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Cytogenetic Analysis of *Alburnus escherichii* (Teleostei: Leuciscidae) in Turkey

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ABSTRACT
In this study, cytogenetic properties of *A. escherichii* were investigated using standard Giemsa staining, C-banding, and Ag-NOR staining techniques. The karyotype of Sakarya bleak, the diploid chromosome number was 2n = 50, consists of six pairs of metacentric, 12 pairs of submetacentric, and seven pairs of acrocentric autosomes (NF=86). No morphologically distinguishable heteromorphic sex chromosomes were detected in the karyotype. While most autosomes had centromeric and pericentromeric C-heterochromatin (some chromosomes slightly) blocks, the other autosomal pairs were C-negative. Also, the short arm of the eleventh submetacentric chromosome pair was entirely C-positive. The nucleolar organizer regions were localized in medium-sized submetacentric autosomal pairs. Active NORs associated with the C-heterochromatin were observed in the whole of the short arm of the eleventh chromosome pair, and their active NORs were homomorphic. The banded karyotypes of *A. escherichii* were reported in this study for the first time. Significant findings obtained in this research may contribute to cytotaxonomy of *Alburnus* species in Anatolian and Europe.

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Türkiye'deki *Alburnus escherichii*'nin (Teleostei: Leuciscidae) Sitogenetik Analizi

ÖZET
Bu çalışmada *A. escherichii*'nin sitogenetik özellikleri, standart Giemsa boyama, C-bantlama ve Ag-NOR boyama teknikleri kullanılarak araştırılmıştır. Diploid kromozom sayısı 2n = 50 olan Sakarya İnci balığının karyotipi altı çift metasentrik, 12 çift submetasentrik ve yedi çift akrosentrik otozomdan (NF = 86) oluşmaktadır. Karyotipte morfolojik olarak ayırt edilebilir heteromorfik cinsiyet kromozomu tespit edilmedi. Çoğu otozom, sentromerik ve perisentromerik C-heterokromatin (bazı kromozomlar hafif) bloklarına sahipken, diğer otozomal çiftler C-negatiftir. Ayrıca, on birinci submetasentrik kromozom çiftinin kısa kolu tamamen C-pozitifti. Nükleolar düzenleyici bölgeler, orta büyüklükte submetasentrik otozomal çiftler halinde lokalize idi. C-heterokromatin ile ilişkili aktif NOR'lar, on birinci kromozom çiftinin kısa kolunun tamamında gözlendi ve aktif NOR'lar homomorfikti. *A. escherichii*'nin bantlı karyotipleri ilk kez bu çalışmada rapor edilmiştir. Bu araştırmada elde edilen önemli bulgular, Anadolu ve Avrupa'daki *Alburnus* türlerinin sitotaksonomisine katkı sağlayabilir.

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INTRODUCTION
The genus *Alburnus* (Rafinesque, 1820), is known to be bleak, is represented by 43 species in Europe and Western Asia (Eschmeyer, et al., 2016). Of 20 species belonging to the genus *Alburnus* (Bektaş et al., 2020), 18 species are endemic in Turkey's inland waters (Çiçek et al., 2018). *Alburnus nasreddini* (Battalgil, 1943) was a local endemic in Akarçay basin (Eber, Akşehir and Karamık lakes; Selevir and Seyitler reservoirs; Kalli, Adıyan and Akşehir streams), Ilgın

