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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Karyotypic analysis of *Chondrostoma regium* (Teleostei: Leuciscidae) distributed in the Karasu River (Erzurum)

Karasu ırmağında (Erzurum) yayılış gösteren *Chondrostoma regium*'un (Teleostei: Leuciscidae) karyotipik analizleri

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Abstract: In this study, the karyotypic characteristics of *Chondrostoma regium* (Heckel, 1843) have been investigated. Fish samples were caught from the Karasu River (Euphrates River Basin) with fishing net. The live fish were transported to the laboratory and kept in aerated aquaria before the analyses. The karyotype analysis was performed in fish kidney and gill epithelium cells. It was determined that *C. regium* had 2n=50 chromosomes. In detail, the karyotype formula of *C. regium* was determined as 9 metacentric, 7 submetacentric, 1 subtelocentric and 8 telocentric chromosome pairs (18M+14SM+2ST+16T), and fundamental arm number was calculated as 82. Constitutive heterochromatin regions were determined on telomeres of the chromosomes. Nuclear orgnizer regions were detected on 21st chromosome. Karyotype symmetry/asymmetry index was calculated as 2.32. The karyotypes of gill and kidney cells were the same. No sex chromosomes were cytologically detected.

Keywords: *Chondrostoma regium*, fish cytotaxonomy, chromosome banding

Öz: Bu çalışmada *Chondrostoma regium*'un (Heckel, 1843) karyotipik özellikleri araştırılmıştır. Balık numuneleri Karasu Irmağından (Fırat Nehri havzası) serpme ağlarla yakalanmıştır. Analizlerden önce canlı balıklar laboratuvara taşınmış ve havalandırmalı akvaryumlarda tutulmuştur. Karyotip analizi balıkların böbrek ve solungaç epitel hücrelerinde yapılmıştır. *C. regium*'un 2n=50 kromozoma sahip olduğu belirlenmiştir. Ayrıntılı olarak *C. regium*'un karyotip formülü 9 metasentrik, 7 submetasentrik, 1 subtelosentrik ve 8 telosentrik kromozom çifti (18M+14SM+2ST+16T) olarak belirlenmiş olup, temel kol sayısı 82 olarak hesaplanmıştır Kromozomların telomerlerinde konstitütif heterokromatin bölgeleri belirlenmiştir. 21. kromozom üzerinde nüklear organizer bölge tespit edilmiştir. Karyotip simetri/asimetri indeksi 2,32 olarak bulunmuştur. Solungaç ve böbrek hücrelerinin karyotipi aynı olup sitolojik olarak hiçbir eşey kromozomu tespit edilmemiştir.

Anahtar kelimeler: *Chondrostoma regium*, balık sitotaksonomisi, kromozom bantlama

INTRODUCTION

It is known that 391 fish species are living in freshwaters (Saygun, 2021) and 512 fish species in the seas (Bilecenoğlu et al., 2014) in Turkey. According to the cytotaxonomic studies, it is seen that the karyotype of approximately 89% of these species has not been determined. From a methodological point of view, although molecular cytogenetic methods have become widespread in the world, traditional cytotaxonomic methods (Giemsa, GTG-, AgNOR-, C-, Q- and RE-banding made by air drying and culture methods) are used even in the latest studies (Araya-Jaime et al., 2020; Goes et al., 2020; Moreva, 2020). Finally, there are studies in Turkey in the field of cytogenetics that examine only some studies on carp (Saygun, 2021). However, no study has yet been conducted on the karyotype of *Chondrostoma regium*.

Chondrostoma is a genus of the family Leuciscidae and this genus consists of about 26 species (Freyhof, 2014). A total of thirteen *Chondrostoma* species (*C. beysehirense, C. ceyhanensis, C. colchicum, C. cyri, C. holmwoodii, C. meandrense, C. nasus, C. kinzelbachi, C. regium, C. smyrnae C. toros, C. turnai* and *C. vardarense*) are reported to be found in various regions of Turkey (Küçük et al., 2007; Özcan, 2008; İlhan, 2009; Freyhof and Özuluğ, 2009; Kuru et al., 2014; Çiçek et al., 2015; Küçük et al., 2017; Güçlü et al., 2018; Çiftçi et al., 2020; Küçük et al., 2021; Küçük et al., 2023).

