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Comparative cytogenetics of four endemic *Capoeta* (Teleostei: Cyprinidae) species from Anatolia, Türkiye

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Abstract. The genus *Capoeta* is an important taxon covering a wide distribution in Türkiye. However, only a few genetic studies on *Capoeta* species reported from Türkiye. There is no cytogenetical study in *Capoeta aydinensis* Turan, Küçük, Kaya, Güçlü & Bektaş, 2017, *Capoeta bergamae* Karaman, 1969, *Capoeta erhani* Turan, Kottelat & Ekmekçi, 2008 and *Capoeta pestai* (Pietschmann, 1933). Thus, in this study, we karyotyped through classical cytogenetic techniques (Giemsa staining, Ag-NORs, and C-banding) the four endemic *Capoeta* species. The diploid chromosome number invariably was 150 in the four species. However, chromosome morphologies in the karyotypes had some differences between them. The number of biarmed chromosomes in the karyotypes was higher in all studied species. Their karyotypes contained respectively: 54 metacentric, 42 submetacentric and 54 subtelo-acrocentric in *C. aydinensis*, 56 metacentric, 30 submetacentric and 64 subtelo-acrocentric in *C. bergamae*, 50 metacentric, 42 submetacentric and 58 subtelo-acrocentric in *C. erhani* and 44 metacentric, 40 submetacentric and 66 subtelo-acrocentric chromosomes in *C. pestai*. C-bands were on the pericentromeres of most chromosomes in the four species. Three chromosome pairs carry rDNA genes in all studied species. The chromosomal locations of these sites were varied between the species. This study provides new insights into the chromosomal data of the hexaploid cyprinids. Moreover, obtained cytogenetic results should be conclude the cytotaxonomy of the genus *Capoeta* that distributed in Türkiye.

Keywords: Ag-NOR, C-banding, chromosome morphology, chromosome number, scraper.

INTRODUCTION

Türkiye has one of the most diverse and species-rich freshwater ichthyofaunas according to the different eco-regions that formed in Anatolian freshwaters (Küçük et al., 2009; Bektaş et al., 2017). The endemic species are

much higher than in Western Asia or Europe (Küçük et al., 2009). The members of the genus *Capoeta* (Valenciennes, 1842) (Cyprinidae, Barbinae) distribute from East Europe to West Asia, including Anatolia (Bektaş et al., 2017). Seventeen species named *Capoeta antalyensis*, *C. aydinensis*, *C. baliki*, *C. banarescui*, *C. barroisi*, *C. bergamae*, *C. caelestis*, *C. capoeta*, *C. damascina*, *C. ekmekciae*, *C. erhani*, *C. oguzelii*, *C. pestai*, *C. sieboldii*, *C. tinca*, *C. trutta* and *C. umbla* of this genus are presently recognized in the inland waters of Türkiye. Except for six species (*C. barroisi*, *C. capoeta*, *C. damascina*, *C. ekmekciae*, *C. trutta* and *C. umbla*) the other *Capoeta* members are endemic to Anatolia (Bektaş et al., 2019).

Taxonomic problems still exist in Anatolian *Capoeta* species and the species diversity of this genus has not been resolved (Turan et al., 2017). Özuluğ and Freyhof (2008) collected an additional species of *C. trutta* from Seyhan River in Türkiye. *C. turani* was described as a new species from this drainage according to the different morphological characters (Özuluğ and Freyhof, 2008). *C. erhani* was described in Ceyhan River of Türkiye by Turan et al. (2008). It was distinguished from the other members of *C. trutta* in the scope of morphological characters (Turan et al., 2008). Otherwise, *C. pestai* was described from Eğirdir Lake and it was also recorded from Lake Beyşehir. In fact, Beyşehir population of *C. pestai* was described as a new species called *C. mauricii* by Küçük et al. (2009) according to the different morphological characters. However, according to the molecular phylogeny study (cyt b gene sequences) in the genus *Capoeta* by Bektaş et al. (2017), *C. turani* was synonymized to *C. erhani*. Also, *C. mauricii* was synonymized to *C. pestai* (Bektaş et al., 2017). Otherwise, *C. bergamae* distributes in the western basins of Türkiye, as well as *C. aydinensis* was described as a new species in the recent years and is presently known from the Büyük Menderes River drainages (Turan et al., 2017).