Lake basin (Aşağı Çiğil Creek-Ilgın), and Sarayönü-Beşgöz system (Gülle et al., 2017). There are still some taxonomic problems of some species exist within this genus (Bektaş et al., 2020). In recent years, systematics of *A. nasreddini* have been investigated by morphological (Gülle et al., 2017; Bayçelebi et al., 2020) and molecular studies (Mangıt and Yerli, 2018; Bektaş et al., 2020). Gülle et al. (2017) stated that *A. nasreddini* and *A. escherichii*, scattered in neighbouring basins, have similar morphology, but *A. nasreddini* is a valid species in terms of having a deeper body structure, shorter nose, and wide eye diameter. *A. escherichii* (Sakarya Basin), and *A. nasreddini* (Akarcay Basin), they did not suggest *A. nasreddini* as synonymous due to unique haplotypes. According to Mangıt and Yerli (2018), despite the low genetic distance between *A. escherichii* (Sakarya Basin), and *A. nasreddini* (Akarcay Basin), they did not suggest *A. nasreddini* as synonymous due to unique haplotypes. On the contrary, Bayçelebi et al. (2020) stated that no morphological characters would distinguish *A. nasreddini* from *A. escherichii* and synonymous. According to Çiçek et al. (2020), *A. nasreddini* is endemic to the ichthyofauna of Turkey. However, Bektaş et al. (2020) proposed that *A. nasreddini* the synonym of *A. escherichii* due to its close genetic relationships.

Karyological properties of *Alburnus alburnus* (Cataudella et al., 1977; Sofradzija et al., 1979; Hafez et al., 1978,1981; Vujosevic et al., 1983; Klinkhardt et al., 1995; Arkhipchuk, 1999; Ziegler et al., 2003; Bianco et al., 2004; Schmid et al., 2006; Ráb et al., 2008; Khosravanizadeh et al., 2011), *A. akili* (Arkhipchuk, 1999), *A. adanensis* (Ünal and Gaffaroğlu, 2016), *A. filippii* (Gül et al., 2006; Nazari et al., 2009), *A. heckeli* (Simovic et al., 1994; Gül et al., 2004), *A. mossulensis* (Gül et al., 2000; Yüksel and Gaffaroğlu, 2008), *A. orontis* (Vasil'yev, 1980), *A. tarichi* (Gül et al., 2003), and *A. albidus* (Bianco et al., 2004), *A. arborella* (Fontana et al., 1970) have been reported so far. As far as we know, cytogenetic studies on *Alburnus* species are limited to traditional Giemsa staining. For this reason, detailed cytotaxonomic studies using C- and Ag-NOR banding techniques are critical. In this study, banded chromosomal analysis of *A. escherichii* is investigated using C- and Ag-NOR banding techniques.

MATERIALS and METHODS

Four females and two male specimens of *A. escherichii* were collected from the natural habitat in the Sarayönü Beşgöz system (38°16′ N, 32°20′ E), Konya, Turkey (Figure 1). The study was conducted with the permission of the Ministry of Forest, and Water Works (21-264211-288.04-E.1031530), Republic of Turkey. This permission also replaces the permission of the local ethics committee. The fish specimens were collected from the bank of the Beşgöz system by a small ladle and transported to a well-aerated aquarium in the research laboratory. A karyological analysis was carried out based on the protocol for the air-drying technique of Bertollo et al. (2015),.