The genus *Chondrostoma* also disributed in the Caspian Sea, Isfahan, Tigris-Euphrates and Kor River basins. Four *Chondrostoma* species (*C. cyri, C. esmaeilii, C. regium* and *C. orientale*) are in Iranian inland waters (Eagderi et al., 2017). There are also records of *C. regium* being found in Greater Zab River, west of the Erbil city, Iraq (Al-Marjan, 2016). Various studies have been conducted on the general biology, taxonomy, karyology and morphology of the species in Iran (Esmaeili et al., 2010; Jouladeh-Roudbar, 2014). Karyotype analyzes of *Chondrostoma* species were also described from various geographical regions of Europe. For example, karyotype analysis of *Iberochondrostoma lusitanicum* (formerly *Chondrostoma lusitanicum*) in Portugal has been reported by Collares-Pereira and Ráb (1999). Chromosome studies of *C.*

nasus were performed by Ráb et al. (2008) and Boroń et al. (2020). Karyotype analyzes of *Chondrostoma* species were also defined from different regions of Turkey (Arslan and Gündoğdu, 2016). There are only two karyotype studies on *C. regium*. One of these studies was reported from Iran (Esmaeili et al., 2010) and the other from Turkey (Kaya, 2009). Moreover, nucleolar organizer region and constituve heterochromatin characteristics in the *C. regium* were not reported. In this study, karyotypic properties of *C. regium* were determined by using C-banding, Ag-NOR and giemsa staining techniques. Chromosomal banding findings were compared with those obtained from previous studies on *Chondrostoma* species.

MATERIAL AND METHODS

Chondrostoma regium specimens (12♀♀, 10♂♂) (Figure 1) were caught from Karasu River, Erzurum province in eastern Turkey. The fish were brought alive to the laboratory and placed in well-aerated aquaria.

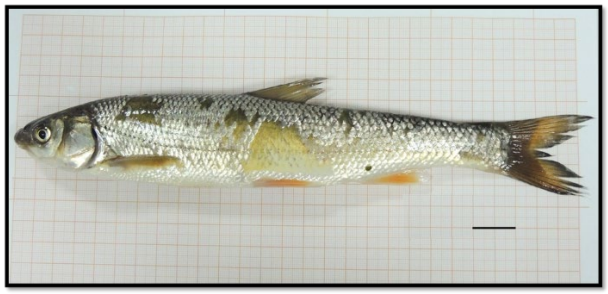

Figure 1. *Chondrostoma regium* Karasu River (Erzurum), 236 mm SL

For karyotypic analysis, Collares-Pereira's (1992) air drying technique was modified and applied. On the other hand, C-banding technique of Sumner (1972) was performed to detect constitutive heterochromatin regions in chromosomes. Ag-NOR staining was performed to detect nucleolar organizer regions (NOR) in chromosomes. For this, the method of Howell and Black (1980) was used. The preparations prepared in two ways were examined under a Leica DM750 light microscope and a computer-aided microscope unit with a Leica ICC50 HD camera at x100 magnification, and the best metaphase plaque spreads were photographed. At least 10 preparations from each tissue of each of the fish caught in the study (Denton, 1973) were evaluated. Karyotypes were prepared by classifying them according to their chromosome lengths. For this, chromosome arms were measured. Arm measurements were made using the Leica LAS EZ 3.0 image analysis program. The arm ratio (AR), centromeric index (CI) and relative arm length (RAL) of each chromosome were calculated. This process was performed in accordance the method of Levan et al. (1964). Karyotype symmetry and asymmetry were also calculated according to Eroğlu (2015).

