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Research article/Araştırma makalesi

Karyotype of the Black Sea Turbot, *Scophthalmus maeoticus* (Pallas 1814) (Pisces: Pleuronectiformes)

Serkan SAYGUN [*1]

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

Abstract

In this study, using conventional staining method, chromosome structures and numbers of Black Sea turbot *Scophthalmus maeoticus* (Pallas 1814), a species of flatfish living in the Black Sea, have been examined. The specimens of the fish were obtained through fishing in regions between the coasts of West and Middle Black Sea of Turkey. It was determined that *S. maeoticus* had a diploid number chromosomes of 2n= 44 and a fundamental number of NF= 48. The karyotpe of turbot contained 2 pairs of metacentric, 7 pairs of subtelocentric and 13 pairs of acrocentric chromosomes.

Key words: *Scophthalmus maeoticus*, turbot, The Black Sea, chromosome, cytotaxonomy

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Karadeniz Kalkan Balığının, *Scophthalmus maeoticus* (Pallas 1814) (Pisces: Pleuronectiformes) Karyotipi

Özet

Bu çalışmada geleneksel boyama metou kullanılarak Karadeniz'de yaşayan yassı balıkların bir türü olan Karadeniz Kalkanı *Scophthalmus maeoticus* (Pallas 1814)'un kromozom yapıları ve sayıları incelenmiştir. Balık örnekleri Türkiye'nin Batı ve Orta Karadeniz kıyıları arasında kalan bölgeden avcılık yoluyla elde edilmiştir. *S. maeoticus*'un 2n=44 diploid kromozoma sahip olduğu ve kromozom kol sayısının da NF=48 olduğu tespit edilmiştir. Kalkan balığının karyotipinin 2 çift metasentrik, 7 çift subtelosentrik ve 13 çift akrosentrik kromozom içerdiği belirlenmiştir.

Anahtar kelimeler: *Scophthalmus maeoticus*, kalkan balığı, Karadeniz, kromozom,sitotaksonomi

1. Introduction

Fish species are classified in many ways including the use of morphometric measurements and ratios, meristic counts, anatomical characteristics, color, reproductive isolation tests as well as the karyotype and DNA analyses. Karyotype analysis is one of the methods that has been used in ichthyology since the mid-20th century that is especially applied for the classification of the taxon but this may lead to some identical problems, confused with the other turbot species, especially Atlantic turbot (*Scophthalmus maximus*) due to some morphological similarities. Karyotype of species represents the physical demonstration of the genetic system. The number and morphology of chromosomes are conserved to a further and better extent relative to such other traits (Watson et al., 2013).

Traditionally, flatfish are classified as halibut and flounder with a right and left eye. It is argued hypothetically that halibut and scald fish evolved from ancestors analogous to Psettods and the form of a right and left eye emerged from a rather primitive ancestor (Berendzen and Dimmick, 2002). As knowledge on this subject grows, the process of understanding the inter-relation of flatfish becomes more complicated. The side with the eye stands as a significant characteristic in terms of classification. The first scientist to claim the alternative hypothesis was Chapleau (1993) who stated the side with the eye is mostly determinative for flatfish but the condition of the eye is not an exact determinative of the inter-relations within the group. As a result of a molecular study conducted by Berendzen and Dimmick (2002) the conclusion was reached that the knowledge or the determination of the side with the eye does not suffice to derive phylogenic knowledge.

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: 90454235053; Fax.: 90454235053; E-mail: serkan_saygun@hotmail.com
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In the world, about 772 alive species are identified in approximately 129 genera and 14 families within the Pleuronectiformes order (Nelson et al., 2016). Turbot were classified as part of the Bothidae family until the 1970s; however they are currently classified in Scophthalmidae (turbot) (Nelson et al., 2016). While four genera, these being *Lepidorhombus*, *Phrynorhombus*, *Scophthalmus* and *Zeugopterus* are represented in eight or nine species worldwide (Nelson et al., 2016; Froese and Pauly, 2017), 5 species of them in 3 genera were reported to be in Turkey's seas (Akşıray, 1987; Bilecenoğlu et al., 2014). There are twelve studies known to be on chromosomes of three species of the Scophthalmidae family (Klinkhardt, 1995; Arai, 2011). As a result of the taxonomic, morphological and phylogenic analysis conducted (Froese and Pauly, 2017; Borsa, and Quignard, 2001), it was reported that the Black Sea turbot, which is systematically recognized and classified as *Psetta maxima* (Suzuki et al., 2004), was, in fact, *Scophthalmus maeoticus* (Pallas 1814) because this taxon name is used to as usual synonymous of *S. maeoticus* (Froese and Pauly, 2017; GBIF, 2017). However, it was used *Rhombus maeoticus*, one of old synonymous of *S. maeoticus*, in previous two old studies conducted by Ivanov (1969) and Vasiliev (1985).

