each were made at 10-min intervals. Then a few drops of the suspension were trickled on hot (heated on a hot plate after rinsing in alcohol) and cold (placed in a freezer after rinsing in alcohol) slides. The slides were air dried and stained with 7% Giemsa solution (pH=7) for analysis of chromosomal morphology. Slides were rinsed in distilled water and dried at room temperature for 3-4 h. Using Olympus light microscope chromosomes were counted in 69 metaphase plates of *E.lucius*. Photos were obtained and chromosomes were classified according to standard method (Levan et al., 1964). Karyotype was prepared by organizing chromosomes in pairs by size. The idiogram were prepared to supply the common depict of the pike chromosomes in Microsoft Excel 2003 software.

RESULTS

The chromosome feature of the *E.lucius* specimens was 2n = 50 chromosomes with 6 pairs of metacentric (M), 12 pairs of submetacentric (Sm), 7 pairs of subtelocentric (St) chromosomes, with a fundamental number (NF) equal to 86 (Fig. 1).

A total of 69 numberings were made of chromosome spreads at metaphase from four males and four females. Similarity of the karyotypes of females and males did not show the presence of any chromosomal differences (data not displayed). After combining the data the frequency of these numberings in relation to the discerned chromosome number is demonstrated in figure 2. This obviously proves that the modal number was 2n= 50 with 79% of the cells checked having this chromosomes number. In figure 1 one of the metaphase spreads is depicted and the karyotype presented from this metaphase is demonstrated in figure 3.

The karyotype formula suggested for *E.lucius* was 2n=50, 12M + 24Sm+14St, and the arm number (number of chromosome arms) was NF=86, which was estimated by alloting a value of two arm for M/Sm chromosomes and one arm for the St/A chromosomes (Fig. 3).