RESULTS

Total 580 and 644 metaphase plaques were counted in the gill and kidney tissues taken from female and male *C. regium* species. The most repeated value was found to be 2n=50 in both tissues. In the karyogram prepared from the metaphase plaques, it was determined that 9 pairs of chromosomes were metacentric (M), 7 pairs were submetacentric (SM), 1 pair were subtelocentric (ST) and 8 pairs were telocentric (T) (18M+14SM+2ST+16T) (Figure 2).

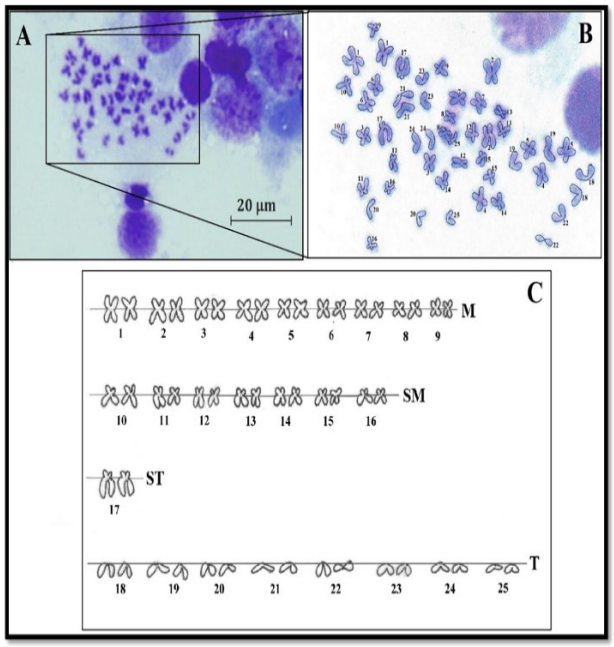

Figure 2. A) Metaphase spreads and standard giemsa staining of *Chondrostoma regium*. B) Chromosomes determined by image processing, C) Karyotype: 18M+14SM+2ST+16T (M: metacentric, SM: submetacentric, ST: subtelocentric, T: telocentric)

Chromosome arm lengths were found to vary between 0.9 and 3.9 µm, the longest chromosome was metacentric, the shortest was telocentric, and the Fundamental Arm Number (FN) was 82 (Table 1).

The ideogram prepared according to the measurements was shown in Figure 3. No sex chromosomes were detected in this species. The chromosomal symmetry/asymmetry index value was calculated as 2.32 (S/A_I= (1×18) + (2×14) + (3×2) + (4×16) / 50), and it was understood that the karyotype type of *C. regium* was between symmetric and asymmetric.

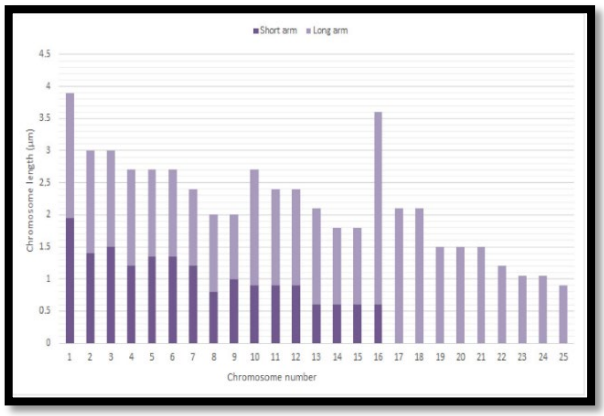

Figure 3. Haploid ideogram of *Chondrostoma regium*

Table 1. Measurements (in µm) and classification of the chromosomes of *Chondrostoma regium* (M: metacentric, SM: submetacentric, ST: subtelocentric, T: telocentric)