The cytogenetic studies have played an important role in describing the main features in cytotaxonomy and for understanding chromosome evolution in fish species (Gaffaroğlu et al., 2020). However, the karyotypes of fishes are poorly studied compared to the other vertebrates in response to the richness of this group. The karyotype of many fish species is still undescribed due to the difficulty of sampling the individuals, the necessity of having alive individuals, in troubling to obtain karyotypes from cell-culture and unsuccessful in obtaining good metaphase spreads (Rossi, 2021). In this context, having too many chromosomes is another reason for this problem .

Knowledge of karyotype is necessary for fish cytogenetics. Detailed investigations of the chromosomes with Giemsa stained karyotypes have only been performed on only seven species namely, *C. trutta*, *C. umbla* (Kılıç-Demirok and Ünlü, 2001), *C. capoeta*, *C. barroisi* (Kaya, 2003), *C. damascina* (Unal and Gaffaroğlu, 2016), *C. antalyensis* and *C. baliki* (Karasu-Ayata et al., 2017) from Türkiye. The chromosomal banding properties have been reported only in *C. damascina* (Unal and Gaffaroğlu, 2016) and *C. antalyensis* (Gaffaroğlu et al., 2012). Due to the lack of chromosomal reports, this study aimed to investigate karyotypes with Giemsa staining, C-banding and Ag-NOR staining in four Anatolian endemic *Capoeta* species.

MATERIAL AND METHODS

Cytogenetic analyses were performed on four *Capoeta* species from Türkiye (Table 1, Figure 1). The alive samples were carried to the laboratory. The individuals were treated in vivo for mitotic chromosome preparation by Bertollo et al. (2015). Chromosome preparations were obtained from the cephalic kidney cells after injection of 0.1% colchicine. After hypnotization with 0.075 M KCl, fixation steps (methanol: acetic acid, 3:1) were repeated at least three times in cell suspension. At least 10 metaphase slide was prepared from each individual. All the experiments followed ethical protocols and after sacrificing, the individuals were deposited in 70% ethanol in the laboratory. The process was approved by the Local Animal Ethics Committee of Türkiye (Protocol Number: 68429034/05/17). The Ag-NORs and C-banding were analysed according to the methods reported by Howell and Black (1980) and Sumner (1972).

At least 100 metaphase spreads per individual were analysed to confirm the diploid chromosome number. Images were photographed using Leica DM 3000 microscope (Leica Microsystems GmbH, Germany) with AKAS software (Argenit Mikrosistem, Türkiye). Chromosomes were measured by digital calliper and classified as metacentric, submetacentric and subtelo-acrocentric according to the arm ratios (Levan et al., 1964). Karyotypes were arranged manually. To count the fundamental arm number (FN) meta- and submetacentrics were considered as biarmed whereas subtelo-acrocentrics as uniarmed.

RESULTS

All studied *Capoeta* species have diploid chromosome number 2n = 150 (*Figs. 2A, 3A, 4A, 5A*) with karyotypes composed of mainly biarmed chromosomes. Karyotype formulas were as follows: 54 metacentric, 42