Since the 1980s cytogenetic studies, which have been carried out intensively in human-beings and other organisms, have also been carried out at the same rapidity in fishes. Of these, approximately twelve studies related to flatfish were focused to determine chromosomes of species belonging to the Scophthalmidae family. Some of them are not only related directly as karyotype purpose but also used to determine the ploidy of fishes in aquaculture because of reporting only chromosome number of experimented species in these studies.

This study intends to determine the karyotype of the turbot, *Scophthalmus maeoticus* (Pallas 1814) of the Scophthalmidae family, and to reveal the species-specific differences in the chromosome structures.

2. Materials and methods

The study was conducted along the coastline between Cape Çam, Ordu in the East (41°06′998N–37°47′169E) and Cape Ölüce, Zonguldak (41°18′826N–31°23′833E). The specimens were collected in Zonguldak, Bartın, Ayancık and Sinop from fishermen (Figure 1). Six turbot specimens obtained from the hatchery of the Trabzon Central Fishery Research Institute (CFRI) were also used in the study.

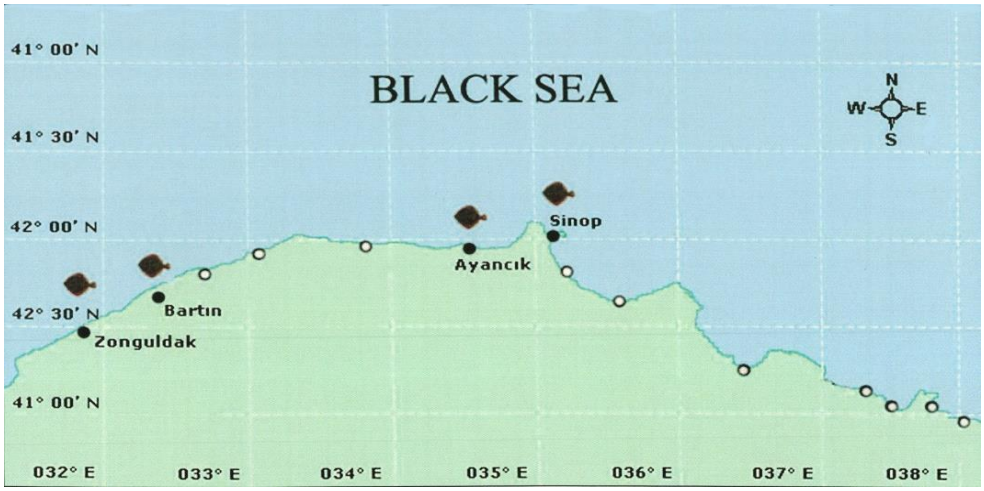

Figure 1. In the study, the map shows the sampling stations of Black Sea turbot

The mitotic chromosomes were analyzed in total 26 turbot specimens of various sizes. The specimens were transferred to laboratories alive and kept in well-aired containers. Figures 2 a, b and c demonstrate the thorn-like bone structures on both sides, which are specific to the *S. maeoticus* species sampled as a part of this study. Bony tubercles generally developed on both sides, which are always larger than eye as shown in Figure 2. d, e, f and g, as defined in some references (Nielsen, 1986; Samsun et al., 2005). By modifying the method followed by Denton (1973), we undertook our preliminary trials in research, mitotic inhibition, dissection and the hypotonic application process. Kligerman and Bloom's (1977) method was used for chromosome preparation of the solid tissues(gills and fins) ; the dried preparations were stained with 6% Giemsa solution (pH 6.8 phosphate buffer) for 15 minutes. After this process, a microscopic examination of the Giemsa stained slides was carried out. With the aim of counting and determining type of chromosomes, the Nikon Eclipse™ EC600 phase contrast microscope was used for the observations of at least the best ten metaphase on the slides prepared from each specimen. The suitable metaphase plates in the preparations were identified in 10× magnification and then in 100× magnification with immersion oil and metaphase chromosomes were observed (Denton, 1973).