Chromosome number	Short arm	Long arm	Chromosome length	Arm ratio	Centromeric index	Relative arm length (%)	Chromosome type	Arm number
1	1.95	1.95	3.9	1.0	50	7.27	M	4
2	1.4	1.6	3.0	1.14	46.7	5.59	M	4
3	1.5	1.5	3.0	1.0	50	5.59	M	4
4	1.2	1.5	2.7	1.25	44.44	5.03	M	4
5	1.35	1.35	2.7	1.0	50	5.03	M	4
6	1.35	1.35	2.7	1.0	50	5.03	M	4
7	1.2	12	2.4	1.0	50	4.47	M	4
8	1.1	1.1	2.2	1.0	50	3.94	M	4
9	1.0	1.0	2.0	1.0	50	3.73	M	4
10	0.9	1.8	2.7	2.0	33.33	5.03	SM	4
11	0.9	1.5	2.4	1.66	37.5	4.47	SM	4
12	0.9	1.5	2.4	1.66	37.5	4.47	SM	4
13	0.8	1.5	2.3	1.88	34.78	4.12	SM	4
14	0.6	1.5	2.1	2.5	28,57	3.91	SM	4
15	0.6	1.2	1.8	2.0	33.33	3.35	SM	4
16	0.6	1.2	1.8	2.0	33.33	3.35	SM	4
17	0.6	3.0	3.6	5.0	16.66	6.71	ST	4
18	0	2.1	2.1	0	0	3.91	T	2
19	0	2.1	2.1	0	0	3.91	T	2
20	0	1.5	1.5	0	0	2.79	T	2
21	0	1.5	1.5	0	0	2.79	T	2
22	0	1.5	1.5	0	0	2.79	T	2
23	0	1.2	1.2	0	0	2.23	T	2
24	0	1.05	1.05	0	0	1.95	T	2
25	0	0.9	0.9	0	0	1.67	T	2
Total	16.85	37.25	55.8	-	-	-	-	82

C-Banding method was also used to determine the karyotypic features in detail. In the analysis, it was determined that the constitutive heterochormatine regions of *C. regium* were located at the ends of the 1st and 6th chromosome pairs, and the 13th and 16th chromosome pairs were localized at the end of the long arm (Figure 4). One active Ag-NORs was detected in the species. NORs were observed in the telomeres of the long arms of 21st chromosome (Figure 5).

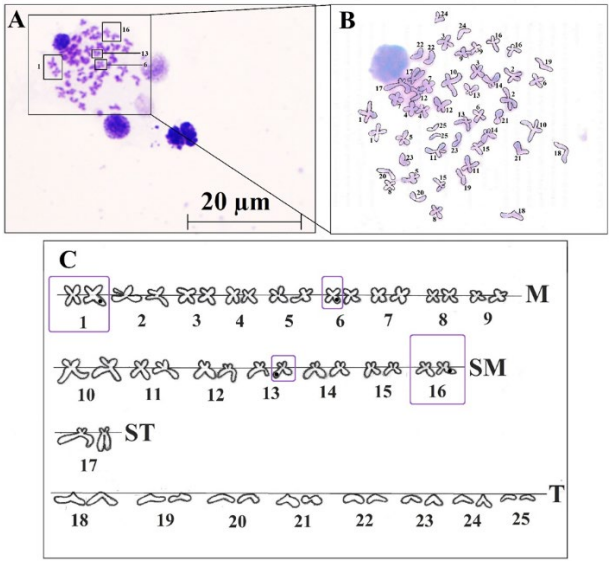

Figure 4. A) C-banded metaphase plate of *Chondrostoma regium*. B) Chromosomes determined by image processing. C) Karyotype. Heterochromatin regions were shown in the square