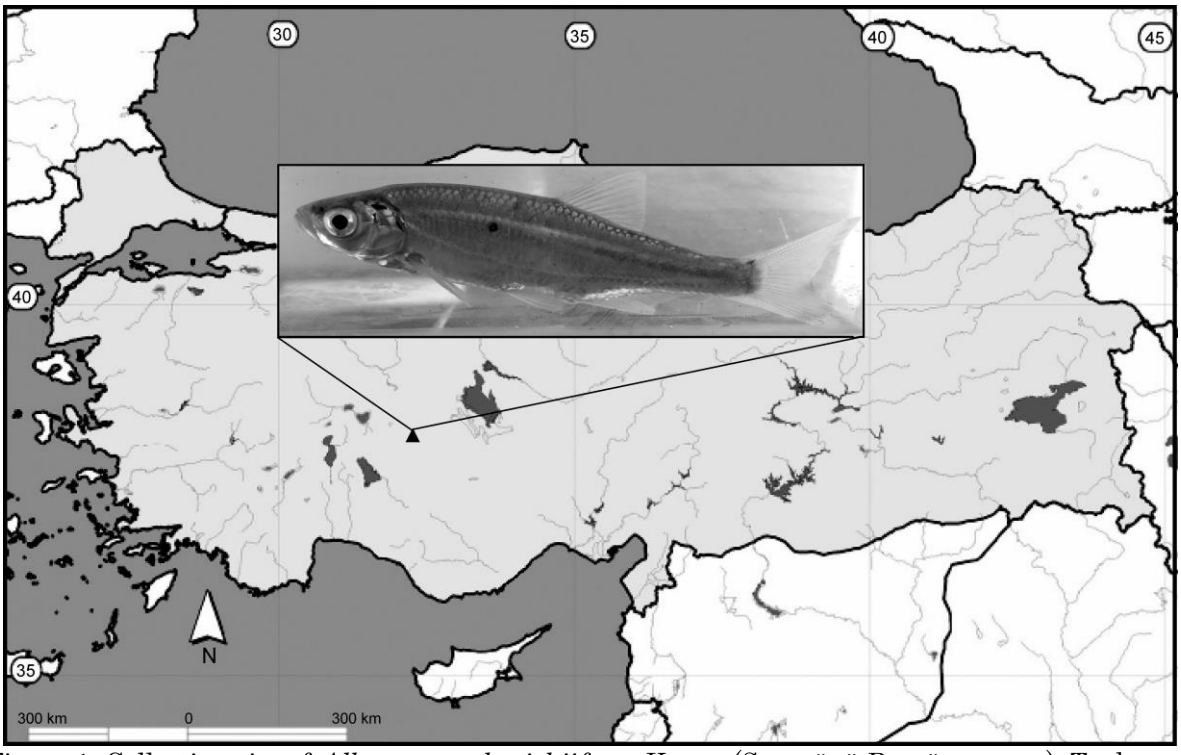

Figure 1. Collecting site of *Alburnus escherichii* from Konya (Sarayönü Beşgöz system), Turkey
Şekil 1. Alburnus escherichii'nin Konya'daki (Sarayönü Beşgöz sistemi) toplama alanı

Colchicine was injected into abdominal cavities (1g / 0.006g) to stabilize mitotic activity. 3-3.5 hours after the colchicine treatment, kidney tissue with high-mitotic activity was removed and kept in an oven at 36.5 ° C for 45 minutes in the KCI (0.046 g) solution at room temperature (25°C). After that, the upper hypotonic supernatant was discarded by centrifugation at 2000 rpm for 10 minutes. For the fixation process, fixation (3:1, methanol: acetic acid) was added to 10 ml of a mixer, and the upper supernatant was discarded by centrifugation at the same speed. This process was repeated twice. The suspension dried by dropping into the slides and was stained by the Giemsa (10 %) solution for 10 minutes. C- banding and Ag-NOR staining were performed according to Sumner (1972) and Howell and Black (1980), respectively. Well-spread metaphases were photographed under a microscope, and their karyotypes were made. Definition of the shapes of the chromosomes was established according to Levan et al. (1964). The fundamental number of autosomal arms (NF) were calculated.

RESULTS

The diploid chromosome number (2n) of *A. escherichii* is 50. The karyotype consists of six pairs of metacentric (nos: 1-6), 12 pairs of submetacentric (nos: 7-18), and seven pairs of acrocentric (nos: 19-25) autosomal chromosomes (NF = 86) (Figure 2). The heteromorphic sex chromosome of the species was not detected in the karyotype.

