www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 9/3 (2016) 1-7

Research note/Araştırma notu

The first study in Turkey about the chromosomes of tongue fish, *Pegusa lascaris* (Risso, 1810) (Soleidae, Pleuronectiformes), living in the Black Sea

Serkan SAYGUN [*1], Filiz SAYGUN [1]

[1] Department of Fisheries Technology Engineering, Fatsa Faculty of Marine Sciences, Ordu Univ., Fatsa, Ordu, Turkey

Abstract

In this study, It was examined the chromosome structures, numbers and other cytogenetic features of sole *Pegusa lascaris* (Risso, 1810) being one of the flatfish species live in the Black Sea, by conventional banding methods. The Sole specimens were obtained from fishermen along the coast of West and Central Black Sea (between Zonguldak and Ordu). Gill and fin epithelia tissues derived by dissecting fish samples which used colchicine (0.05%) treatment for six hours were incubated in hypotonic solution (0.4% KCl) and then treated with Carnoy's fixative. As a result of karyological examination, it was determined that the chromosome number of *P. lascaris* showed 2n= 42 including 6 metacentric, 8 submetacentric, 12 subtelocentric and 16 acrocentric chromosomes (NF= 56).

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Key words: Tongue fish, Pegusa lascaris, Pleuronectiformes, flatfishes, karyotype

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Karadeniz'de yaşayan dil balığı, *Pegusa lascaris* (Risso, 1810) (Soleidae, Pleuronectiformes) kromozomları konusunda Türkiye'deki ilk çalışma

Özet

Bu çalışmada; Karadeniz'de yaşayan yassı balık (Pleuronectiformes) türlerinden dil balığının *Pegusa lascaris* (Risso, 1810) kromozom yapıları, sayıları ve sitogenetiksel özellikleri rutin bantlama yöntemleri kullanılarak incelenmiştir. Araştırmada dil balığı örnekleri Batı ve Orta Karadeniz sahili boyunca (Zonguldak ve Ordu illeri arası) bazı bölgelerden avcılıkla elde edilmiştir. Altı saat süre ile kolşisin (% 0,05) muamelesi uygulanan balık örneklerinden diseksiyonla çıkarılan solungaç ve yüzgeç epitel dokuları % 0,4 KCl solüsyonunda inkübe edilmiştir ve sonrasında Carnoy fiksatifi ile muamele edilmiştir. Yapılan karyolojik incelemeler sonucunda *P. lascaris* balığının 42 diploit sayıda ve 6 metasentrik, 8 submetasentrik, 12 subtelosentrik ve 16 akrosentrik kromozomdan meydana gelen karyotipi (NF=56) olduğu tespit edilmiştir.

Anahtar kelimeler: Dil balığı, Pegusa lascaris, Pleuronectiformes, yassı balıklar, karyotip

1. Introduction

The object in the classification of fish, similar to other living being groups, is to make a classification based on their natural relations and degree of relationship. However, as sufficient information regarding these subjects has not been acquired so far, performing a totally natural classification has not been possible and the classifications have been made partly artificially. Moreover, there is a great conflict between the ichthyologists in terms of selecting the optimal morphological features in the phylogenetic examination of the fish. A wide range of systems have emerged as some consider cranial osteology, some consider osteology of the caudal region, some consider scales, some consider the structure of the fins, and some consider the structure of the soft sections of the body of prime importance. There are four different systems used for the comparison of the big groups of fish living today. These are Berg (1947), Romer (1960), Helfman et al. (2009) and Nelson et al. (2016).

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +904524235053 Ext4733; Fax.: +904524235053; E-mail:serkan_saygun@hotmail.com

It is known that flatfish (e.g. turbot, flounder, etc.), one of the most valuable fish in terms of the economic significance thereof among the fish living in the seas of Turkey, have interesting similarities on the basis of species (Akşıray, 1987). Today, other flatfish, mainly turbot, which are in danger of extinction due to overfishing and pollution, are taxonomically in the order called Pleuronectiformes (Flatfishes). Production of several (turbot, flounder, etc.) of these genus members, which have been emphasized in the aquaculture studies many times in Turkey and around the World are performed successfully to a great extent.