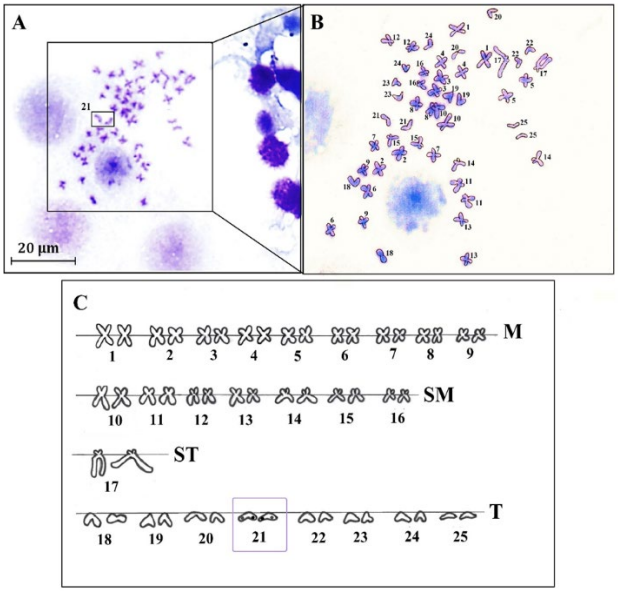

Figure 5. A) Ag-NOR stained metaphase plate showing NOR regions of *Chondrostoma regium*. B) Chromosomes determined by image processing. C) Karyotype. NOR region was shown in the square

DISCUSSION

Since the 1960s, significant contributions have been made to the fields of genetics, taxonomy and environmental toxicology by performing karyological analyzes of fish species belonging to Teleostei (Cucchi and Baruffaldi, 1990). Karyotype provides basic information about chromosome

morphology, size and number, and is used to distinguish controversial species and to construct evolutionary family trees (Tan et al., 2004). In addition, fish karyotype studies provide important datas for polyploidy, gynogenesis, androgenesis, and the generation of new aqua-hybrid species (Dai and Han, 2018). In recent years, an increase in the number of cytogenetic studies applied to fish has been observed. The karyotypes of 3.425 fish species or subspecies are known (Arai, 2011). Leuciscidae is one of the richest and most important fish family. The species of the family have a wide living space in Turkey. In the study, karyotypic analysis of *C. regium*, which is found in the native fauna of the Karasu River (Erzurum, Turkey) and belongs to the Leuciscidae family, was carried out.

There is no cytogenetic study of *C. regium* in the inland waters of Eastern Anatolia to date. In the current study, the chromosome number of *C. regium* inhabiting in Karasu River was determined as 2n=50. As a result of the karyotype analysis, it was determined that there were 9 metacentrics, 7 submetacentrics, 1 subtelocentric and 8 telocentrics chromosome pairs (18M+14SM+2ST+16T) in the species. The arm number was found to be FN=82. Except for this study, there is no previous karyotype study of this species in our country. In the world, only the karyotype of *C. regium* from Iran's Fahlian River has been reported (Esmaeili et al., 2010). In the study, the diploid chromosome number of this species was determined as 2n=52. The karyotype consists of 21 submetacentric and 5 subtelocentric chromosome pairs (42SM+10ST). The FN was determined as 58. Cytologically, no heteromorphic sex chromosomes were found. When the study was compared with the current study results, it was understood that the Karasu river fish specimens was not karyotypically similar to the Persian fish specimens. In addition, it was concluded that the number of arms in Persian fish was calculated incorrectly. Because if even the number of arms of subtelocentric chromosomes is not taken into account, there must be at least 84 arms in 42 submetacentric chromosome sets. Therefore, there is a significant difference between the two species. The difference in normal comparison is due to the separate habitats.

Results of the current study are partially in agreement with the results obtained from other studies with *Chondrostoma* genus (Table 2). There are differences in the number of chromosome arms in the comparative karyotype, which is parallel to the current study in terms of chromosome number. The reason for the difference in karyotype may also be population-related conditions other than the reason mentioned above. Interspecific polymorphism or species at the sampling site, or even inter-population differences can lead to this condition. In addition, other factors such as chromosome losses during preparation, abnormal positions of fixed cells, chromosomes from nearby cells, unidentified microarms in chromosomes, insufficient sample number, errors in chromosome arm measurement, and incorrect determination of chromosome type may cause differences between studies (Arai, 2011; Khosravanizadeh et al., 2011). Therefore, giemsa stained karyotype studies should be supported by other cytogenetic techniques. For this reason, C-banding method was used in the research. C-bands are constitutive regions of heterochromatin containing repetitive DNA sequences that are transcriptionally inactive (Gold et al., 1986). C-banding studies are few in Cyprinidae. The characteristic C-band patterns are mostly specific to Teleostei. Heterochromatin difference can be used in the chromosome evolution of Cyprinidae and the differentiation of species (Gaffaroğlu and Yüksel, 2009). In our study, constitutive heterochromatin was detected in four pairs of chromosomes. To date, no C-banding studies on *C. regium* have been found in the literature.

