Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi
Menba Journal of Fisheries Faculty
ISSN 2147-2254 | e-ISSN: 2667-8659

Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi 2023; 9(1): 1-6 Araştırma Makalesi/Research Article

Chromosomal analysis of *Sander lucioperca* (L., 1758) (Perciformes: Percidae) from Turkey

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Article Info

Received:
15/09/2022
Accepted:
16/02/2023

Keywords:
- Pikeperch
- Karyology
- Constitutive heterochromatin region
- Nucleolus organizer region

Abstract

Sander lucioperca (pikeperch) is a percid fish species of high commercial value and potential for being aquaculture in Turkey. However, karyological studies are deficient for population of Turkey. So, the aim of this study is to carry out diploid chromosome number, karyotype formula, fundamental arm number and chromosomal banding properties (with C-banding and Ag-NOR staining) of *S. lucioperca*. Specimens of *S. lucioperca* were captured from Konya, Turkey and alive specimens carried to the laboratory. Chromosome obtaining was provided by using air-drying technique from the head kidney. Chromosome slides were prepared and banding procedures were applied. Result of the analysis, diploid chromosome number was found as 48 and karyotype of the pikeperch consist of 32 biarmed and 16 uniarmed chromosomes. Constitutive heterochromatin regions were observed on the pericentromeres of some of the chromosomes. Ag-NORs were determined on one pair of submetacentric chromosome. This report is the first that determines chromosomal properties of *S. lucioperca* from Turkey. This study may contribute the cytogenetic information of this species.

Atıf bilgisi/Cite as: Ünal-Karakuş S., Gaffaroğlu M., Karasu-Ayata M.(2023). Chromosomal analysis of *Sander lucioperca* (L., 1758) (Perciformes: Percidae) from Turkey. Menba Journal of Fisheries Faculty, 9(1), 1-6. https://doi.org/10.58626/menba.1175728

INTRODUCTION

Totally 384 fish species distribute in the inland waters of Turkey. A total of 32 species have been introduced deliberately or accidentally (Çiçek et al., 2020). The family Percidae has three subfamilies, these are Percinae, Luciopercinae and Etheostomatinae. The subfamily Luciopercinae Jordan and Evermann, 1896 has three valid genera (*Sander*, *Zingel* and *Romanichthys*) and 10 valid species. The genus *Sander* Oken, 1817 belongs to the subfamily Luciopercinae. This genus has five species named as *Sander canadensis*, *S. lucioperca*, *S. marinus*, *S. vitreus* and *S. volgensis* (Fricke et al., 2021). From this species, *S. lucioperca* is a fish species originating from Europe and was introduced into the inland waters of Turkey since 1950's (Küçük, 2012). Pikeperch is distributed widely and highly a popular fish in Turkey with good export prospects (Ablak & Yılmaz, 2004; Küçük, 2012). Turkey is listed as one of the top pikeperch producers (Küçük, 2012). Otherwise, this piscivorous fish has destructive effects on the native fish taxa especially on the endemic and restricted species (Küçük, 2012). Endemic fish species of Turkey like *Alburnus akili* and *Pseudophoxinus handlirchii* are extinct. One of the reasons for this extinction is the introduction of predatory *S. lucioperca* in Eğirdir and Beyşehir Lakes (Küçük, 2012). Also, the population of *Pseudophoxinus anatolicus* is extinct due to the introduction of *S. lucioperca* in Beyşehir Lake (Sasi, 2011).

The methodologies used for chromosomal obtaining and karyotype analysis have been developed so much in the last years. Fish cytogenetic is a useful area in cytotaxonomy, fish breeding in aquaculture, in phylogenetic studies and detecting variations within and among the populations (Martins et al., 2011).