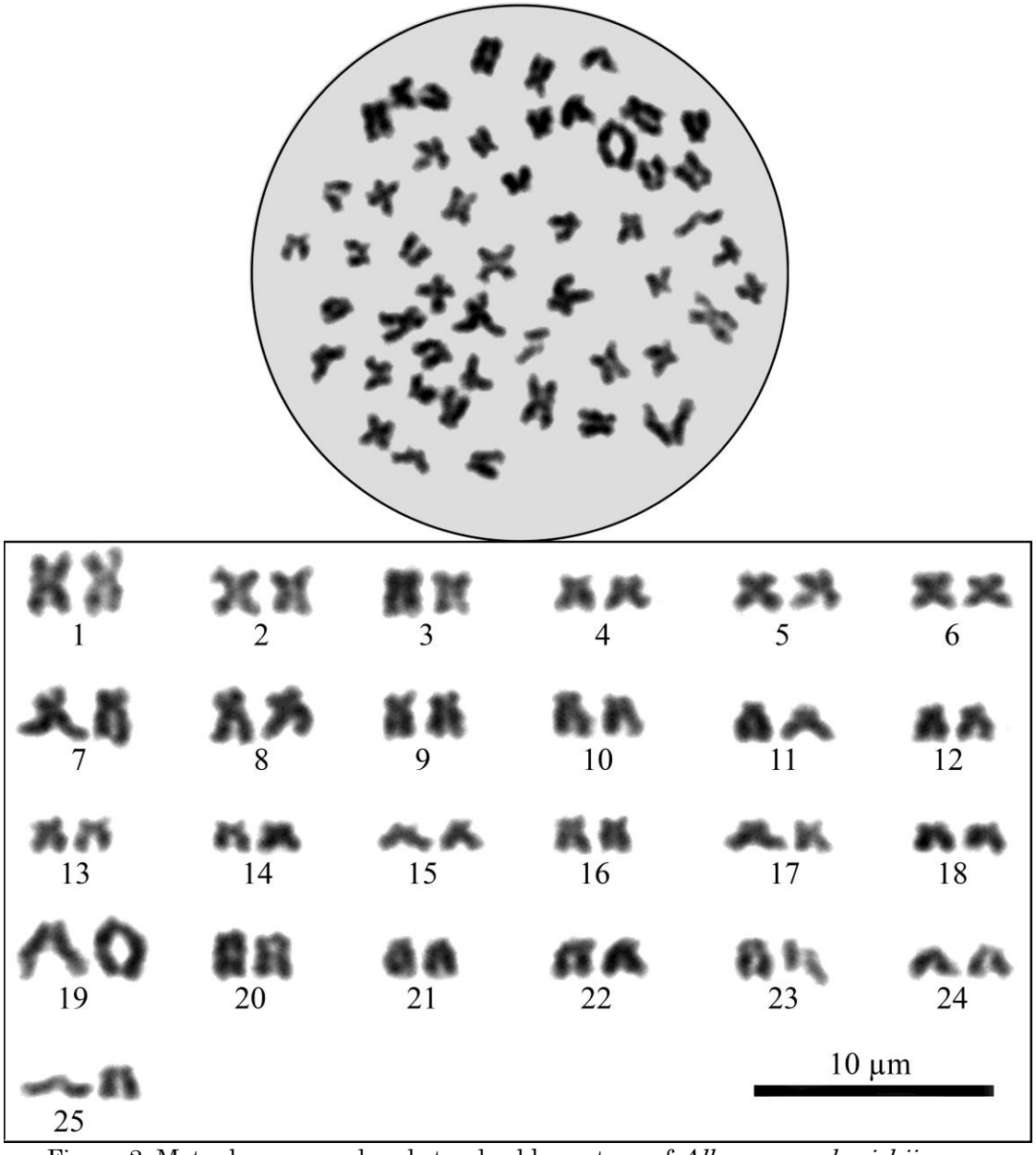

Figure 2. Metaphase spread and standard karyotype of *Alburnus escherichii*
Şekil 2. Alburnus escherichii'nin metafaz yayılımı ve standart karyotipi

C-banded metaphase plate and karyotype of *A. escherichii* is shown in Figure 3. Centromeric and pericentromeric bands were detected in three metacentrics (1 to 3), three submetacentric (nos: 8, 11, 18), and five acrocentric chromosome pairs (nos: 20, 21, 22, 24, 25). Slight centromeric and pericentromeric bands were identified in one metacentric (no: 4), six submetacentric (nos: 9, 10, 14, 15, 17), and two acrocentrics (nos: 19 and 23) chromosome pairs. Other chromosomes were C-negative. The whole of the short arms of chromosome 11 are C-positive and associated with NOR.