Table 2. Karyotypic features of *Chondrostoma* species living in Turkey and the World-wide

Species	Locality	Chromosome numbers (2n) – FN- symmetry/asymmetry index	Chromosome formula	C-band	NOR	Reference
C. regium	Karasu River (Erzurum)	50 – 82 – 2.4 S/A	18M+12SM+2ST+18T	1., 6., 13., 16.	21.	This study
C. regium	Göksu River (Mersin)	50 – 86 – 2.24 S/A	22M+8SM+6ST+14T	-	-	Kaya, 2009
C. regium	Fahlian River (Iran)	52 – 58 – 2.19 S/A	42SM+10ST	-	-	Esmaeili et al., 2010
C. beysehirense	Lake Beyşehir (Konya)	50 – 92 – 2.14 S/A	20M+22SM/ST+8T	1., 3., 4., 7., 8., 15.	6.	Arslan and Gündoğdu, 2016
C. meandrense	Menderes River (Aydın)	52 – 82 – 2.61 S/A	18M+6SM+6ST+22T	-	-	Uysal, 2011
C. nasus	Drian River (Bosnia)	50 – 88 – 2.28 S/A	38M/SM+12T	-	-	Barshiene, 1977
C. knerii	Neretva River (Yugoslavia)	50 – 86 – 2.56 S/A	36M/SM+14T	-	-	Berberović et al., 1970
C. prespense	Lake Prespa (Italy)	50 – 92 – 2.0 S	16M+26SM+8T	-	-	Bianco et al., 2004
C. soetta	Po River (Italy)	50 – 94 – 2.2 S/A	16M+14SM+14ST+6T	-	-	Cataudella et al., 1977
C. phoxinus	Neretva River (Yugoslavia)	50 – 86 – 2.56 S/A	36M/SM+14ST/T	-	-	Berberović et al., 1970

NORs are regions where nucleoli are in contact on chromosomes and contain genes that transcribe rRNA. Systematic and phylogenetic comparisons between species can be made with chromosomal NOR information (Amemiya and Gold, 1988). The number and location of NORs are also used as chromosomal markers in fish systematics (Pereira et al., 2012; Rossi et al., 2012; Nabais et al., 2013). NORs are usually located at the end of the short arm of submetacentric chromosomes (Gromicho and Collares-Pereira, 2007). Rarely, it can be observed at the end of the long arm of telocentric chromosomes (Rab, et al., 1990). Changes in the NOR region and activity can be observed between and within the species (Ulupınar and Alaş, 2002). These differences are due to the differentiated activation of rDNA cistrons, unequal amplification including rDNA segments, and paracentric inversion of chromosomal segments carrying rDNA cistrons (Porto-Foresti

et al., 2007). In our study, NOR was observed on the one telocentric chromosome (21st chromosome). The NOR placement is consistent with the literature. There are no NOR banding studies with *C. regium*. In a study with another *Chondrostoma* species, active NOR regions were observed in the short arm of the sixth submetacentric chromosome pair of *Chondrostoma beysehirense* in Beyşehir Lake (Gündoğdu, 2016). In the *Chondrostoma prespense* species, two pairs of NOR regions were determined on the chromosomes (Bianco et al., 2004). When all these results are compared, the studies partially agree with our research findings (Table 2).

Kaya (2009) reported that the karyotype of *C. regium* caught from Göksu River (Mersin, Turkey) was 2n=50 with 11 pairs of metacentric, 4 pairs of submetacentric, 3 pairs of subtelocentric and 7 pairs of telocentric chromosomes (22M+8SM+6ST+14T) and FN=86. Although the Göksu River fish specimens was later identified to be *C. toros*, we can say that the species are similar to each other in terms of chromosome type and chromosome arm numbers.