Cytogenetic studies especially in endemic freshwater fish species are very popular in Turkey (Karasu-Ayata et al., 2021; Unal-Karakuş, 2021). Also this studies have been carried out on the Turkey populations of conventional distributed fish species like *Carassius auratus* (Ölmez-Aydın & Kuru, 2001), *Gobius paganellus* (Ergene-Gözükara & Çavaş, 2002), *Oncorhynchus mykiss* (Örs, 2003), *Alburnoides bipunctatus* (Kılıç-Demirok & Ünlü, 2004), *Anguilla anguilla* (Turan et al., 2005), *Silurus glanis* (Aydın, 2005), *Pseudorosbora parva* (Karasu-Ayata et al., 2016), *Cyprinus carpio* (Unal & Gaffaroğlu, 2016), *Rhodeus amarus* (Karasu-Ayata et al., 2021) and *Esox lucius* (Arslan & Alpaslan, 2020). Above mentioned studies were conducted in the determination of the diploid chromosome number, chromosome morphology and conventional chromosomal banding techniques (especially C-banding and silver staining of NORs). Some cytogenetic studies available from different countries in *S. lucioperca* (Ráb et al., 1987; Mirinargesi et al., 2007; Jankun et al., 2014) to date. However, cytogenetics of *S. lucioperca* population from

Turkey have not been studied. It is necessary to study *Sander lucioperca*'s chromosomal characteristics since the Turkish population has not been studied and it harms Anatolian endemic species in particular. So, the aim of this study is to determine cytogenetic properties of *S. lucioperca* with conventional cytogenetic techniques.

MATERIALS AND METHODS

Seven specimens (four females, three males) of *S. lucioperca* were collected from Kayabaşı Stream, Beyşehir, Konya, Turkey (37°30′N, 31°31′E) by electrofishing. The individuals were carried alive to the laboratory and kept in well aerated aquarium until analysis. The fishes were treated with the guidelines of the local ethics committee of Kırşehir Ahi Evran University (Protocol Number: 68429034/05). The air-drying technique of Bertollo et al. (2015) was performed on the head kidney for chromosome preparation. The fish were injected intraperitoneally with 0.1% colchicine solution (1 ml per 100 g body weight) and kept in aerated aquaria for 2 h. Then the head kidneys of the specimens were removed and placed in hypotonic KCl solution (0.075 M) for 40 min at 37 °C. After this step, the cell suspension was centrifuged for 10 min at 1200 rpm, after which the supernatant was discarded. The cells were fixed with 5 ml fixative solution (3:1, methanol: glacial acetic acid) for 30 min at 4 °C. Then the cells were centrifuged, and supernatant was discarded again. These last two steps were repeated two to three times. The cell suspensions were then dropped onto cleaned slides. Air-dried slides were stained by 10% Giemsa for 20 min. Then slides were rinsed with distilled water and allowed to dry at room temperature. 10 to 20 slides were prepared from each specimen. All analysed specimens are deposited in the Genetic Laboratory of Kırşehir Ahi Evran University, Turkey (MGSUMKA 300-307).

The C-banding technique of Sumner (1972) was performed for determining constitutive heterochromatin regions whereas Ag-staining technique of Howell & Black (1980) was followed for determining NORs. For C-banding, slides were treated with 0.2 N HCl for 30 min at room temperature, then rinsed with distilled water and air-dried. The slides were then incubated with 5% $Ba(OH)_2$ for 15-20 min at 37 °C, followed by rinsing and drying. Slides were incubated with 2 × SSC for 2 h at 70 °C and rinsed and dried once again. Then slides were stained by 10% Giemsa for 30 min. For Ag-staining, two drops of colloidal developer and four drops of 50% $AgNO_3$ solution were added onto the slides. The coverslip was used to cover the slide and then placed in an incubator at 70 °C. When the slide colour changed to golden brown, the coverslip was removed. Then slide was rinsed and dried.

The chromosome slides were scanned via Leica DM3000 research light microscope (Leica Microsystems, GmbH, Germany) and photographs of metaphases were taken under AKAS software (Argenit Mikrosistem, Turkey). At least 10 metaphases were examined per individual. Karyotypes were arranged manually. Chromosomes were measured by a digital calliper. Chromosomes were classified according to Levan et al. (1964). For calculating fundamental arm number (FN) meta-submetacentrics were taken as biarmed whereas subtelo-acrocentrics were considered as uniarmed.