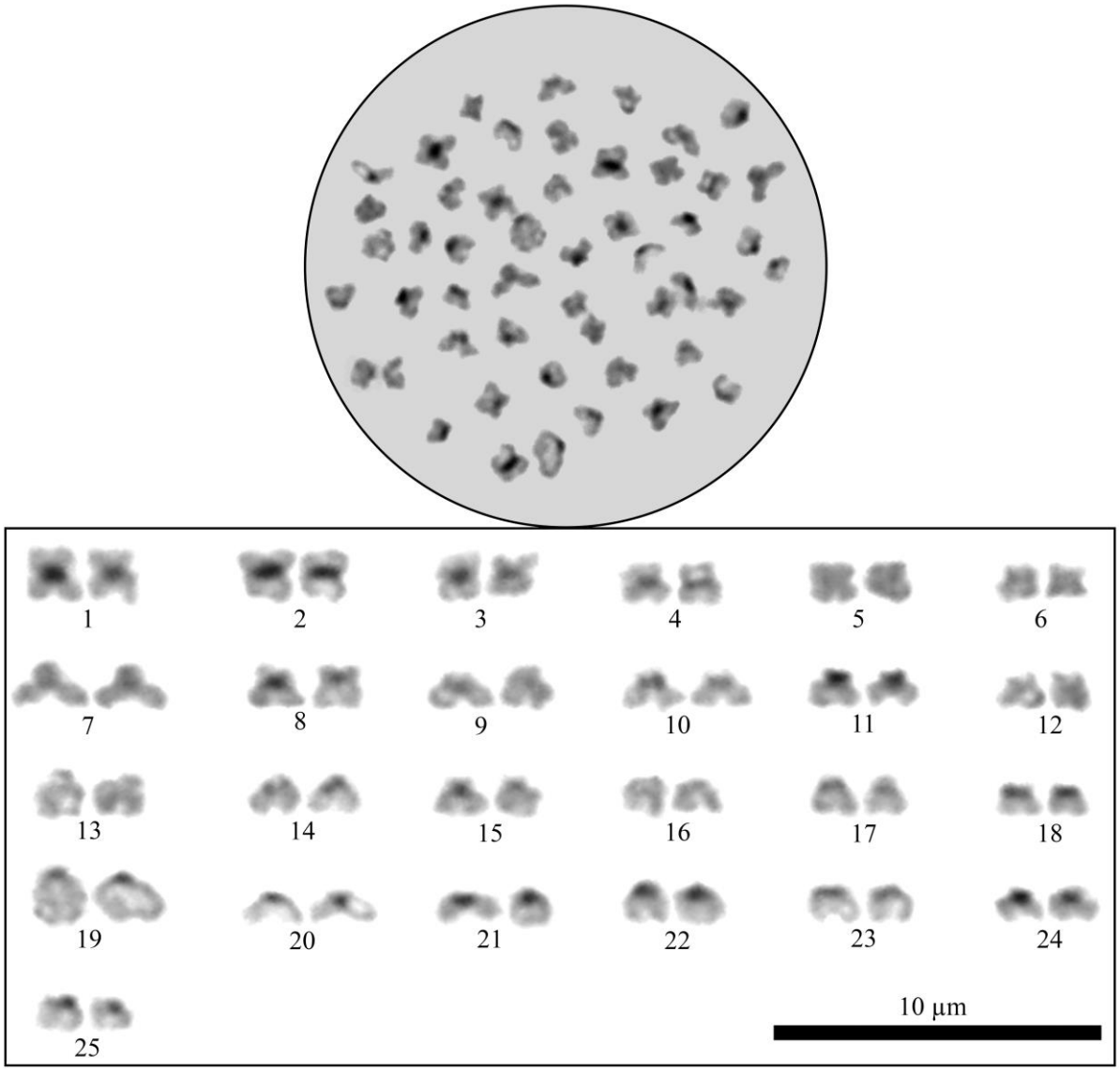

Figure 3. C-banded metaphase spread and arranged karyotype of *Alburnus escherichii*
Şekil 3. Alburnus escherichii'nin C-bantlı metafaz yayılımı ve karyotipi

Active Ag-NOR is localized on the whole of the short arms of the medium-sized submetacentric (no: 11) chromosome pair and is associated with C-heterochromatin (Figure 4). The NORs were homomorphic in the studied specimens, and no heteromorphism was observed in NOR sizes.