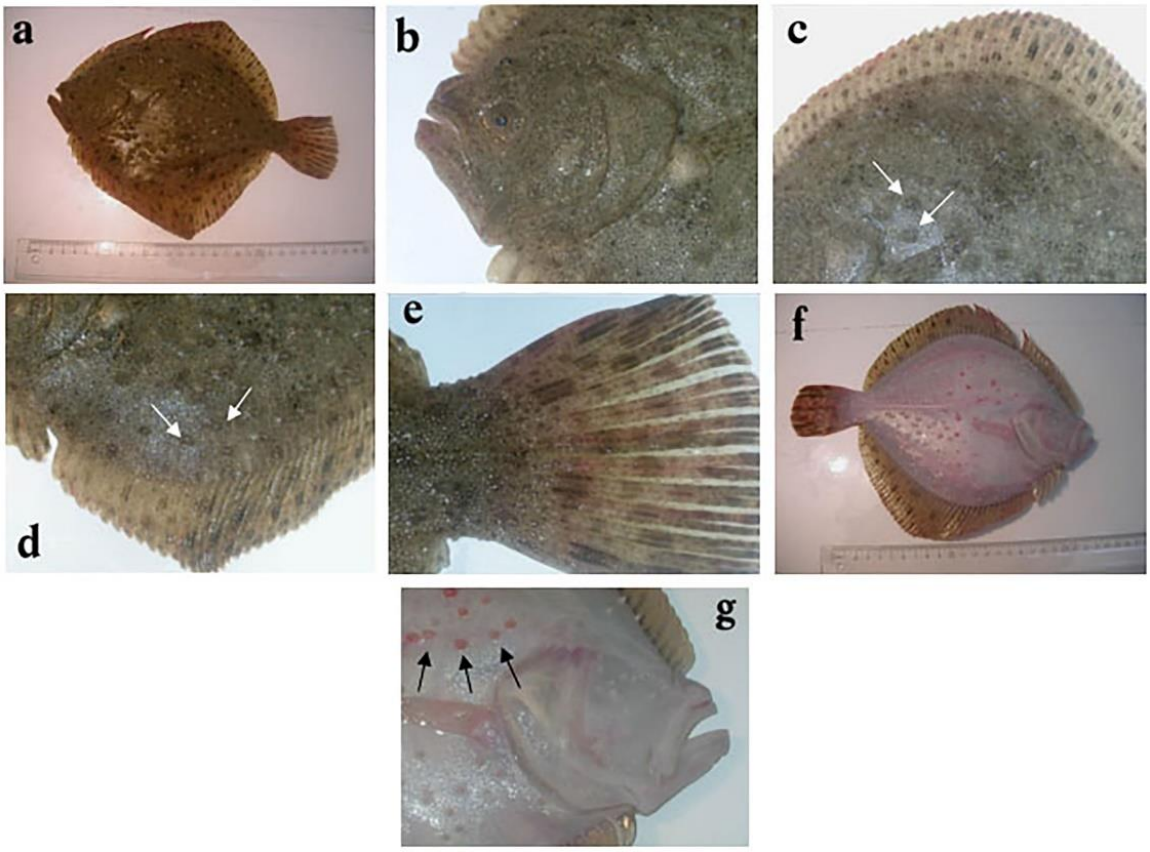

Figure 2. Morphology of the turbot sampled in the study: a- The upper left side (eyed side); b- Head and eyes; c-, d-, e- (respectively) Dorsal, anal and caudal side and fins; f- The lower right side (eyeless side); g- Gills (eyeless side) (species specific thorns-bony scales located on both sides of the fish were indicated by arrows)

Photographs of each sample were taken at a minimum 100 metaphase (Thorgaard and Disney, 1990) via a CCD camera (Pixelink™ Megapixel FireWire Camera, Vitana Corp.), which was connected to a microscope and then transferred to a computer during the microscopic investigation. The chromosomes were counted on the best metaphase images, with the data converted into graphic expressions; and the numbers of diploid chromosomes of the turbot samples at each sampling stations were determined (Denton, 1973; Thorgaard and Disney, 1990).