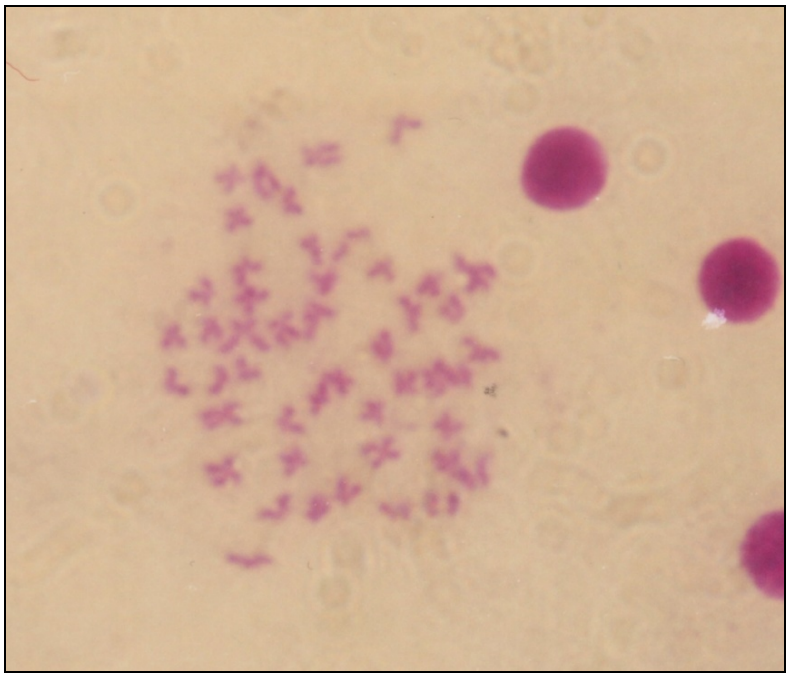

FIGURE 1. Metaphase chromosomes of pike (*Esox lucius*) in this study

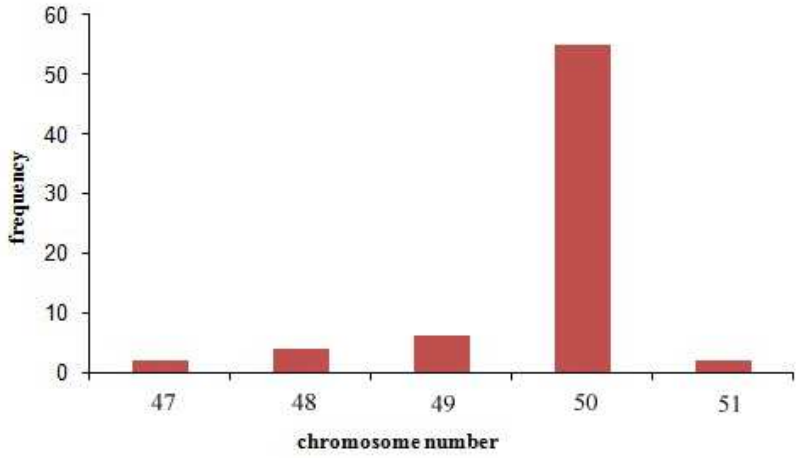

FIGURE 2. Distribution of the chromosome numbers from 69 metaphase plates from *E.lucius*

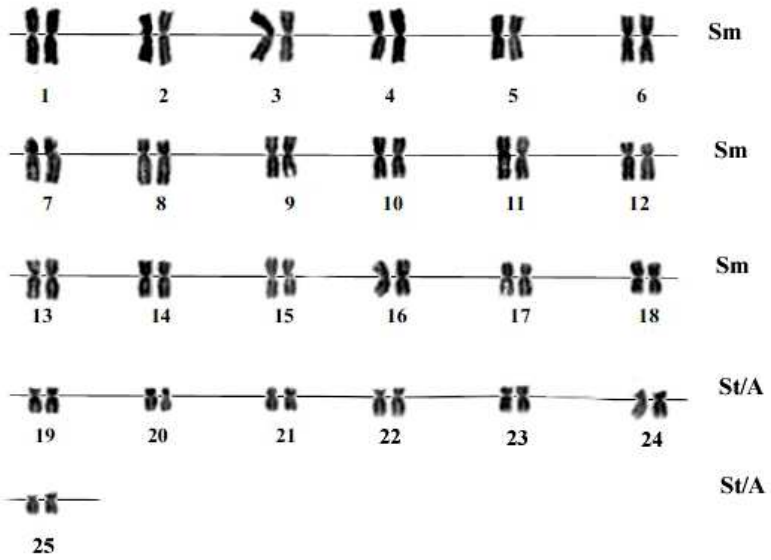

FIGURE 3. Karyotype in pike (*Esox lucius*) (2n=6M + 12 SM + 7St/a)

