Caryologia

International Journal of Cytology, Cytosystematics and Cytogenetics

ISSN: 0008-7114 (Print) 2165-5391 (Online) Journal homepage: https://www.tandfonline.com/loi/tcar20

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To cite this article: Sevgi Unal & Muhammet Gaffaroğlu (2016) Karyology of six cyprinid fishes from Seyhan and Ceyhan rivers in Anatolia, Caryologia, 69:4, 362-369, DOI: 10.1080/00087114.2016.1247328

To link to this article: https://doi.org/10.1080/00087114.2016.1247328

Published online: 02 Nov 2016.

CARYOLOGIA: INTERNATIONAL JOURNAL OF CYTOLOGY, CYTOSYSTEMATICS AND CYTOGENETICS, 2016
VOL. 69, NO. 4, 362–369
http://dx.doi.org/10.1080/00087114.2016.1247328

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Karyology of six cyprinid fishes from Seyhan and Ceyhan rivers in Anatolia

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ABSTRACT

Karyological properties of *Cyprinus carpio*, *Capoeta damascina*, *Luciobarbus pectoralis*, *Pseudophoxinus zekayi*, *Squalius seyhanensis* and *Alburnus adanensis* from the Seyhan and Ceyhan river systems were investigated by examining metaphase chromosomes through Giemsa staining, C-banding and Ag-NOR techniques. The diploid number of chromosomes in *A. adanensis*, *P. zekayi* and *S. seyhanensis* were 50, in *C. carpio* and *L. pectoralis* 100, and in *C. damascina* 150. Sex chromosomes were not observed in any of the examined species. Several chromosomes presented C-bands in pericentromeric and/or paracentromeric position, near the centromere. Some species had centromeric and pericentromeric heterochromatic blocks. NORs were observed in one pair of submetacentric chromosomes in *L. pectoralis*, *P. zekayi*, *S. seyhanensis* and *A. adanensis*, in one pair of metacentric chromosomes in *C. carpio* and three pairs of chromosomes in *C. damascina*. The cytogenetic features of endemic fishes *A. adanensis*, *L. pectoralis*, *P. zekayi* and *S. seyhanensis* were detected for the first time in this study.

ARTICLE HISTORY
Received 19 January 2016
Accepted 9 October 2016

KEYWORDS
Cyprinidae; karyotype; C-banding; nucleolus organizer regions (NOR); polyploidy; Seyhan and Ceyhan river systems

Introduction

Investigations of fish chromosomes have necessity in aquaculture, conservation and response to pollutants, and systematics and comparative genetics among fishes and other vertebrate groups (Salvadori et al. 2015). Cytogenetic studies in fishes have not been as successful or widespread as in other vertebrate groups because of the large number of small chromosomes and insufficiency of karyotype data (Ramasamy et al. 2010). Most karyological knowledge in fishes concerns cyprinids (Arai 2011). The diploid chromosome number for several cyprinid fishes is 50 and this is considered a model chromosome number for cyprinid fishes. Polyploid chromosome state has also been observed in some cyprinid fishes (Oellermann and Skelton 1990). Polyploidy, the multiplication of entire sets of chromosomes beyond the normal set of two, has occurred extensively, independently, and is often repeated in many groups of fish (Leggatt and Iwama 2003).

Chromosome complements ($2n$), chromosome numbers and individual chromosome sizes vary in fishes. Independently of the level of ploidy, chromosome numbers are expressed as $2n$ for the somatic chromosome number, and as n for the gametic chromosome number. The fundamental chromosome number for a species or family is expressed as x; $2n = 2x$ represents diploid individuals, $2n = 3x$ represents triploid individuals and $2n = 4x$ represents tetraploid species (Gregory and Mable 2005). While the number of chromosomes in most of these Cyprinidae species varies between $2n = 40$ and $2n = 50$, their ancestral diploid chromosome number is generally accepted as $2n = 48$ (Chrisman et al. 1990). Species with a diploid chromosome number of $2n = 98$ and 100 are the product of the polyploidization of $2n = 48$ and 50 species (Bhatnagar et al. 2014). Within this species part of subfamily Cyprininae and part of subfamily Leuciscinae, belong family Cyprinidae, has been reported may be of polyploid origin (Arai 2011).