Among the photographs taken with the microscope, the relative arm lengths of the most available metaphase chromosomes were measured using MicroMeasure© (Version 3.3 PC Software) (Reeves, 2001; Jankun et al. 2003, Karahan, 2016). Chromosome morphology was ascertained on the basis of arm ratio as suggested by Levan et al. (1964) and the chromosomes were classified as metacentrics (m), submetacentrics (sm) and acrocentrics (a) or telocentrics (t). NF (chromosome arm number) was determined considering m/sm chromosomes to have two arms and t/a chromosomes to have one arm (Denton, 1973; Thorgaard and Disney, 1990; Oliveira and Gosztonyi, 2000). Adobe Photoshop® was used for the preparation of the karyograms and ideograms (Çetin et al., 2010).

3. Results

In this study, the number and shape of the diploid chromosomes of turbot, *Scophthalmus maeoticus* (Pallas 1814), which is the only Scophthalmidae species among the flatfish (Pleuronectiformes) species inhabiting in the Black Sea were determined.

A total of 727 metaphase plates obtained from twenty six turbot specimens collected from study area and reared in Institute of CFR were examined in this study. The karyotype of *S. maeoticus* had a diploid chromosome number of 2n= 44 chromosomes, and a fundamental number of NF =48. It consisted of 2 pairs of metacentric (m), 7 pairs of subtelocentric (st) and 13 pairs of acrocentric (a) chromosomes. Metaphase plate and karyogram of the species are given in Figures 3 and 4.

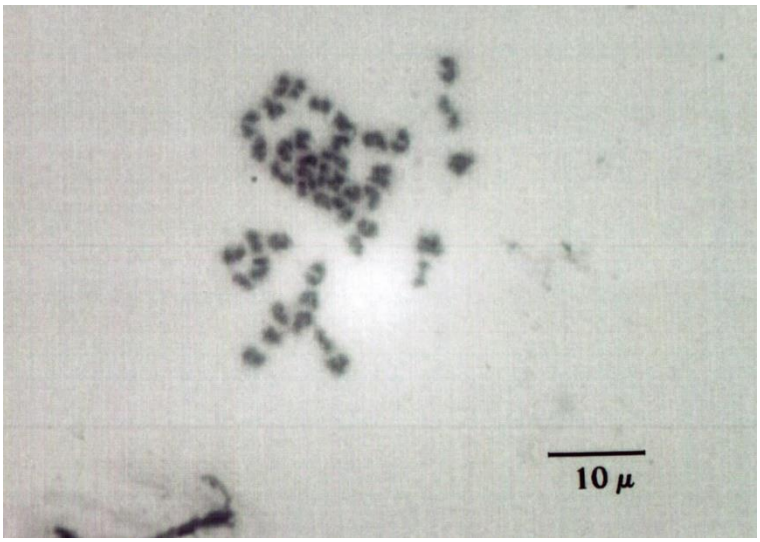

Figure 3. The metaphase plate of *Scophthalmus maeoticus*

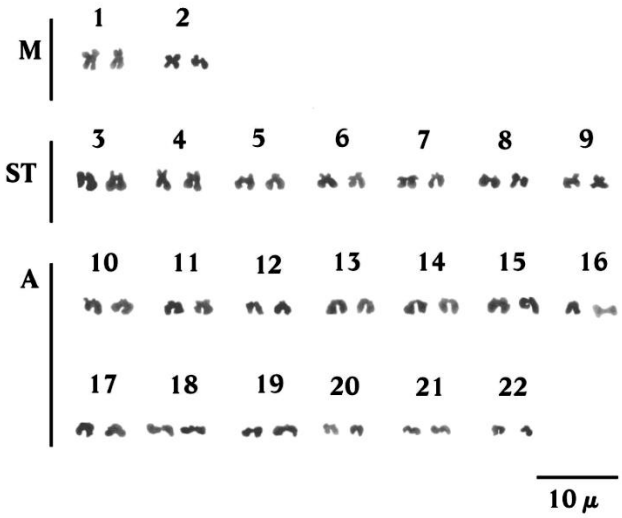

Figure 4. Karyotype of *Scophthalmus maeoticus*

The ideogram of *S. maeoticus* was created by the relative arm length ratios of chromosomes. Figure 5 shows the ideogram drawn up with respect to the relative short arm (p) and relative long arm (q) lengths of the chromosomes