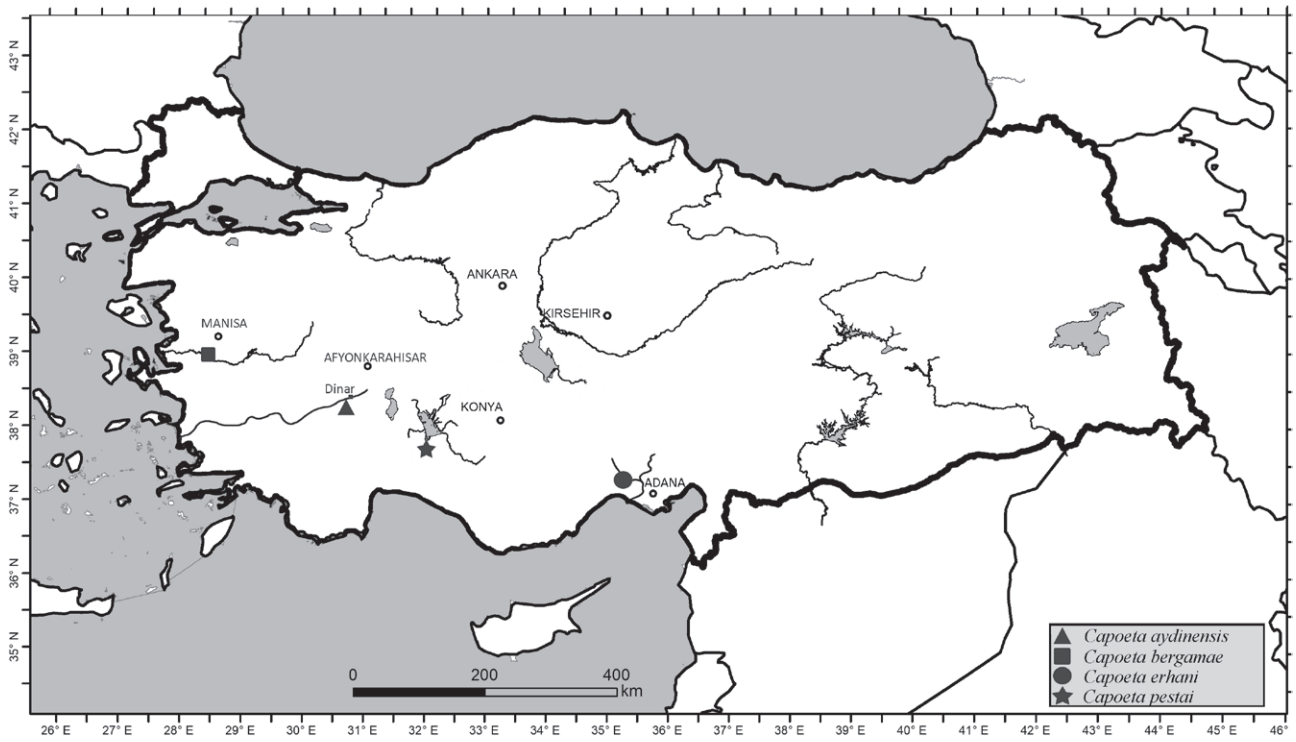

Figure 1. Map of the collected specimens of *Capoeta* species.

submetacentric and 54 subtelo-acrocentric in *C. aydinensis* (*Fig. 2B*); 56 metacentric, 30 submetacentric and 64 subtelo-acrocentric in *C. bergamae* (*Fig. 3B*); 50 metacentric, 42 submetacentric and 58 subtelo-acrocentric in *C. erhani* (*Fig. 4B*) and 44 metacentric, 40 submetacentric and 66 subtelo-acrocentric chromosomes in *C. pestai* (*Fig. 5B*). FN was calculated as 234 in *C. pestai*, 236 in *C. bergamae*, 242 in *C. erhani* and 246 in *C. aydinensis*. Morphologically differentiated sex chromosomes were not detected in all studied species.

In terms of C-bands, *C. aydinensis* contains very few C-bands (*Fig. 2C*). These C-bands were located on the pericentromeres of chromosome pairs 18, 21, 64 and 69 (*Fig. 2D*). Thirteen chromosome pairs of *C. bergamae* has intense pericentromeric C-bands of chromosome pairs 2, 8, 37, 44, 48, 53, 56, 59, 61, 65, 66, 69, 71 (*Fig. 3D*). *C. erhani* has slightly pericentromeric C-bands of chromosome pairs 1, 2, 3, 9, 16, 17, 26, 28, 31, 42, 48, 56, 58, 60, 66 and 73 (*Figs. 4C, D*). Intense pericentromeric C-bands of chromosome pairs 1, 3, 7, 19, 24, 25, 30, 34, 40, 43, 45, 46, 49, 51, 55, 56, 62, 64 and 73 were found in *C. pestai* (*Fig. 5D*). Some of the other chromosomes also have less intense pericentromeric C-bands in *C. pestai* (*Fig. 5C*) and *C. bergamae* (*Fig. 3C*).