DISCUSSION and CONCLUSION

A nearly invariant number of diploid (2n = 50) chromosomes, the dominance of metacentric and submetacentric, and the presence of the largest st/a chromosome pair were characteristic of all leuciscine genera examined to date (Ráb and Collares-Pereira, 1995; Ráb et al., 2008; Pereira et al., 2009). The karyotype of the Sakarya bleak specimens had the characteristic features of the leuciscin species. The diploid chromosome number of the genus, except for the Kızılırmak population of *A. mossulensis* (48), is 50 (Table 1). Detection of the heteromorphic chromosome system in cyprinids, especially in leuciscin species with small chromosomes such as *Alburnus*, *Phoxinus*, and *Pseudophoxinus*, is quite difficult. So, heteromorphic sex chromosomes were not detected in

the karyotype of *A. escherichii*.

C- heterochromatin bands define constitutive heterochromatin regions containing transcriptionally inactive highly repetitive DNA sequences. The difference in heterochromatin localization can be used as a cytogenetic marker for species differentiation and chromosome evolution in taxons (Ren et al., 1992). In most species, heterochromatin bands are in the centromeric and/or telomeric regions of chromosomes. Besides, heterochromatin bands can be found in the whole of the short and long arms of chromosomes (metacentric, submetacentric, and acrocentric) (Gold et al., 1986). Leuciscidae, such as *Abramis* (Ocalewicz et al., 2004), *Chondrostoma* (Arslan and Gündoğdu, 2016), *Leuciscus* (Boroń et al., 2009), *Phoxinus* (Boroń, 2001), *Pseudophoxinus* (Ergene et al., 2010; Ünal et al., 2014; Ayata et al., 2016; Ünal and Gaffaroğlu, 2016), *Scardinius* (Bianco et al., 2004), *Squalius* (Ünal, 2011; Ünal and Gaffaroğlu, 2016; Doori, 2019; Ayata, 2020) and *Vimba* (Rábová et al., 2003), in species C-heterochromatin bands are localized in centromeric and pericentromeric regions. C-heterochromatin band properties of *Alburnus escherichii* are like *A. albidus* (Schmid et al., 2006). While C-heterochromatin bands of *A. escherichii* are similar to *A. adanensis* (Ünal and Gaffaroğlu, 2016), paracentric heterochromatin bands have not. The silver-staining technique for nucleolar organizer regions (Ag-NOR) is widely used to determine the species karyotypic character and stain