RESULTS

The diploid chromosome number of *S. lucioperca* was 2n = 48 (Figure 1a). Karyotype was consisted of one pair of metacentric, 15 pairs of submetacentrics and eight pairs of subtelo-acrocentric chromosomes (Figure 1b). FN was calculated as 80. The largest chromosome in the karyotype was a submetacentric. Morphologically differentiated sex chromosomes were not detected. Constitutive heterochromatin regions were observed on the pericentromeres of some of the chromosomes (Figure 1c). Moreover, heterochromatic blocs were determined on three pair of chromosomes (second and third submetacentric pairs and fifth subtelo-acrocentric pair) (Figure 1d). Also, some C-bands were observed on the long arms of six chromosome pairs (fourth and thirteenth submetacentric and first, second, third and seventh subtelo-acrocentric) (Figure 1d). Otherwise, Ag-NORs were determined on the terminal regions of the short arms of fifth submetacentric chromosome pair (Figure 1e, f). One of this NOR had a weaker signal compared to another. Also, on some silver-stained metaphases only one Ag-NOR was observed.

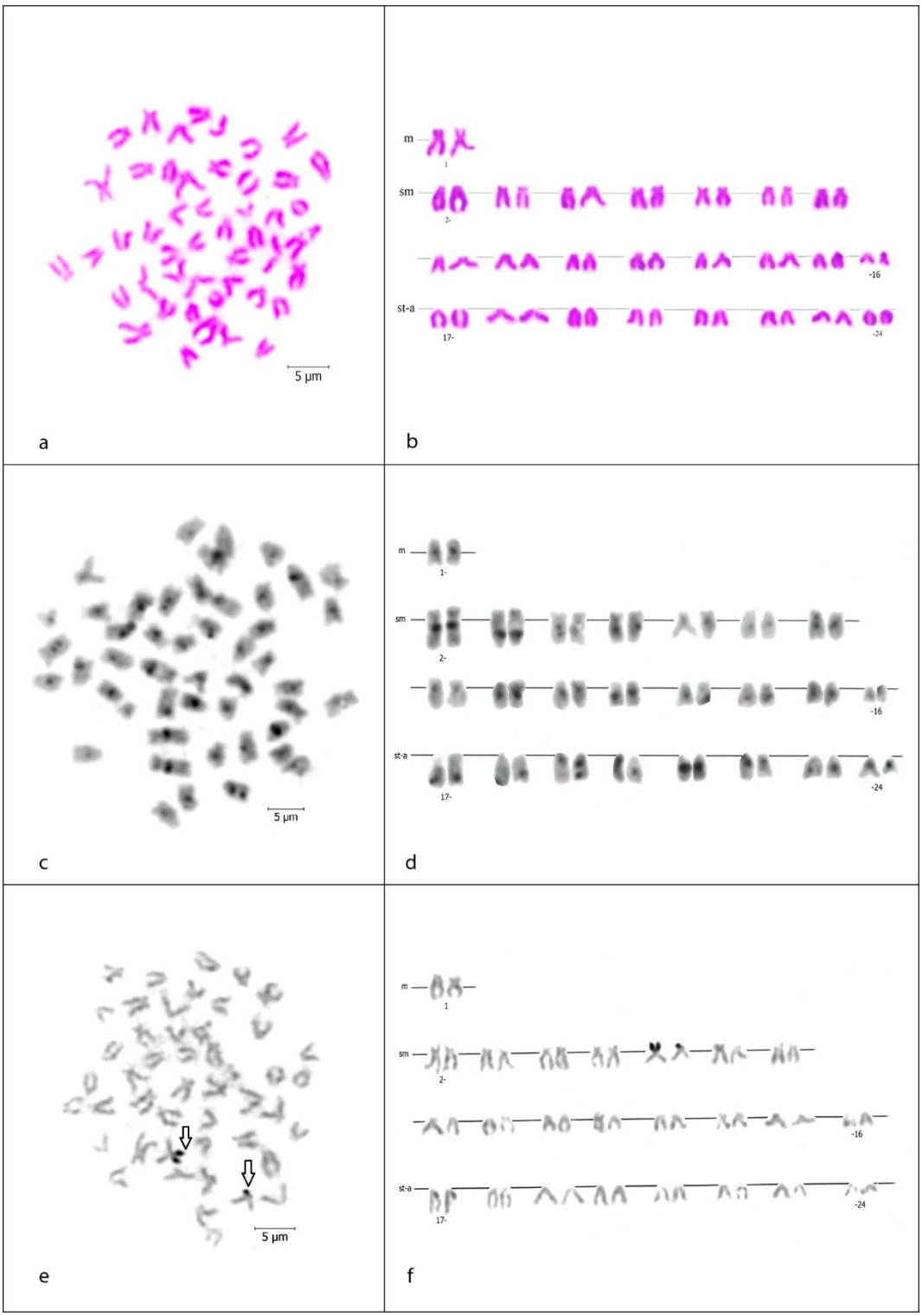

Figure 1. Giemsa stained metaphase (a), arranged karyotype (b), C-banded metaphase (c), arranged karyotype (d), silver stained metaphase (e) and arranged karyotype (f) of *Sander lucioperca*. Arrows indicate the Ag-NORs. Scale bar = 5 µm.