The Soleidae family is monophyletic with the Achiridae and Cynoglossidae families. It is differentiated from these two groups by a slight morphological difference. Skin is present in the lower jaw and interoperculum continuing ventrally of the three families (Nelson et al., 2016). According to Nelson et al. (2016) and Froese and Pauly (2016) it is reported that there are 32 genera living in total in the Soleidae family and 175 species belonging to these species. However, it is reported that there are 8 species of *Solea* genus in Turkey (Demirsoy, 2005), and in another source there are 5 species (Akşıray, 1987). As a result of the allozyme and cytochrome *b* gene analyses made by Borsa and Quignard (2001), genetic information relating to some soleid species was obtained. In their studies, they detected that *Pegusa* (*Solea*) *impar*, *Pegusa* (*Solea*) *lascaris*, *Solea aegyptiaca*, *S. senegalensis* and *S. solea* fish, which were compared by providing an expansion of the gene regions affected by allozyme enzymes, contain cytochrome *b* gene different from one another. Moreover, they reported that *lascaris* and *impar* species would be named in *Pegusa* genus and *Solea* genus would definitely be synonymous. According to Arai (2011), it is stated that karyological analysis of 7 species belonging to 5 genera out of 130 species (130 species in total) was conducted. However, it was specified that diploid chromosome numbers of Soleidae family members are between 42 and 48 in this reference. It is seen that today, this number increases only to eight as a result of the literature search (Tab. 1).

In this study, it is aimed to detect karyotype of the *P. lascaris* (Soleidae: Pleuronectiformes) species which are among the flatfish found commonly in the Black Sea which have not been studied in terms of karyology in Turkey yet, and to determine the differences specific to species in the structures of chromosomes by means of various staining and banding methods.

2. Materials and methods

Research was conducted on the shoreline from Cape Çam, Ordu in the east (41°6.995′N– 37°47.189′E) to Cape Ölüce, Zonguldak in the west (41°27.33′N – 31°45.535′E) in Turkey (Fig. 1). Samples were obtained from the fishermen fishing by means of various fishing gear and equipment (bottom trawl and gill-nets of various types) in nine stations (Emiroğlu et al., 2013).

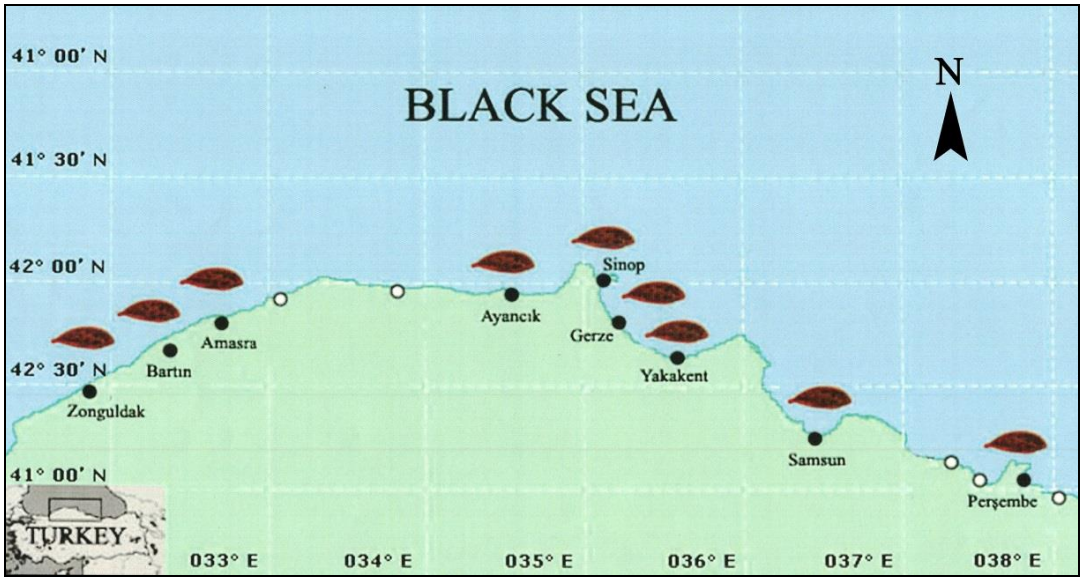

Figure 1. In the study, map showing the sampling stations of sole fishes, *P. lascaris*