The family Cyprinidae is the largest family of freshwater fishes and the most species-rich family of vertebrates (Nelson 2006). The family is represented by 36 genera and 193 species in Turkish inland waters (Kuru et al. 2014). Seyhan and Ceyhan rivers are inland waters of Turkey and have fish habitats with high biogeographical diversity (Yerli 2015). The ichthyofauna of the Seyhan and Ceyhan river systems consist of 29 species and of these 18 species belong to the Cyprinidae family (Erk'akan and Ozdemir 2011; Turan et al. 2013). The members of this family have different chromosome numbers. While some species have a diploid chromosome number, some species are polyploid. Cytogenetic studies are important in obtaining chromosomal data of species, as well as information about karyoevolution and phylogenetic relationships among relative species, and for evaluating polyploidy and hybridization. No faunal

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karyological studies have been carried out in the Seyhan and Ceyhan rivers.

The aim of this study is to reveal the diploid chromosome number, karyomorphology, C-band and NOR characteristics of endemic leuciscines *Alburnus adanensis* Battalgazi, 1944, *Pseudophoxinus zekayi* Bogutskaya et al. 2007, *Squalius seyhanensis* Turan et al. 2013, barbin *Luciobarbus pectoralis* (Heckel 1843) and nonendemic cyprinins *Cyprinus carpio* Linnaeus, 1758, *Capoeta damascina* (Valenciennes, 1842) from the Seyhan and Ceyhan river systems (by conventional techniques). The rivers are vulnerable to water pollution, flooding, climate change, mass fish deaths and overfishing, and the chosen species are assessed as vulnerable (*C. carpio*, *P. zekayi* and *A. adanensis*), least concern (*C. damascina* and *L. pectoralis*) and data deficient (*S. seyhanensis*) (IUCN 2015). The karyological investigations of *A. adanensis*, *P. zekayi*, *S. seyhanensis*, and *L. pectoralis* were performed for the first time in this study. This research constitutes fundamental cytogenetic data of multiple cyprinid fishes for advanced genetics and taxonomical studies.

Materials and methods

Seventy-four specimens of six species were collected by electrofishing in different localities from Seyhan and Ceyhan river systems, Anatolia (Table 1). Individuals of each species were transported as alive to the laboratory and kept in well aerated aquaria until analysis. Identified species in the study were deposited at the Ahi Evran University Genetics Research Laboratory, Turkey.

Chromosome preparation and conventional staining

Chromosomes preparations were carried out as described by Collares-Pereira (1992). Cell suspensions were obtained after injecting 0.1% colchicine intraperitoneally (0.01 ml g^{-1} body weight) 45 min before sacrificing. Kidney, spleen and gill tissues were removed, homogenized and hypotonized by KCl 0.075 M for 15 min at 37°C. Suspensions were centrifuged at 1000 rpm for 10 min. Supernatant was removed and cold fresh fixative (3:1 methanol and glacial acetic acid) was added for fixation of cells. This process was repeated three or five times depending on density of cells. Slides (separately 10 slides for each species) were stained for 15–25 min with 10% Giemsa, pH 6.8.

Chromosome banding and imaging

Constitutive heterochromatin regions and heterochromatin blocks were analyzed with a modified C-banding technique (Sumner 1972). Chromosome slides were treated with 0.2 N HCl for 45 min at room temperature, then incubated in 5% $Ba(OH)_2$ for 15–20 min at 37°C in a waterbath, and incubated with 2 × SSC for 70 min at 60 °C. Then dried slides were stained by Giemsa for 45 min. Nucleolus organizer regions (NORs) were detected with silver nitrate staining modified to species according to Howell and Black (1980). For staining of AgNORs, two drops of protective colloidal developer and four drops of the silver nitrate are pipetted onto chromosome slide. The slides were covered with a coverglass and stabilized at 70°C. The coverglass was removed from onto slide and slide was rinsed off under deionized water. The slide was dried and covered by a coverglass.

Karyotyping

Chromosome morphologies were determined according to the ratio of the arms (Levan et al. 1964). Arm ratios (q/p) of the classified chromosomes were obtained by dividing the length of the long arm (q) by the length of the short arm (p). The biarmed chromosomes and arm ratios of 1–1.7 were classified as metacentric (m), arm ratios of 1.71–3 were classified as submetacentric (sm), and arm ratios of 3.01–7 were classified as subtelocentric (st). Chromosomes uniarmed had a single arm and/or a little p arm were assessed as subtelocentric (st) and acrocentric (a) or subtelo-acrocentric (st-a).

All images were photographed by a Leica DM 3000 microscope (Leica Microsystems GmbH, Germany) equipped with a camera and AKAS software (Argenit Microsystems, Istanbul, Turkey).

Results

Karyomorphology

Alburnus adanensis *(Battalgazi, 1944)*

The analysis of 50 mitotic metaphase cells of *Alburnus adanensis* showed that diploid chromosome number

Table 1. Collection data of studied species.