Multiple Ag-NORs were found in the studied species. The common Ag-NOR number was six in four *Capoeta* species (*Figs. 2E, 3E, 4E, 5E*). These Ag-NORs were located on the terminal regions of metacentric chromosomes 1 and 5 as a strong signal and additionally weaker signals of chromosomes 12, 25 and 72 in *C. aydinensis* (*Fig. 2F*). Ag-NORs were detected on the terminal regions of the short arms of three submetacentric chromosome pairs 31, 33 and 37 in *C. bergamae* (*Fig. 3F*). Ag-NORs were located on the terminal regions of the short arms of 7th metacentric, 34th and 37th submetacentrics in *C. erhani* (*Fig. 4F*). Ag-NORs were found on the terminal regions of the short arms of three submetacentric chromosome pairs 26, 28 and 30 in *C. pestai* (*Fig. 5F*). Also, Ag-NOR number polymorphisms were detected in *C. bergamae* (*Figs. 6A, B*), *C. erhani* (*Figs. 7A, B, C, D*) and *C. pestai* (*Figs. 8A, B, C, D*) in some silver stained metaphases.

DISCUSSION

In the subfamily Barbinae a large number of species are polyploid. This subfamily may represent a more complicated polyploid system than other vertebrates. Polyploidy (whole genome duplication), has played an important role in the evolution of cyprinids (Yang et al., 2022). From the subfamily Barbinae (which includes

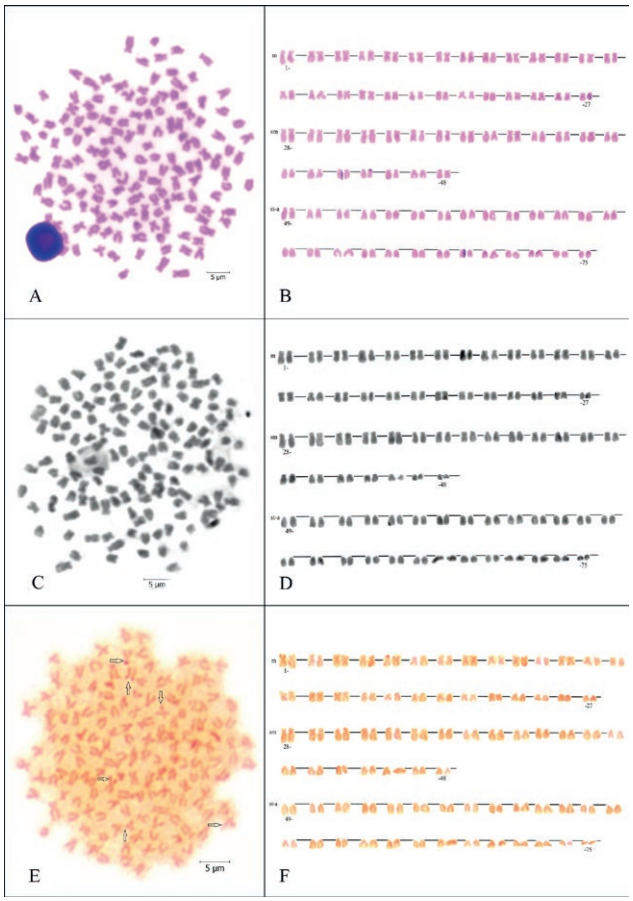

Figure 2. Metaphase plates of *Capoeta aydinensis* by Giemsa stained (A), C-banded (C) and Ag-stained techniques (E) and arranged karyotypes (B, D, F). Arrows indicate the Ag-NORs bearing chromosomes. Scale bars = 5 μm.