In the study, 25 sole fish specimens (16 males, 9 females) with various sizes were used. Chromosomes were analyzed from the tissue preparations obtained from these tongue fish specimens. Vouchers were transferred to the building, where tissue preparation would be performed, in aired containers such that the fish would stay alive.

It was determined that the sole fish obtained as a result of the taxonomic and morphological analysis were *P. lascaris* (Risso, 1810) (Fig. 2) (Borsa and Quignard, 2001; Froese and Pauly, 2016).

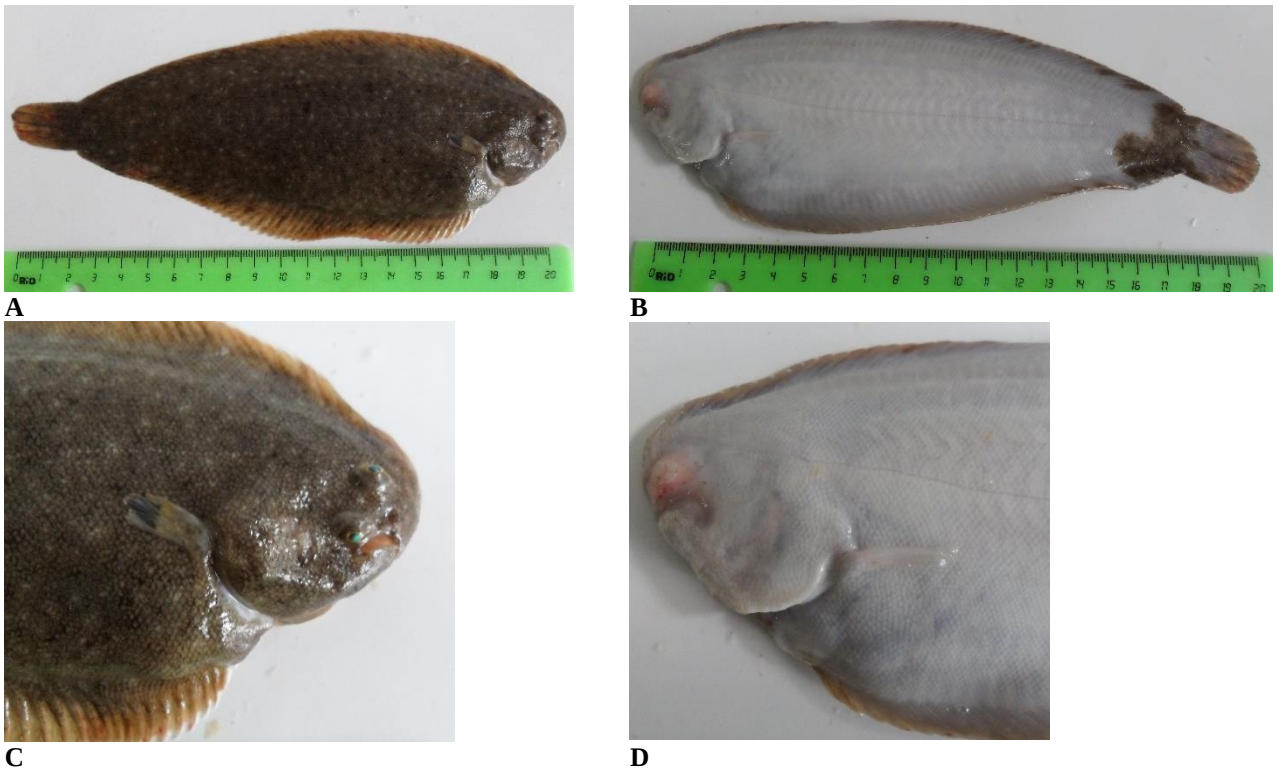

Figure 2. Morphology of the tongue fish sampled in the study, *Pegusa lascaris* (Risso, 1810), **A**- Right side (Upper side); **B**- Eyeless left side (Lower side); **C**: Head and gill (Right Side); **D**: Head and Gill (Left side) - Original