DISCUSSION

Although the advances in the cytogenetics have been developed the basic features of karyotypes have been observed under the conventional staining's (Martins et al. 2011). In this context, the determination of diploid number, chromosome morphology and FN are most popular in fish species (Martins et al., 2011). The diploid chromosome numbers 2n = 48 have been reported in all studied species of Percidae (Arai, 2011). In this regard, 2n of *S. lucioperca* is determined as 48 in this study as reported by Ráb et al. (1987), Mirinargesi et al. (2007), Arai (2011) and Jankun et al. (2014) from different countries. Arai (2011) reported that chromosome morphologies and FN's show some differences between the species of Percidae. Ráb et al. (1987) suggested that karyology of percid chromosomal evolution has been connected with rearrangements of the centromere position rather than chromosome number change. Percid karyotypes are dominated by submeta and subtelo-acrocentric chromosomes (Suciu & Ráb, 1992) as observed in this study. Chromosome morphology of *S. lucioperca* in this study is the same with the

reports of Ráb et al. (1987) and Jankun et al. (2014). However, chromosome morphologies of Turkey population of *S. lucioperca* is different from South Caspian Sea (Mirinargesi et al., 2007) and Hungary (Arai, 2011) populations. The number of biarmed chromosomes of South Caspian Sea (Mirinargesi et al., 2007) and Hungary (Arai, 2011) populations are less than this study. So, FN's of this populations are lower than this study. The differences on these studies should be the result of chromosome contraction. The largest chromosome pair in the karyotype was submetacentric in this study however no information about the largest chromosome was given in previous *S. lucioperca* studies.

From the other four species of the genus *Sander* only *S. volgensis* Ráb et al. (1987) and *S. vitreus* (Arai, 2011) have been studied karyologically. The 2n of this species is the same with populations of *S. lucioperca*. Also, the karyotype of *S. volgensis* Ráb et al. (1987) is the same with this study. However, *S. vitreus* (Arai, 2011) differs from *S. lucioperca* about having all chromosomes as subtelo-acrocentric. From the same subfamily Luciopercinae, *Zingel zingel* and *Zingel streber* (Ráb et al., 1987) have the same diploid chromosome number as *S. lucioperca*. But the number of biarmed chromosomes of this species (Ráb et al., 1987) are less than *S. lucioperca*. Moreover, from the subfamily Percinae, *Perca fluviatilis* (Ráb et al., 1987) and *Percarina demidoffi* (Suciu & Ráb, 1992) have the same diploid chromosome number as *S. lucioperca*. Ráb et al. (1987) reported 30 biarmed chromosomes in *P. fluviatilis* whereas 32 biarmed chromosomes were determined in this study. Otherwise, *P. demidoffi* has 28 biarmed chromosomes and half of them were categorized as metacentrics (Suciu & Ráb, 1992). In this study only one pair of metacentric was observed. One small pair of metacentric was concluded as a percid marker chromosome in *P. demidoffi* (Suciu & Ráb, 1992) but in our karyotypes of *S. lucioperca* metacentric pair is not a small chromosome pair (medium-sized). A pair of large submetacentric and a pair of small metacentric in six percid species including *S. lucioperca* was reported as a marker chromosome by Ráb et al. (1987). This marker submetacentric pair is observed in this study too.

Otherwise, morphologically differentiated sex chromosomes were not determined in *S. lucioperca* like the other studies in percids (Ráb et al., 1987; Klinkhardt & Buuk, 1991; Jankun et al., 2014).