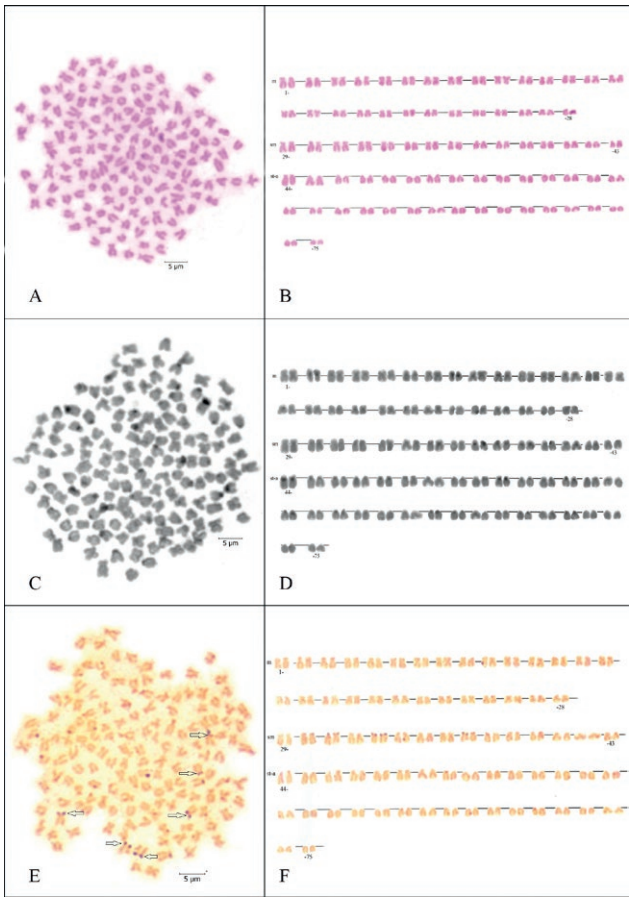

Figure 3. Metaphase plates of *Capoeta bergamae* by Giemsa stained (A), C-banded (C) and Ag-stained techniques (E) and arranged karyotypes (B, D, F). Arrows indicate the Ag-NORs bearing chromosomes. Scale bars = 5 μm.

only four genera), *Barbus* and *Luciobarbus* are tetraploid (2n = 4x) genera (Gaffaroğlu et al., 2013; Karasu-Ayata and Gaffaroğlu, 2019) where the genus *Capoeta* (2n = 6x) is hexaploid (Unal and Gaffaroğlu, 2016). Only the genus *Cyprinion* is diploid (2n = 2x) from this subfamily (Gaffaroğlu and Yüksel, 2004). Yang et al. (2022) reported that according to the mitochondrial and nuclear trees the polyploidy was allopolyploid in the subfamily Barbinae.

Cytogenetic analyses may provide a useful tool for understanding the karyotype changes in the evolution of the species (Gaffaroğlu et al., 2020). Especially according to the high chromosome number (2n = 150) cytogenetic studies are very limited in the genus *Capoeta* from Türkiye (Table 2) and also from the other countries (Arai, 2011). Cytogenetic data are available for only seven Anatolian *Capoeta* species (Kılıç-Demirok and Ünlü, 2001; Kaya, 2003; Unal and Gaffaroğlu, 2016; Karasu-Ayata et al., 2017). The diploid chromosome number has been conserved in the species of the genus *Capoeta* in the previous studies (Table 2). The chromosome number 2n = 6x = 150 in this study is consistent with previous reports (Table 2). However, karyotypes showed a pattern considered basal for the genus, or with small variations due to the pericentric inversions and/or translocations in Anatolian *Capoeta* species (Table 2). The number of biarmed chromosomes is higher than uniarmed chromosomes in the Anatolian *Capoeta* species (Table 2) except *C. trutta* (Kılıç-Demirok and Ünlü, 2001). This feature is detected in this study as well. We conclude that this karyotype structure with mainly biarmed chromosomes is typical for the genus *Capoeta*.

In detail, *C. aydinensis*, *C. bergamae*, *C. erhani* and *C. pestai* show very similar karyotype morphologies with some differences. The number of biarmed chromosomes is as follows 96 in *C. aydinensis*, 92 in *C. erhani*, 86 in *C. bergamae* and, 84 in *C. pestai*. Otherwise, the