Subfamily	Species	Collection sites	River system	Coordinate	Number and sex of analyzed specimens
Cyprininae	*Cyprinuscarpio*	Hacıbeyli Village, Kozan, Adana	Ceyhan	37°38′ N 36°02′ E	2♀, 3♂
	Capoeta damascina	Dağlıca Village, Kozan, Adana	Ceyhan	37°33′ N 35°50′ E	7♀, 15♂
		Çulluuşağı Village, Adana	Seyhan	37°38′ N 35°51′ E	
	Luciobarbus pectoralis	Hemite Village, Kadirli, Osmaniye	Ceyhan	37°11′ N 36°04′ E	1♀, 4♂
Leuciscinae	*Pseudophoxinus zekayi*	Alapınar Village, Kozan, Adana	Ceyhan	37°20′ N 35°50′ E	2♀, 5♂
	Squalius seyhanensis	Karahalka Village, Kayseri	Seyhan	38°53′ N 36°49′ E	8♀, 14♂
Alburninae	*Alburnus adanensis*	Koçyurdu Village, Kadirli, Osmaniye	Ceyhan	37°38′ N 36°02′ E	3♀, 6♂
		Çulluuşağı Village, Adana	Seyhan	37°38′ N 35°51′ E	

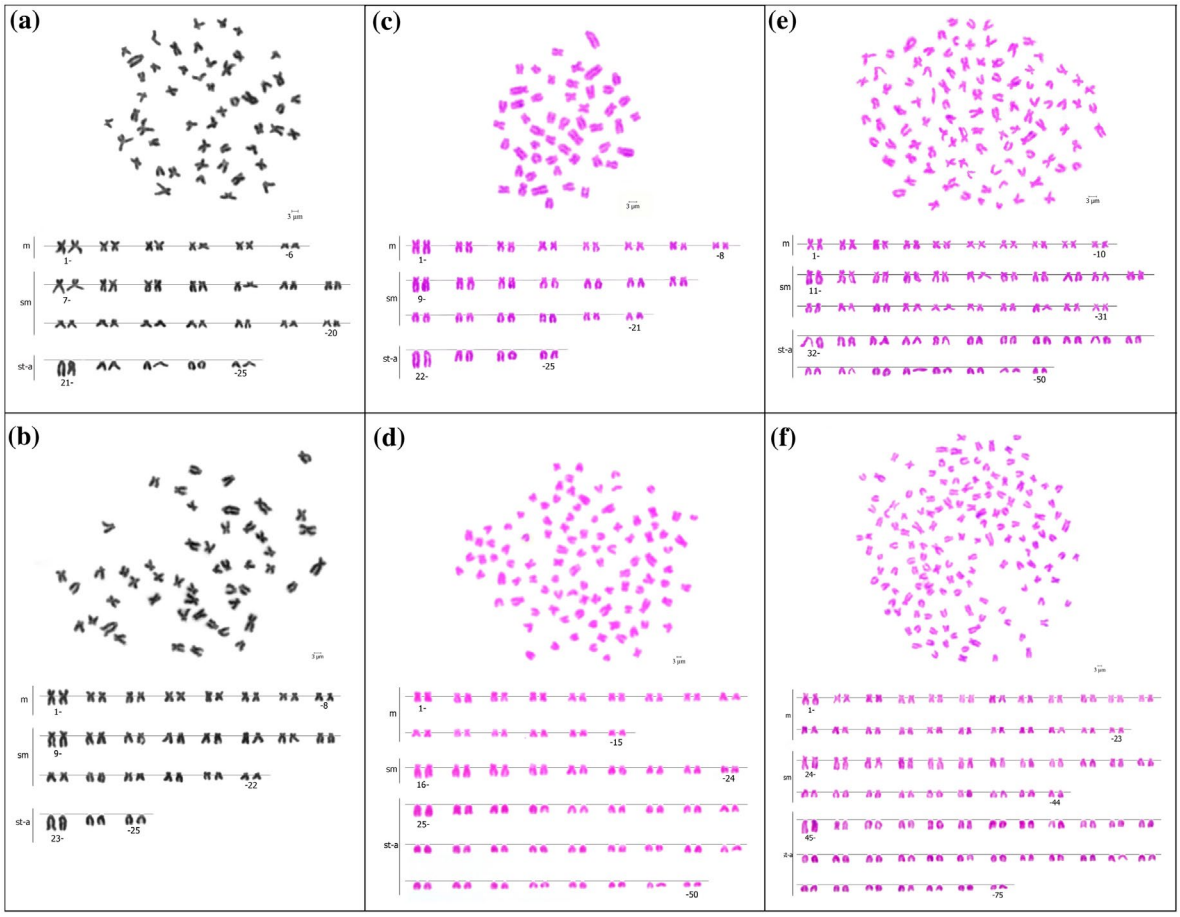

Figure 1. Giemsa stained metaphases and karyotypes of (a) *Alburnus adanensis*; (b) *Squalius seyhanensis*; (c) *Pseudophoxinus zekayi*; (d) *Cyprinus carpio*; (e) *Luciobarbus pectoralis*; (f) *Capoeta damascina*. Scale bar = 3 µm.

varied between $2n = 48$ and 52, with $2n = 2x = 50$ being the most frequently (80%) observed number for the species (Figure 1(a)). The karyotype was composed of 12 m, 28 sm, and 10 st-a chromosomes, with a fundamental number (FN) = 90. The largest chromosome pairs of the karyotype was determined as st-a.