C-banding and silver staining are the most popular chromosomal banding techniques in fish species (Martins et al., 2011). C-banding reveals the constitutive heterochromatin regions concerning the repeated DNAs whereas silver staining detects the active ribosomal sites named as NORs (Arslan & Alpaslan, 2020; Martins et al., 2011; Karasu-Ayata et al., 2021; Unal-Karakuş, 2021). The determination of Ag-NOR number and location, and the location of C-bands have been studied in many fish species from Turkey (Arslan & Alpaslan, 2020; Karasu-Ayata et al., 2021; Unal-Karakuş, 2021). This chromosomal banding features are usually contribute to fish cytotaxonomy (Unal-Karakuş, 2021). As our knowledge, silver staining on the chromosomes of *S. lucioperca* was not applied in the previous cytogenetic studies (Ráb et al., 1987; Mirinargesi et al., 2007; Arai, 2011; Jankun et al., 2014). Only, Ráb et al. (1987) reported a pair of large satellited submetacentric (i.e., NORs carrying) chromosome after Giemsa staining in *S. lucioperca*. It was stated that achromatic regions on the end of its short arms were corresponded to the NOR (Ráb et al., 1987). In this silver-stained karyotype study, Ag-NORs were on the terminal regions of the short arms of middle-sized submetacentric chromosomes as stated by Ráb et al. (1987). Also, the same situation about NOR was reported for *S. volgensis* too (Ráb et al., 1987). So, it is similar in this respect to *S. lucioperca*. No studies have been reported on the Ag-NORs in the other species of the genus *Sander*. One of the most studied percid species, *P. fluviatilis* (Mayr et al., 1985; Klinkhardt & Buuk, 1991) is similar to *S. lucioperca* about having a single Ag-NOR. However, the location of Ag-NORs were on subtelocentric chromosomes (Mayr et al., 1985; Klinkhardt & Buuk, 1991) whereas they were localized on submetacentric chromosomes in this study. Reported heteromorphism between the two NORs in *P. fluviatilis* (Mayr et al., 1985) were observed in this study too. Additionally, observed one Ag-NOR on some silver-stained metaphases of *P. fluviatilis* (Mayr et al., 1985) was detected in some silver-stained metaphases of *S. lucioperca*. Moreover, *P. demidoffi* (Suciu & Ráb, 1992) is like *S. lucioperca* in having single Ag-NOR. However, about the location on the subtelo-acrocentric chromosomes (Suciu & Ráb, 1992) it seems different from *S. lucioperca*.

Otherwise, Jankun et al. (2014) reported the C-banded karyotype of *S. lucioperca* from Poland. In their report the Poland population had no centromeric C-bands. However, there are some centromeric C-bands in the Turkey population. C-bands on the long arms were reported for eight chromosome pairs in Poland population (Jankun et al., 2014) whereas this C-bands are on six chromosome pairs in our study. Heterochromatic blocs were determined on two chromosome pairs in Poland population (Jankun et al., 2014) whereas they were on three pair of chromosomes in Turkey population. There is no C-band information on the other studies of *S. lucioperca* (Ráb et al., 1987; Mirinargesi et al., 2007) and also other species of the genus *Sander* (Ráb et al., 1987), so they cannot be compared with this study.

As compared with samples of other populations of *S. lucioperca*, this study has better resolution results (Giemsa, C-banded and, Ag-NOR metaphases) according to the sensitivity of the method. Chromosome formula is not usually change among the populations of fish (Gaffaroğlu et al., 2013). However, chromosomal banding results should show differences among the populations. Especially number and location of the Ag-NORs are very polymorphic.

CONCLUSION

The karyological investigation of *S. lucioperca* was determined via basic genetics methods. Also, determination of chromosomal banding properties and associated karyotypes were characterized for the first time for Turkey population. Outcomes of the study provide a suitable resource for new cytotaxonomically projects.

COMPLIANCE WITH ETHICAL STANDARDS

a) Authors' Contributions

M. G., S. U. K., and M. K. A.: Designed the study and interpreted data

S. U. K.: Performed the survey work.

M. K. A.: Drafted the paper.

b) Conflict of Interests:

The authors declare that there is no conflict of interest.

c) Statement on the Welfare of Animals:

All procedures used in exeriments involving animals (fish) were in compliance with the "Kırşehir Ahi Evran University Ethical Committee's (numbered 68429034/05)" ethical standards.

d) Statement of Human Rights

This study does not involve human participants.

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