The method, which was determined by Denton (1973) and Kligerman and Bloom (1977) modified as a result of the preliminary tests, was applied in the research. Colchicine solution was injected to the big flatfish (250-1500 g) in a body weight ratio of 25-50 µg/g and in a ratio of 0.05%. Small fish (50-250 g) were maintained in an aired aquarium or fish tank in the colchicine solution in a ratio of 0.005% for 6 hours; After gill and fin tissue was derived, it was kept in 0.4% KCl in petri dishes between 30-40 minutes; each tissue piece was kept in Carnoy's fixative for 30 minutes and after this process was repeated a few times, it was left and kept in a refrigerator.

Microscope slides, where chromosome preparations were laid, were stained in a vertical chalet which was filled with Giemsa prepared with phosphate buffer (pH 6.8) for 15 minutes. The slides were then washed with tap water. The washed slides were kept in xylene for 10 minutes and the extra dye on the slides was removed (Denton, 1973).

In the research, at least 10 preparations (Denton, 1973) from each of the fish sampled from the aforementioned 9 regions were prepared and of these preparations for the detection of chromosomes, Nikon™ Eclipse EC600 phase contrast microscopy was used. Suitable metaphases in the preparations were detected in 10× magnification and metaphase chromosomes were observed in 100× magnification (Denton, 1973; Dutrillaux and Coutourier, 1981). At least 100 metaphases (Thorgaard and Disney, 1990; Rossi et al., 1996) determined for each sample during the examinations was photographed by means of a CCD camera (Pixelink™ Megapixel FireWire Camera, Vitana Corp.) connected to the microscopy, and were computerized. Chromosomes were counted from their images in the metaphase and this data was converted into graphic expressions, and diploid chromosome numbers belonging to flatfish species obtained from each sampling point were determined (Denton, 1973; Thorgaard and Disney, 1990). In the microscopy, relative heights (µ) and arm lengths (µ) of the most suitable metaphase chromosomes among the photographs captured were measured with MicroMeasure© (Version 3.3 PC Software) program (Reeves, 2001; Jankun et al., 2003; Karahan 2016). However, karyotypes which were measured in compliance with the principles indicated by Levan et al. (1964), were determined by being arranged at the centromeric level. The Adobe Photoshop® program was used for the preparation of karyograms. Arm ratios (q/p) of the classified chromosomes were obtained by dividing the length of the long arm (q) to the length of the short arm (p) (Levan et al., 1964; Duran- Gonzalez et al., 1990). The arm number of the chromosomes (NF= Number of Fundamental) was determined by counting the total arms of the double-armed (metacentric and submetacentric chromosomes with p and q arms) chromosomes (Levan et al., 1964; Denton, 1973; Thorgaard and Disney, 1990). Ideograms were drawn which provide monitoring of the chromosomes schematically by arranging the same in the descending order on centromeric axis (according to the p and q arm lengths) in accordance with the class of chromosomes of haploid number (metacentric, submetacentric, subtelocentric and acrocentric) (Denton, 1973; Duran-Gonzalez et al., 1990). Adobe Photoshop® was used for the preparation of the ideograms.

3. Results

In the study, a total of 1113 (∑N) metaphases, which were in a good state among the preparations made from the 25 samples of sole fish sampled from 9 points including Zonguldak (∑n= 104), Bartın (∑n= 144), Amasra (∑n= 110), Ayancık (∑n= 104), Sinop (∑n= 214), Gerze (∑n= 110), Yakakent (∑n= 121), Samsun (∑n= 106) and Perşembe (∑n= 100) were examined. It was observed that the chromosome number belonging to this species varied between 24 and 42 as a result of the chromosome counting performed in the examined metaphases. According to the result of the counting, it was detected that the 2n chromosome number was 42 with a ratio of 26%.