When Table 2 was examined, the most important common point of *Chondrostoma* from Iran to Central Europe was that the number of chromosomes was 2n=50 and the chromosomal symmetry/asymmetry values were between 2.00 – 2.61 and all have a symmetrical/asymmetrical chromosome structure, except one (*C. prespense*). However, there are some differences in the chromosome structure. These differences are usually due to submetacentric and subtelocentric chromosomes. Scientists accept that fish species with more telocentric chromosomes are primitive, while species with metacentric and submetacentric chromosomes are more complex (Geng et al., 2013; Şahin, 2015). Accordingly, as can be seen from Table 2, M and SM chromosomes are frequently seen in *Chondrostoma* species. The data emerging in many studies support the hypothesis that this genus was complex, as M and SM type chromosomes are more than single-armed chromosomes (T) in *Chondrostoma* genomes. Since there are 30 double-armed and 18 single-armed chromosomes in the karyotype (18M+14SM+2ST+16T) of *C. regium* determined in the present study, it can be said that this species is also complex. Likewise, when we consider double-armed chromosomes as M/SM, the sum of M/SM (average 34.5) is more than T (mean 12.2) in all *Chondrostoma* species, as indicated in Table 2. In this study, the sum of M and SM chromosome numbers of *C. regium* was determined as 30. This shows that Karasu River (Erzurum) *C. regium* specimens relatively have the general karyotype features of the genus *Chondrostoma*.

Fundamental arm numbers (FN) vary depending on the karyotype. In some cases, it is also used as an indicator of differentiation between species. Although FN values of chromosomes vary between 58 and 94 in *Chondrostoma*, it was seen that this value mostly varies between 80-86. In the present study, FN = 82 and remained within the most repetitive value range (Table 2). On the other hand, the number and structure of chromosomes in *Chondrostoma* species vary slightly. Even the same researchers working in the same region at different times for a single species report variations in their results. This is an unstable situation that can be seen in the cytogenetic results of fish (Şahin, 2015). It is estimated that studies using molecular cytogenetic methods such as FISH (fluorescence in situ hybridization) will become widespread rapidly and reduce the confusion on this subject.

CONCLUSION

There is no karyotype studies on *Chondrostome regium*, which is distributed in the Euphrates River and its tributaries. In this respect, the work is a first for the Euphrates and Karasu Rivers. Karyotypic analysis of two other fish species (*Squalius cephalus* and *Alburnus mossulensis*) in the parts of the same river within the borders of Erzurum province was previously performed by Kılıç and Şişman (2016) and Şişman et al. (2016), and this is the third study. Thus, the faunistic structure of the Karasu River was clarified a little more. Moreover, the analyses of C-positive regions and NORs were added to the karyotype in this study for the first time. We think that revealing the genome characteristics of fish species living in the Karasu River by using molecular staining (FISH, CMA_3 etc.) techniques will give clearer results.

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AUTHORSHIP CONTRIBUTIONS

Turgay Şişman collected the fish samples, have edited the graphics and figures of the manuscript, and designed and wrote the manuscript. Büşra Yamaç performed the experimental work. All authors approved the submission and publication of this manuscript.

CONFLICTS OF INTEREST

The author declares that there is no conflict of interest on this manuscript.

ETHICS APPROVAL

All necessary legal permissions were obtained before starting the study. The necessary permissions for this were: Atatürk University Animal Experiments Local Ethics Committee Ethics Committee Permission (ATA0107100-1332, 06.09.2011), Research Permit from the Ministry of Agriculture and Rural Affairs (67852565/140-0303-863, 03.04.2013), Research Permit from the Ministry of Environment and Forestry (72784983-48804-63471, 03.04.2013).

DATA AVAILABILITY

The data supporting the conclusions of this paper are available in the main paper.

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