Squalius seyhanensis *(Turan et al., 2013)*

The analysis of 100 mitotic chromosomes of *Squalius seyhanensis* revealed that diploid number varied between $2n = 46$ and 50, with $2n = 2x = 50$ being the most frequently (79%) observed number for the species (Figure 1(b)). The karyotype contained 16 m, 28 sm and 6 st-a chromosomes, with a FN = 94. St-a chromosome pairs were detected as the largest pairs of the karyotype.

Pseudophoxinus zekayi *(Bogutskaya et al., 2007)*

The analysis of 100 metaphase plates of *Pseudophoxinus zekayi* showed that the diploid chromosome number varied between $2n = 45$ and 50, with $2n = 2x = 50$ being the most frequently (85%) observed number for the species (Figure 1(c)). The karyotype consisted of 16 m, 26 sm and 8 st-a chromosomes, FN = 92. The largest chromosome pair of the karyotype was determined as st-a.

Cyprinus carpio *(Linnaeus, 1758)*

The analysis of 80 metaphase plates of *Cyprinus carpio* showed that the diploid number varied between $2n = 97$ and 102 chromosomes, with $2n = 4x = 100$ being the most frequently (75%) observed number for the genus (Figure 1(d)). The karyotype consisted of 30 m, 18 sm and 52 st-a chromosomes, with FN = 148.

Luciobarbus pectoralis *(Heckel, 1843)*

The analysis of 60 mitotic metaphase cells of *Luciobarbus pectoralis* and obtained data showed that the diploid chromosome number varied between $2n = 98$ and 102, with $2n = 4x = 100$ being the most frequently (83.30%) observed number for the species (Figure 1(e)). The karyotype was composed of 20 m, 42 sm, and 38 st-a chromosomes, with FN = 162. The largest chromosome pair of the karyotype was determined as st-a.

Capoeta damascina *(Valenciennes, 1842)*

The analysis of 60 metaphase cells of *Capoeta damascina* revealed that diploid chromosome number varied between $2n = 138$ and 150, with $2n = 6x = 150$ being the most frequently (68.30%) observed number for the species (Figure 1(f)). The karyotype consisted of 46 m, 42 sm and 62 st-a chromosomes, with FN = 238.

Differentiated sex chromosomes were not observed in all studied species.

Constitutive heterochromatin regions

Constitutive heterochromatin was observed in the centromeric regions in most chromosomes of all the analyzed species. Also in *Capoeta damascina*, *Pseudophoxinus zekayi* and *Alburnus adanensis*, heterochromatin blocks were located in the long arms of some chromosomes. Centromeric, pericentromeric and paracentromeric C-bands were detected in *A. adanensis*, *P. zekayi* and *Squalius seyhanensis*. In addition, C-bands were located mostly in the pericentromeric region on *Cyprinus carpio*. Moreover, pericentromeric heterochromatic blocks were observed all of the some chromosomes in *Luciobarbus pectoralis* (Figure 2).

Nucleolus organizer regions

NORs were located in the terminal regions on the short arms of one pair of sm chromosomes in *Luciobarbus pectoralis*, *Squalius seyhanensis* and *Alburnus adanensis*. NORs were observed on different localities of three pairs of chromosomes (on short arms of four sm chromosomes, on short and long arm of a sister chromatid and on long arms of a sm pair) in *Capoeta damascina*. In *Cyprinus carpio*, NORs were observed on short arms of a m chromosome pair (Figure 3). NOR number polymorphisms were detected in *Pseudophoxinus zekayi*. NOR number varied between two and four, with two NORs the most frequently (61%) observed number for the analyzed metaphases (Figure 3(f)). There were 14% with three NORs (Figure 3(g)), and 25% with four NORs (Figure 3(h)).