The ratios of percentage change of the diploid chromosome number according to the regions were observed as 30% (n= 31) in Zonguldak, 21% (n= 30) in Bartın, 23% (n= 25) in Amasra, 25% (n= 26) in Ayancık, 28% (n= 60) in Sinop, 25% (n= 28) in Gerze, 30% (n= 36) in Yakakent, 32% (n= 34) in Samsun and 23% (n= 23) in Perşembe.

In the study, as a result of the karyological analyses, the chromosome number of the *P. lascaris* was 2n= 42, karyotype thereof was detected as 3 pairs of metacentric, 4 pairs of submetacentric, 6 pairs of subtelocentric and 8 pairs of acrocentric chromosomes and the arm number thereof was detected as 56 (NF). In figure 3 and 4, metaphase and karyogram belonging to this are shown.

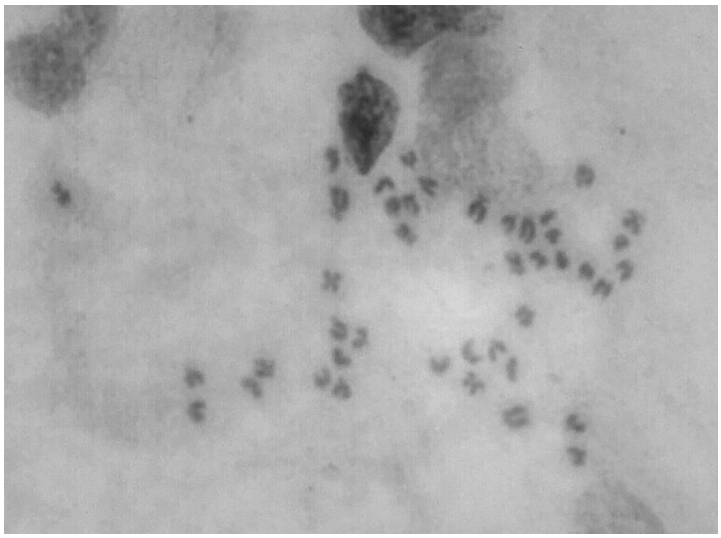

Figure 3. The metaphase plate of *P. lascaris*

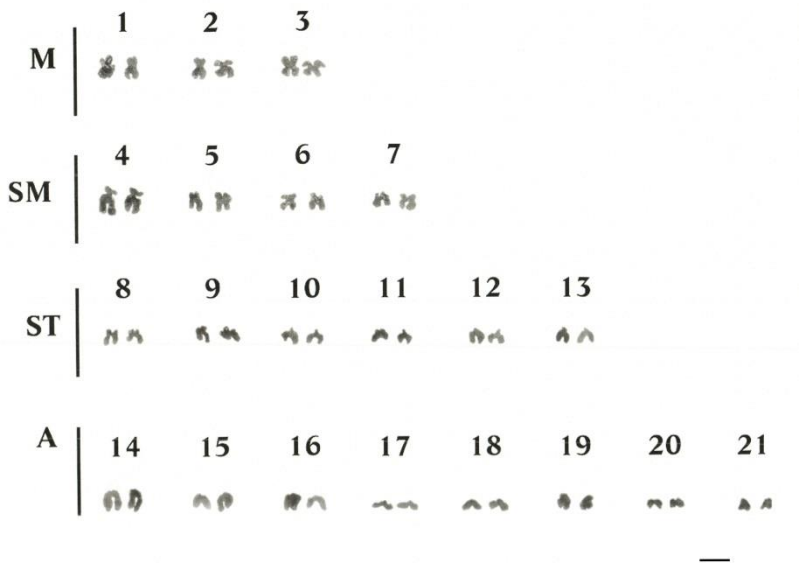

Figure 4. The Karyotype of *P. lascaris*, Bar-10 µ

Chromosome arm lengths of chromosome pairs of *P. lascaris* measured with MicroMeasure software. The classification of chromosome pairs of *P. lascaris* was done according to chromosome arm length ratios (q/p) determined in Levan et al. (1964)'s nomenclature. The ideogram, which was prepared according to the relative short arm (p) and long arm (q) lengths of the chromosomes determined as measurements, is shown in figure 5.