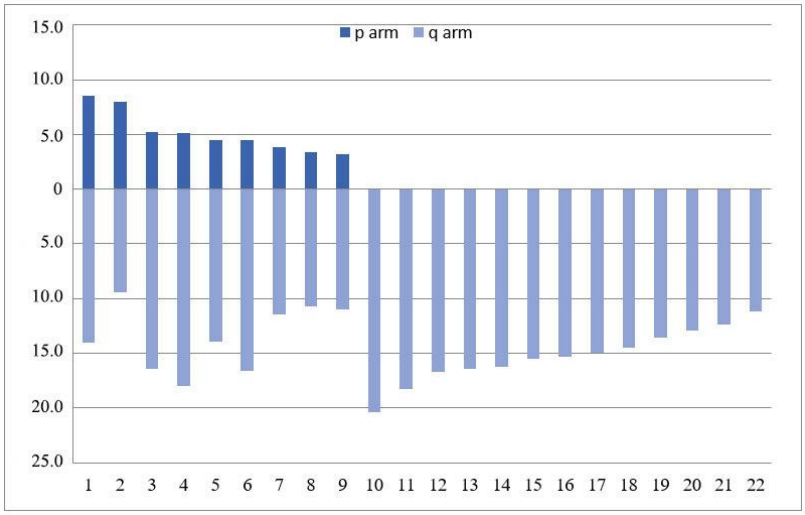

Figure 5. The ideogram of *Scophthalmus maeoticus*

4. Conclusions and discussion

It has been known to be some taxonomic problems in the solution of the relationships within the Pleuronectiformes order which has a structure that makes it difficult to work morphologically and in making a classification that reflect their affinities (Berendzen and Dimmick, 2002). However, it has been understood that the studies enabled the determination of cytotaxonomic information will make a significant contribution to the taxonomy, in addition to the systematic and molecular genetic information of the Scophthalmidae family. As a result of the cytogenetic studies, important and uncertain differences among the species of the family were detected, as can be seen in Table 1. This study, conducted on the Black Sea turbot, determined; the number of diploid chromosomes of the studied species was 2n= 44, while the karyotype consists of four metacentric, fourteen subtelocentric and twenty six acrocentric chromosomes, and the number of arms was determined as NF= 48.

Only three turbot species out of the Scophthalmidae family were studied cytogenetically (Table 1). One of these studies was reported *Rhombus maeoticus* as synonymous of the species belonging to *Scophthalmus* genus inhabit in the Black Sea and determined the number of diploid chromosomes as 2n= 40 - 48, and indefinitely determined the number of chromosome arms as NF= 60 (Ivanov, 1969), another study determined the same as 2n= 40 NF= 48 (Vasiliev, 1985). While results were observed for *Scophthalmus maximus*, which is the most intensively studied species, as 2n= 44, K= 4m+ 2sm-st+ 10st+ 28a (NF= 48) (Bouza et al., 1994; Piferrer et al., 2000); 2n= 44, K= 4m+ 22st+ 38a (27), 2n= 44, K= 4m+ 12st+ 28a (Chen et al., 2005) and 2n= 44 diploid (Castro et al., 200; Wang et al., 2010); the values found as 2n= 44, K= 4m+ 12st+ 28a and NF= 48 (Pardo et al., 2001) for *S. rhombus*. The results were reported by Fan et al. (2010) as a consequence of the cytogenetic study carried out on the basis of cell culture on *S. maximus* and were reported as 2n= 44 chromosomes and the karyotype as 4m+ 2sm+ 10st+ 28t. However, Taboada et al. (2014) explained the number of diploid chromosomes as 2n= 44 and the karyotype as 3 pairs of m/sm and 19 pairs of st/a of *S. maximus* as a result of the study conducted by the latest and most advanced cytogenetic analysis method available (FISH with BAC clones) for the mapping of the chromosomes. The study results are similar to the diploid chromosome numbers yielded through both studies and are consistent with the karyotype determined (Table 1).

Table 1. An outline of some cytogenetic and karyological studies reported in Scophthalmidae family (2n: Diploid chromosome number, NF: Number of Fundamental= Total arm number)