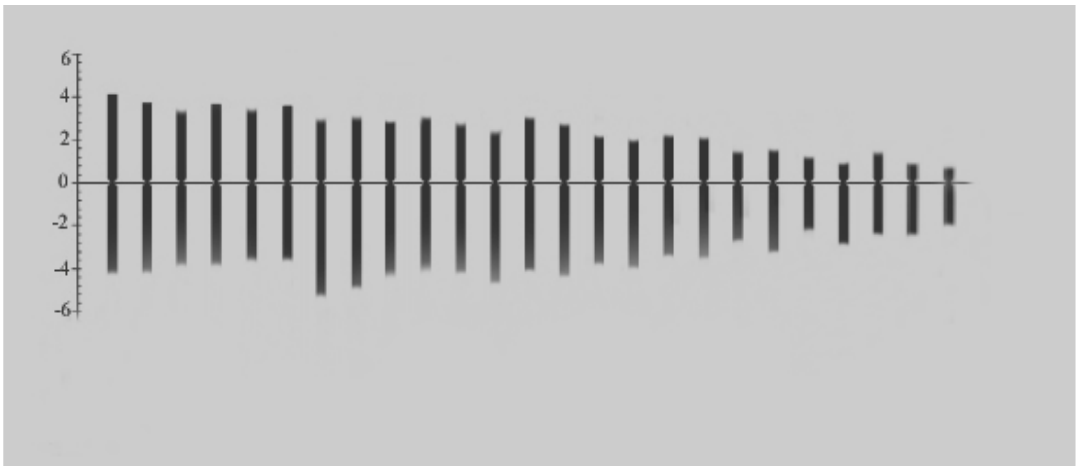

FIGURE 4. Haploid idiogram of pike (*Esox lucius*) from Anzali lagoon

DISCUSSION

Based on the results obtained the chromosome number of the species *Esox lucius* was 2n=50 which similar to the chromosome number reported for other species belonging to the genus *Esox*. In the genus *Esox*, the chromosome numbers 2n=50 was confirmed by Rab & Crossman (1994) who studied in *E. lucius*, *E.americanus, E. niger* and *E. masquinongy*. Furthermore, Nygren (1968) informed a diploid number of 2n = 50 acrocentric chromosomes in *Esox lucius* from two Swedish lakes: Mälaren and Uttran; the same 2n numbers were reported by Rab & Mayr (1987), and Jankun et al. (1998). In this study we observed that both male and female specimens of *E. lucius* obtained from Anzali lagoon had a diploid number of chromosomes 2n = 50 and the number of chromosome arms were NF = 86, which different with the results of the previous studies (Rab & Crossman, 1994, NF = 76; Jankun et al., 1998, NF = 86; Zou & Li, 2006, NF = 50) (Table 1).

TABLE 1. Chromosome formula, chromosome number (2n) and chromosome arms in different species belonging to the genus *Esox*.

No.	Species	2n	Chromosome formula	NF	Reference
1	*Esox lucius*	50	14M + 36SMSt	_	Nygren, 1968
2	*Esox lucius*	50	24M /Sm + 14St + 12t	76	Rab and Mayr, 1987
3	*Esox lucius*	50	24M /Sm + 14St + 12t	88	Rab & Crossman,1994
4	*Esox lucius*	50	-	50	Jankun et al., 1998
5	*Esox lucius*	50	50 St	50	Zou and Li, 2006
6	*Esox lucius*	50	12M + 24Sm + 14St /t	86	Presnt study
7	*Esox masquinongy*	50	12M + 14Sm + 24St/ A	78	Rab & Crossman,1994
8	*Esox niger*	50	12M + 12Sm + 26St/ A	74	Rab & Crossman,1994
9	*Esox americanus*	50	12M + 10Sm + 28St/ A	72	Rab &Crossman,1994

Although chromosome numbers of the present study and the previous studies were similar, but differences in chromosome formula and number of arms (NF) were observed. Chromosome analysis of the species *Esox masquinongy* (Linneaus, 1758) was reported as 2n=50 and NF=76 (Rab & Crossman, 1994) and for the species *E. niger* belong to north American rivers was reported as 2n=50 and NF=74 (Rab & Crossman, 1994). Zou and Li, 2006 described a karyotype of 2n=50 consisting 25 pairs of telocentric chromosomes with NF=50 for white spot pike *E. lucius* from Ertix river in China (Table 1). Some explanations for these discrepancies in number and type of chromosome may be due to the presence of different populations, races and/or sub species in sampling area arising from mutation, race improvement and hybridization with other indigenous species (Crossman & Rab, 1996; Vasil'ev & Vasil'eva, 2008; Nazari et al*.,* 2010). Sex, age as well as the hygienic state of the species, the concentration of colchicines and also the time of mitotic arrest may also influence chromosome characteristics particularly the chromosome arm number (NF) (Kalbasi et al., 2006; Pourkazemi et al*.,* 2010).

It has been presented that the successful gynogenesis in northern pike produced only females by heat shock (Luczynski et al*.,* 1997); this lead to the deduction that the chromosomal sex detection organization in *E. lucius* is of the type of XY, as females are homogametic (Jankun et al*.,* 1998). Unluckily, procedures of chromosome polymorphism test utilized in this report did not show sex chromosomes in the pike. Further sensitive techniques for cytogenetic studies in *E. lucius* should include take advantage of restriction enzymes, the use of differential staining methods combined with molecular techniques (e.g. fluorescent *in situ* hybridization with telomeric probe), will be required to acquire a better comprehension of chromosomal studies of this species.

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