Discussion

The karyological features of endemic fishes *Alburnus adanensis, Luciobarbus pectoralis, Pseudophoxinus zekayi* and *Squalius seyhanensis were* investigated in this study for the first time. Although the karyotypes of *Cyprinus carpio* and *Capoeta damascina* were determined in previous studies (Pekol 1999; Gorshkova et al. 2002) C-band and NOR phenotypes of these species were examined for the first time in this research. The diploid chromosome numbers of *A. adanensis* (FN = 90), *P. zekayi* (FN = 92) and *S. seyhanensis* (FN = 94) are 50, those of *C. carpio* (FN = 148) and *L. pectoralis* (FN = 162) are 100, and that of *C. damascina* (FN = 238) is 150 (Table 2). Although the species have the same diploid chromosome number they differ with regard to chromosome morphologies. However, *P. zekayi* and *S. seyhanensis* have the same number of m chromosomes. The largest chromosome pair of the $2n = 50$ Leuciscinae sets is characteristically a st-a chromosome pair, and is thus propounded as a

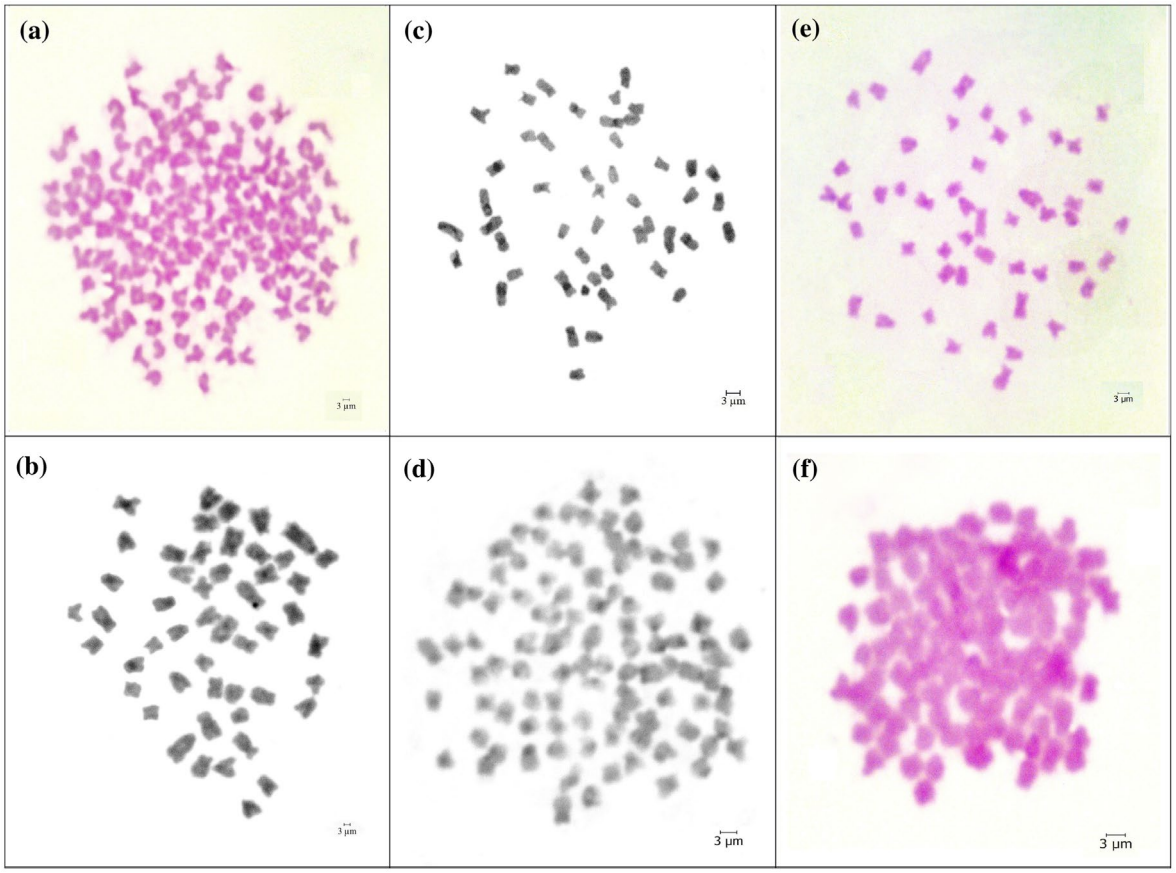

Figure 2. Constitutive heterochromatin regions of (a) *Capoeta damascina*; (b) *Pseudophoxinus zekayi*; (c) *Alburnus adanensis*; (d) *Cyprinus carpio*; (e) *Squalius seyhanensis*; (f) *Luciobarbus pectoralis.* Scale bar = 3 µm.