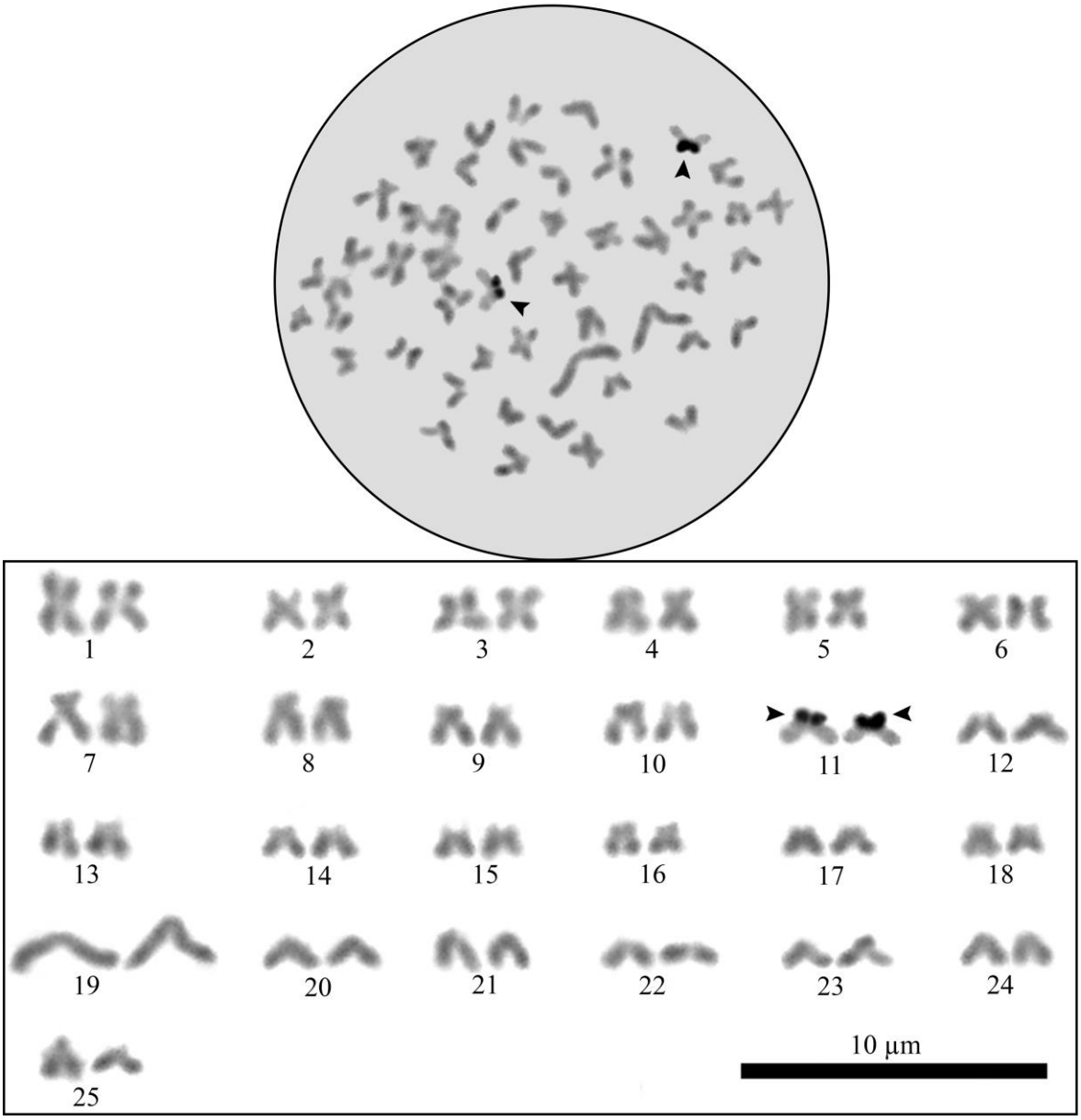

Figure 4. Silver-stained metaphase spread and arranged karyotype of *Alburnus escherichii*. Arrows indicate the Ag-NORS

Şekil 4. Alburnus escherichii'nin gümüş boyalı metafaz yayılımı ve karyotipi. Oklar Ag-NOR bölgelerini göstermektedir

Table 1. Chromosomal records of *Alburnus* species (Abbreviations; 2n-diploid chromosome number, NF-fundamental number of chromosomal arms, M-metacentric, SM-submetacentric, A-acrocentric)

Çizelge 1. Alburnus türlerinin kromozom kayıtları (Kısaltmalar; 2n-diploid kromozom sayısı, NF-temel kromozom kol sayısı, M-metasentrik, SM-submetasentrik, A-akrosentrik)