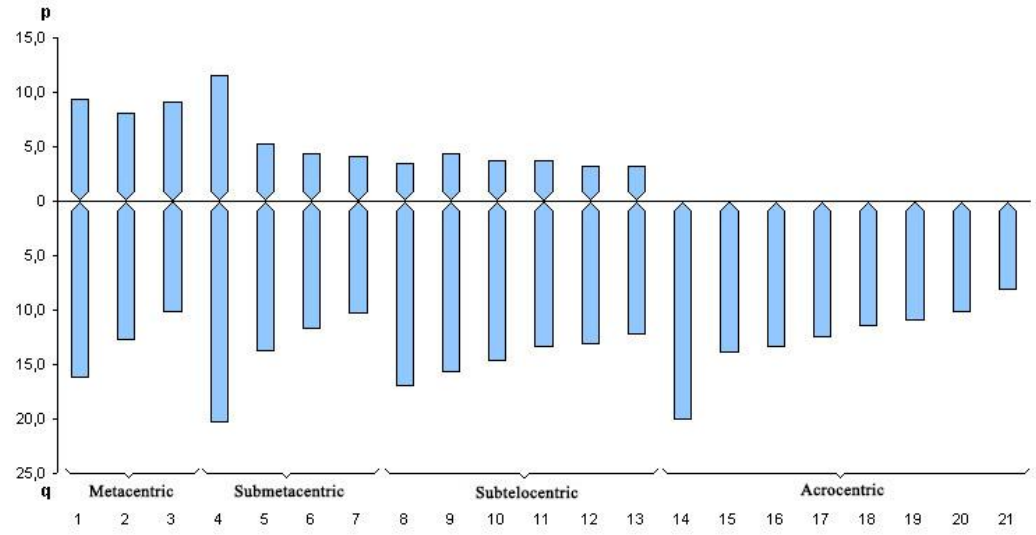

Figure 5. The Ideogram of *P. lascaris*' haploid chromosomes.

4. Conclusions and discussion

It is known that there are about 772 extant species in approximately 129 genera and 14 families belonging to the Pleuronectiformes order living in the world (Nelson et al., 2016). In this part, it was focused on and argued with thirty three studies, cytogenetically worked on seven species of Soleidae family until today. In this study, chromosomes of a species from the Soleidae (sole fish) family living in the Black Sea were detected firstly in Turkey (table 2).

Table 2. A summary of some cytogenetic and karyological studies reported in members of Soleidae family

Species	Location	2n	NF	Karyotype	Reference
Heteromycteris oculus	Bay of Bengal, India	48	54	6msm+ 42a	Patro and Prasad (1981)
Pegusa (*Solea*) *lascaris*	Black Sea, Russia	42	48	6sm+ 36sta	Vasiliev (1978)
Solea lascaris	Spain	42	56 -58	8m+ 8-10sm+ 24-26a	Pardo et al. (2001)
Pegusa lascaris	**Black Sea, Turkey**	**42**	**56**	**6m+ 8sm+ 12st+ 16a**	**In This Study**
Solea lutea	Adria, Cratoria	30	44	14msm+ 16sta	Sofradzija (1985)
Solea solea	England	42	42	42a	Barker (1972)
Solea solea	Spain	42	56 -58	8m+ 8-10sm+ 24 - 26a	Pardo et al. (2001)
Solea solea	Gulf of Venice, Italy	42	60	8m+ 10sm-st+ 24a-t	Libertini et al. (2002)
Solea senegalensis	Spain	42		6m+ 4sm-st+ 8st+ 24a	Vega et al. (2002)
Solea senegalensis	Spain	42		6m+ 4sm-st+ 8st+ 24a	Cross et al. (2006)
Solea senegalensis	Spain	42		6m+ 4sm-st+ 8st+ 24a	García-Cegarra et al. (2013)
Solea senegalensis	Spain	42			Molina-Luzón et al. (2015)
Microchirus ocellatus	Gulf of Palermo Spain	42	56	14msm+ 28sta	Vitturi et al. (1993)
Zebias zebra	East China Sea	46	46	46a	Arai (2011)