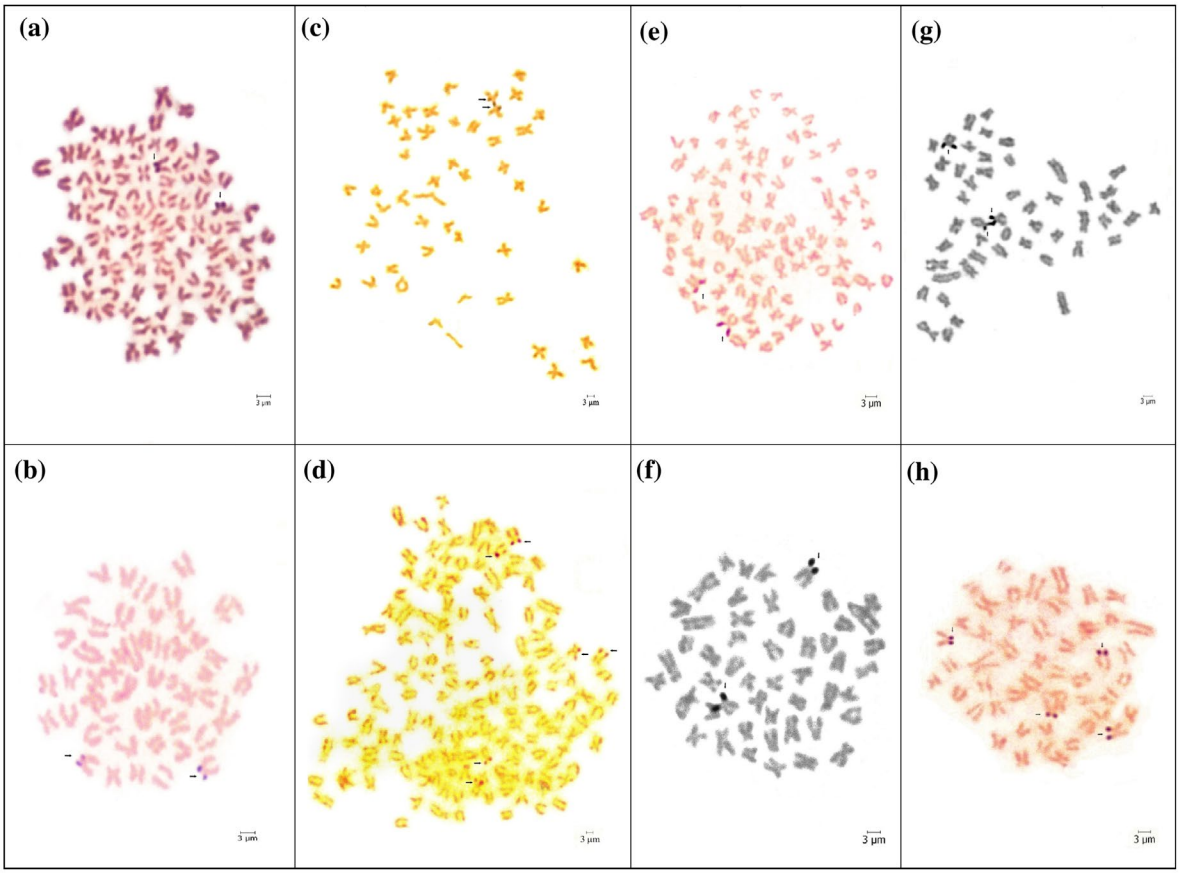

Figure 3. Silver nitrate stained metaphases of (a) *Luciobarbus pectoralis*; (b) *Squalius seyhanensis*; (c) *Alburnus adanensis*; (d) *Capoeta damascina*; (e) *Cyprinus carpio*; (f) *Pseudophoxinus zekayi*; (g, h) *Pseudophoxinus zekayi* with three and four NORs. Arrows show the NORs. Scale bar = 3 µm.

cytotaxonomic marker (Rab et al. 2008). *C. carpio*, *L. pectoralis* and *C. damascina* polyploid species have numerous small chromosome (st-a) and are classified as evolutionary tetraploids and hexaploids of alburnine and leuciscine cyprinids (*A. adanensis*, *P. zekayi* and *Squalius seyhanensis*) according to their karyosystematic characteristics (Rab and Collares-Pereira 1995). In addition, no differentiated sex chromosomes are observed in this study as reported for some species of genus *Alburnus* (Gül et al. 2004), *Pseudophoxinus* (Unal et al. 2014; Karasu Ayata 2015), *Squalius* (Ünal 2011), *Luciobarbus* (Gaffaroğlu et al. 2013), *Capoeta* (Gorshkova et al. 2002) and *C. carpio* (Pekol 1999).