Species	Locality	2n	Karyotype	NF	References
Alburnus akili	-	50	18M + 32SM	-	Arkhipchuk (1999)
A. alburnus	Italy	50	16M + 10SM + 16ST + 8A	-	Cataudella et al. (1977)
	-	50	-	-	Sofradzija et al. (1979)
	France	50	-	-	Hafez et al. (1978, 1981)
	Yugoslavia	50	12M + 16SM + 12T + 10A	90	Vujosevic et al. (1983)
	-	50	16M + 20SM + 14ST-A	86	Klinkhardt et al. (1995)
	-	50	16M + 20SM + 14ST-A	86	Arkhipchuk (1999)
	Germany	50	14M + 14SM + 14ST + 8T	-	Ziegler et al. (2003)
	Germany	50	14M + 14SM + 8T + 14A	-	Schmid et al. (2006)
	Czechia	50	16M + 26/30SM + 8-4ST/A	92/96	Ráb et al. (2008)
	Persia	50	14M + 26SM + 10ST-A	90	Khosravanizadeh et al. (2011)
A. adanensis	Turkey	50	12M + 28SM + 10ST-A	90	Ünal and Gaffaroğlu (2016)
A. albidus	Italy	50	16M + 26SM + 8ST-A	-	Bianco et al. (2004)
A. arborella	Italy	50	-	-	Fontana et al. (1970)
A. filippii	Turkey	50	16M + 16SM + 18A	-	Gül et al. (2006)
	Persia	50	12M + 18SM + 8ST + 12A	88	Nazari et al. (2009)
A. heckeli	-	50	12M + 12SM + 16ST + 10A	78	Simovic et al. (1994)
	Turkey	50	14M + 18SM + 18A	82	Gül et al. (2004)
A. mossulensis	Turkey	48	12M + 20SM + 16A	-	Gül et al. (2000)
	Turkey	50	12M + 16SM + 10ST + 12A	88	Yüksel and Gaffaroğlu (2008)
A. tarichi	Turkey	50	16M + 10SM + 24A	-	Gül et al. (2003)
A. orontis	-	50	-	-	Vasil'yev (1980)
A. escherichii	**Turkey**	**50**	**12M + 24SM + 14A**	**86**	**This study**

transcriptionally active NORs (Zaleśna et al., 2017). The number and location of NOR regions are species-specific, although they may vary between individuals. In studies conducted to date, the number and localization of NORs have been defined as useful chromosomal markers in fish cytogenetics (Rábová et al., 2003). The common NOR phenotype of leuciscins is the submetacentric/subtelocentric-acrocentric (sm/st-a) chromosome pair, which are considered plesiomorphic characters and carry a single NOR (Vasil'yev, 1985; Ráb et al., 1996; Bianco et al., 2004; Ráb et al., 2008; Boron et al., 2009; Pereira et al., 2009; Rossi et al., 2012). Detection of this common NOR phenotype in the medium-sized submetacentric chromosome pair in *Alburnus escherichii* shows a plesiomorphic character. The NOR position in *A. escherichii* (medium-sized sm/st pair) is the same as that of *A. albidus* (Bianco et al., 2004), *A. alburnus* (Schmid et al., 2006), *A. adanensis* (Ünal and Gaffaroğlu, 2016), *A. mossulensis* (Yüksel and Gaffaroğlu, 2008), and *A. filippii* (Nazari et al., 2009). However, in *A. escherichii*, active NORs are localized in the whole of the short arms, unlike the *Alburnus* species studied. The NOR phenotype associated with heterochromatin may be an intra- and/or interspecific chromosomal marker (Ráb et al., 1996). The NOR-bearing chromosome in *A. escherichii* is associated with C-heterochromatin bands, as in *Leuciscus aspius* (Ráb et al., 1990), *L. borysthenicus* (Ráb et al., 1996), *L. leuciscus* (Boron et al., 2009), *Chondrostoma beysehirense* (Arslan and Gündoğdu, 2016), The presence of heterochromatin bands in NOR regions in *A. escherichii* can be considered a cytogenetic marker compared with other *Alburnus* species. As a result, in this study, the chromosomal properties of the *A. escherichii* are determined for the first time. The obtained results may contribute to the cytogenetics and cytotaxonomy of *Alburnus* species in Anatolia and Europe.

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Statement of Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's Contributions

The contribution of the authors is equal.

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