In the study, diploid chromosome number of the sole fish was detected as 2n= 42, karyotype consist of as 6 metacentric, 8 submetacentric, 12 subtelocentric and 16 acrocentric chromosomes and the arm number thereof as NF= 56. As well as the karyological information with respect to the Soleidae family is limited (Tab. 2), the chromosome number was 2n= 42 while karyotype varied between 6sm+ 36sta (NF= 48) (Vasiliev, 1978) and K= 8m+ 8-10sm+ 24-26a (NF= 56-58) (Pardo et al., 2001) in both studies conducted relating to *Solea lascaris*. For *S. lutea* 2n= 30 and K= 14msm (meta- submetacentic) + 16sta (subtelo-acrocentic) (NF= 44) (Sofradzija, 1985), while the chromosome numbers were found to be identical to each other as 2n= 42 for *S. solea* in two different studies, karyotype was between 42a (NF= 42) (Barker, 1972) and 8m+ 8-10sm+ 24- 26a (NF value was between 56 and 58) (Pardo et al., 2001). While the results obtained from our research and the chromosome number in the study made by Vasiliev (1978) was identical, the arm number was different in terms of karyotype consisting of only the submetacentric and acrocentric chromosome. Likewise, the results detected in our research (Tab. 2) were identical with the results obtained by Barker (1972) and Pardo et al. (2001) in terms of the chromosome number, but our results differed from those detected by Barker (1972) and Pardo et al. (2001) in karyotype. In terms of the chromosome arm number, on the other hand, the results were found to be similar to the values determined by (Pardo et al., 2001). Diploid chromosome number, morphology and arm number detected by Sofradzija, 1985 for the *S. lutea*, a Soleidae species close to *P. lascaris*, were totally different from the results of our research. Three researchers reported that they detected the same karyotype (2n= 42, K= 6m+ 4sm-st

(submeta-subtelocentic) + 8st+ 24a) for *S. senegalensis*, another species of sole fish (Vega et al., 2002; Cross et al., 2006; García-Cegarra et al., 2013, Molina-Luzón et al., 2015).

While the results of our study were identical with *Microchirus ocellatus* (Vitturi et al., 1993), which is another species of sole fish (Tab. 2), in terms of chromosome number (2n=42) and arm number (NF= 56) except for the karyotype (K= 14msm+ 28sta), the results were different from *Heteromycteris oculus* (2n= 48, K= 6msm+ 42a and NF= 54) (Patro and Prasad, 1981).

As can be understood from these explanations, either molecular genetic or cytotaxonomic studies show that of the flatfish in the Soleidae family there are great similarities on the basis of species. In this study, it is also aimed to detect the chromosome structures of the sole fish in the Black Sea by making cytogenetic examinations providing comparison on the basis of species.

To conclude, a great majority of the cytotaxonomic thesis and research where analysis of chromosomes have been made so far in Turkey have been conducted in freshwater fish and the study number belonging to sea fish are limited. It is thought that factors such as obtaining the freshwater fish and preserving them alive is easier when compared to sea fish, the easier applicability of the chromosome analysis methods based on this having many number of studies conducted on inland-water fish in the world have an important effect on this. With this study, for the first time a chromosome study has been made on flatfish in Turkey. At the same time, success was achieved in various banding and staining methods applied in the research.

Moreover, cytotaxonomy or cytogenetic will provide an opportunity for collaboration in many fields regarding the subject, especially in taxonomy, ecology, aquaculture and biotechnological research.

Acknowledgements

This study was supported a project with S.068 number [Comparison of Chromosome Structures of Various Flatfish (Pisces, Pleuronectiformes) Living in the Black Sea] by the Department of Science Research Project of Samsun Ondokuz Mayıs University, Turkey.

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(Received for publication 31 October 2016; The date of publication 15 December 2016)