C-banding studies have been revealed phylogenetic relations between species (Arslan and Arslan 2007). Constitutive heterochromatic regions can be determined by this technique and C-positive regions have been considered as chromosome markers in karyological studies on various cyprinid species (Salvadori et al. 2015; Arslan and Gündoğdu 2016). In this study, C-positive bands and heterochromatic blocks are located at the centromeric and pericentromeric regions of most studied species as in the other related species and as in the other cyprinid genus such as *Squalius anatolicus* (Unal 2011), *Tinca* (Arslan and Taki 2012), *Luciobarbus escherichii* (Gaffaroğlu et al. 2013), *Alburnoides* (Gaffaroğlu, Karasu Ayata, Ünal, Kalkan 2014), *Chondrostoma* (Arslan and Gündoğdu 2016), and *Pseudophoxinus* (Karasu Ayata et al. 2016). In the species *C. damascina*, *P. zekayi* and *A. adanensis*, heterochromatic blocks are located in the long arms of some chromosomes as different from other investigated related cyprinid species *L. pectoralis* (in this study), *Capoeta antalyensis, Pseudophoxinus crassus, Pseudophoxinus hittitorum* and *Alburnus albidus* (Bianco et al. 2004; Gaffaroğlu et al. 2012; Unal et al. 2014). In some chromosomes of *A. adanensis*, *P. zekayi* and *S. seyhanensis*, heterochromatin regions are present in the paracentromeric as different from reported in *Pseudophoxinus egridiri, Pseudophoxinus fahrettini* and *Pseudphoxinus antalyae* (Ergene et al. 2010; Karasu Ayata et al. 2016).

In the six species of the family Cyprinidae analyzed in this study, C-bands and heterochromatic blocks were observed in the centromere and pericentromere regions in the most of the chromosomes, which is consistent with similar patterns described in the literature. However heterochromatic blocks were found to be associated to paracentromeric regions in some of the studied species. The presence of heterochromatin and heterochromatin patterns for *C. carpio*, *C. damascina*, *L. pectoralis*, *P. zekayi*, *S. seyhanensis* and *A. adanensis* are described for the first time in this study. The heterochromatin distributional

Table 2. Karyological investigations on some cyprinids of Anatolia.

Species	2*n*	Karyotype	FN	References
Squalius cephalus	50	18 m+12sm+20st-a	80	Pekol 1999
	50	20+12sm+18st-a	82	
Squalius orientalis	50	14 m+20sm+16st-a	84	Kılıç Demirok, 2000
Squalius anatolicus	50	10 m+22sm+10st+8a	82	Ünal, 2011
Alburnus tarichi	50	16 m+10sm+24a	–	Gül et al. 2003
Alburnus heckeli	50	14 m+18sm+18a	82	Gül et al. 2004
Alburnus mossulensis	50	12 m+16sm+10st+12a	88	Gaffaroğlu and Yüksel, 2005
Alburnus filippi	50	16 m+16sm+18a	82	Nur, 2006
Pseudophoxinus antalyae	50	16 m+14sm+12st+8a	92	Ergene et al. 2010
Pseudophoxinus firati	50	38 m-sm+12st	88	Karasu et al. 2011
Pseudophoxinus crassus	50	12 m+30sm+8st-a	92	Unal et al. 2014
Pseudophoxinus hittitorum	50	14 m+26sm+10st-a	90	Unal et al. 2014
Pseudophoxinus elizavetae	50	8 m+34sm+8st	92	Gaffaroğlu, Karasu Ayata, Ünal, Yüksel 2014
Pseudophoxinus battalgilae	50	16 m+28sm+6st-a	94	Karasu Ayata et al. 2016
Pseudophoxinus burduricus	50	18 m+26sm+6st-a	94	Karasu Ayata et al. 2016
Pseudophoxinus egridiri	50	14 m+28sm+8st-a	92	Karasu Ayata et al. 2016
Pseudophoxinus evliyae	50	14 m+30sm+6st-a	94	Karasu Ayata et al. 2016
Pseudophoxinus fahrettini	50	16 m+26sm+8st-a	92	Karasu Ayata et al. 2016
Pseudophoxinus maeandri	50	10 m+32sm+8st-a	92	Karasu Ayata et al. 2016
Cyprinus carpio	100	22 m+30sm+48st-a	152	Pekol, 1999
	100	20 m+30sm+50st-a	150	
Cyprinus carpio	100	12 m+38st+50a	–	Hamalosmanoğlu and Kuru, 2003
Luciobarbus mystaceus	100	22 m+30sm+48st-a	152	Kılıç Demirok, 2000
Luciobarbus escherichii	100	14 m+44sm+42st-a	158	Gaffaroğlu et al. 2013
Capoeta trutta	150	20 m+54sm+76st-a	224	Kılıç Demirok and Ünlü, 2001
Capoeta umbla	150	86 m-sm+64st-a	236	Kılıç Demirok and Ünlü, 2001
Capoeta tinca	150	88 m-sm+62st-a	238	Gaffaroğlu et al. 2010
Capoeta antalyensis	150	84 m-sm+66st-a	234	Yüksel et al. 2011
Alburnus adanensis	50	12 m+28sm+10st-a	90	This study
Squalius seyhanensis	50	16 m+28sm+6st-a	94	This study
Pseudophoxinus zekayi	50	16 m+26sm+8st-a	92	This study
Cyprinus carpio	100	30 m+18sm+52st-a	148	This study
Luciobarbus pectoralis	100	20 m+42sm+38st-a	162	This study
Capoeta damascina	150	46 m+42sm+62st-a	238	This study