Species	**Location**	**2n**	**NF**	**Karyotype**	**References**
Rhombus (*Scophthalmus*) *maeoticus*	Black Sea - Russia	40-48	60?		Ivanov (1969)
Rhombus (*Scophthalmus*) *maeoticus*	Black Sea - Russia	44	48		Vasiliev (1985)
Scophthalmus maeoticus	Black Sea-Turkey	44	48	4m+ 14st+ 26a	In this study
Scophthalmus rhombus	Spain	44	48	4m+ 2sm+ 38a	Pardo et al. (2001)
Scophthalmus maximus	Spain	44	48	4m+ 2sm/st+ 10st+ 28a	Bouza et al. (1994)
Scophthalmus maximus	Spain	44	48	4m+ 2sm/st+ 10st+ 28a	Piferrer et al. (2000)
Scophthalmus maximus	Spain	44	48	4m+ 22st+ 18a	Cunado et al. (2001)
Scophthalmus maximus	China	44	48	4m+ 12st+ 28a	Chen et al. (2005)
Scophthalmus maximus	Spain	44			Castro et al. (2003)
Scophthalmus maximus	China	44			Wang et al. (2010)
Scophthalmus maximus	China	44	60	4m+ 2sm+ 10st+ 28t	Fan et al. (2010)
Scophthalmus maximus	Spain	44		6m/sm+ 38st/a	Taboada et al. (2014)

This study showed a similarity in the results as those conducted on turbot in terms of the diploid chromosome number and fundamental number except the study carried out by Ivanov (1969) in the Black Sea (Russia). It also showed

similar results in regards to the number of metacentric chromosomes (4m) as all of the other studies with *S. maximus* except for the study carried out by Taboada et al. (2014). Differences were also seen in regards to the number of subtelocentric and acrocentric chromosomes compared to the studies undertaken by Cunado et al. (2001) and Chen et al. (2005). However, the karyotype derived from this study was determined to be different with those determined by Bouza et al. (1994), Piferrer et al. (2000) and Pardo et al. (2001) in terms of 1 pair of submeta-subtelocentric (sm/st) and acrocentric (a) chromosomes even though it is fairly similar to the same (Table 1). The results of the study reveal that the Black Sea turbot, *Scophthalmus maeoticus*, is a separated species from the Atlantic turbot (*S. maximus*).

In terms of the number of diploid chromosomes, this study showed similar results as those submitted by Ivanov (1969) and Vasiliev (1985), however, as they failed to definitively determine the karyotypes in their studies a precise comparison could not be made. It was stated within both studies, conducted in the Black Sea, that the species *R. maeoticus* could, taxonomically a synonym, be *Psetta maxima maeotica* or *P maxima*, being a sub-species of *Scophthalmus maeoticus* (Froese and Pauly, 2017).

Suzuki et al. (2004) stated within their study that the results of the genetic analysis (mitochondrial DNA analysis) conducted on the individuals of *Psetta maxima*, commonly known as the Mediterranean Sea turbot, exist in the Atlantic Ocean, the Mediterranean Sea, the Aegean Sea, the Marmara Sea and the Black Sea (the Sea of Azov, Turkish coasts and Romanian coasts) indicate a separated species. They also state that the genetic distance between separate *P. maxima* populations was fairly slight despite the geographic differences, and that the species live in the Black Sea was *P. maxima*, and that the molecular analysis conducted did not support the assumption about a local species or a sub-species, the assumption of which is based on the taxonomic studies (on the basis of the diameter and number of the osteoid apophysis) that have been conducted so far. On the other hand, it is currently reported by taxonomists that the Black Sea turbot is classified into the *Scophthalmus* genus, and is an individual and separated species, referred to as *Scophthalmus maeoticus* (Pallas 1814) (Whitehead 1986; Evseenko, 1996; Eschemeyer, 1998; Froese and Pauly, 2017; GBIF, 2017).

In light of the information above, we believe that the polymorphism in the chromosome numbers and shapes observed in the turbot might have arisen out of the fact that the populations studied were different, the intraspecific and interspecific variations and the fact that there were technical differences as well as differences among the methods and tools used for the purpose of analysis. Since the chromosome sizes are fairly small and issues are encountered in serial banding or staining (Cunado et al., 2001) there are several challenges that occur with the cytogenetic analysis of the flatfish chromosomes (Pleuronectiformes). It was stated that, therefore, it was natural to experience difficulties in the classification of chromosomes, which leads to an expectable outcome of yielding different karyotypes even in the case of the same species (Denton, 1973, Thorgaard and Disney, 1990; Cunado et al., 2001).

In conclusion, there has been a vast plurality of the cytotaxonomic assertions and studies involving chromosome analysis conducted to date in Turkey on freshwater fish species. However, there have been an insignificant number of studies carried out on saltwater fish. This is due to the fact that it is relatively easier to acquire and collect freshwater fish species and to keep them alive compared to saltwater fish species. This study is the first to determine the number of diploid chromosomes of and define the karyotype for the Black Sea turbot, *Scophthalmus maeoticus*, which is a saltwater fish.

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