pattern is nearly similar among the analyzed species, suggesting a common pattern for species in the family Cyprinidae.

Nucleolus organizer region contains genes that produce ribosomal RNA and these regions may hold nucleoli proteins during the entire process of cellular division (Guerra 1988). NORs are usually placed and they are identified by silver nitrate impregnation of the chromosomes. The impregnation marks only nucleoli proteins involved in the transcriptional activity of ribosomal genes (Howell and Black 1980). NORs may be located in a single chromosomal pair, a basal characteristic already reported for different cyprinid species (Arslan and Gündoğdu 2016; Karasu Ayata et al. 2016). Some Anatolian cyprinid species – *S. anatolicus* (Ünal 2011), *P. crassus*, *P. hittitorum* (Unal et al. 2014), *Alburnoides bipunctatus* (Gaffaroğlu, Karasu Ayata, Ünal, Kalkan 2014), *Chondrostoma beysehirense* (Arslan and Gündoğdu 2016), *P. burduricus*, *P. egridiri* and *P. fahrettini* (Karasu Ayata et al. 2016) – have a single NOR in the sm pair, like *A. adanensis*, *S. seyhanensis* and *L. pectoralis*. Further *C. carpio* has one pairs NORs almost all of the short arm of m chromosomes. Although in *C. carpio* Weishun (1985) and Pekol (1999) determined the same number of NORs, localization was different. As far as the European cyprinids are concerned, NOR variability has been insufficiently analyzed, and previous studies on some species have reported only a single pair of NOR-bearing chromosomes. The only cyprinid species reported to have intraspecific variation in the number of NOR-bearing chromosomes (in Chondrostoma lusitanicum) (Rodrigues and Collares-Pereira 1996). The Anatolian cyprinid *P. zekayi* carries intraspecific NOR variation. The number of NOR-bearing chromosomes varies between two and four, e.g. in *Pseudophoxinus battalgilae, Pseudophoxinus burduricus* and *Pseudophoxinus evliyae* (Karasu Ayata et al. 2016). The number of the NORs is considered as an family or subfamily marker (Rab and Collares-Pereira 1995; Rab et al. 2008). Besides data on the NOR numbers, multiple NOR phenotypes appear to be more derived as opposed to a uniform one NOR-bearing chromosome pair (Valic et al. 2010). In this study, *C. damascina* has multiple NOR numbers and locations, like *C. antalyensis* (Gaffaroğlu et al. 2012). *C. damascina* is similar to *C. antalyensis* with respect to the NOR number. However the NORs are located on two pairs of sm and one pair of m chromosomes in *C. damascina*, and on three pairs of sm-st chromosomes in *C. antalyensis.* The number and location of NORs (and NOR number polymorphism in *P. zekayi*) for *C. damascina*, *L. pectoralis*, *P. zekayi*, *S. seyhanensis* and *A. adanensis* are identified for the first time in this study.

In conclusion, when the karyologically investigated species in this study are compared, *P. zekayi*, *S. seyhanensis* and *A. adanensis* had the same diploid number and their NOR numbers and locations were similar to each other. Although *L. pectoralis* and *C. carpio* were

polyploid, their number and location of NORs resembled *P. zekayi*, *S. seyhanensis* and *A. adanensis*. In the polyploid species *C. damascina*, the NOR phenotype was different from other examined species in this study. The study includes the detailed cytogenetic characterization of the six cyprinid species from Seyhan and Ceyhan river systems in Anatolia. Basic karyological data has been presented to contribute to knowledge of cytotaxonomy, chromosomal phylogeny, evolutionary assessment and karyodiversity of cyprinid groups. These data should be corroborated by molecular studies.

Acknowledgments

We would like to thank Dr S Serkan GÜÇLÜ (Süleyman Demirel University) for identifying the species and Dr Muradiye KARASU AYATA (Ahi Evran University) for her support in the laboratory.

Disclosure statement

No potential conflict of interest was reported by the authors.

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