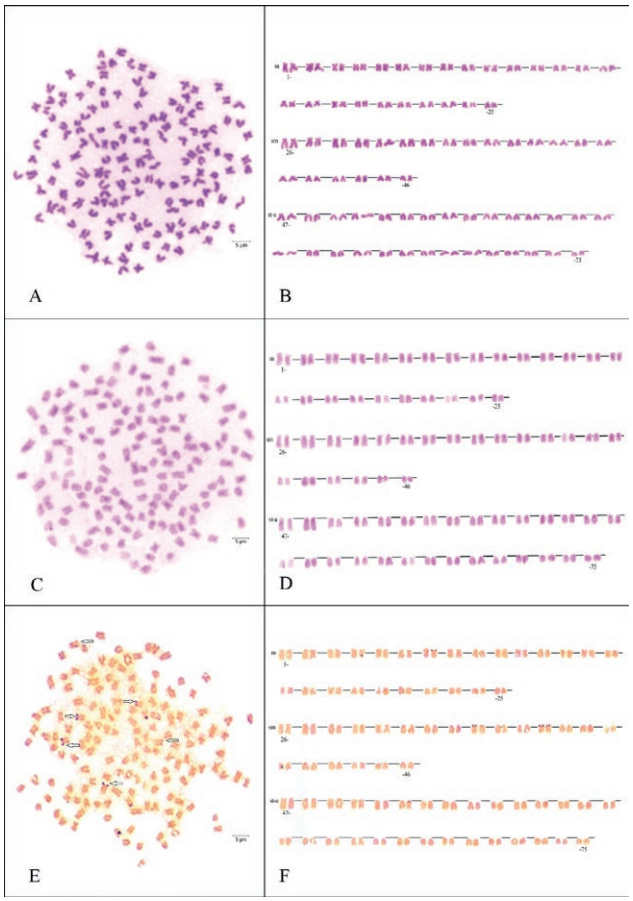

Figure 4. Metaphase plates of *Capoeta erhani* by Giemsa stained (A), C-banded (C) and Ag-stained techniques (E) and arranged karyotypes (B, D, F). Arrows indicate the Ag-NORs bearing chromosomes. Scale bars = 5 μm.

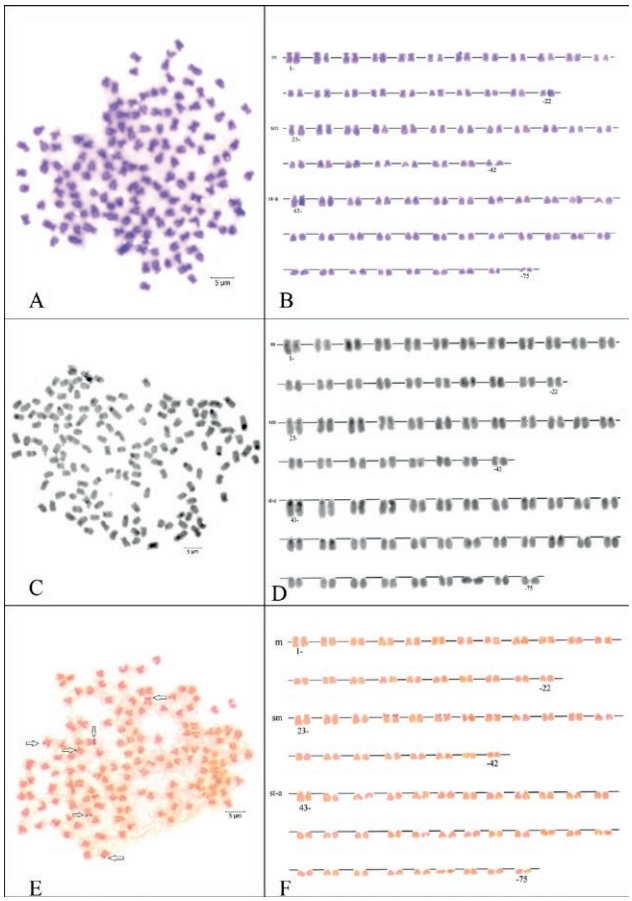

Figure 5. Metaphase plates of *Capoeta pestai* by Giemsa stained (A), C-banded (C) and Ag-stained techniques (E) and arranged karyotypes (B, D, F). Arrows indicate the Ag-NORs bearing chromosomes. Scale bars = 5 μm.

number of uniarmed chromosomes is as follows 54 in *C. aydinensis*, 58 in *C. erhani*, 64 in *C. bergamae* and, 66 in *C. pestai*. The FN ranges from 234 to 246 in this study. Pereira et al. (2012) suggested that distinct FNs with the same chromosome numbers in the species of the genus may be the result of pericentromeric inversions and/or translocations involving centromeres. Karyotypes of four *Capoeta* species in this study showed minor variations in their structures and depending on this having distinct FNs, apparently due to above mentioned chromosomal rearrangements. In addition, karyotypes with higher FNs are regarded to represent a derived condition (Ganai et al., 2011). According to this hypothesis, *C. pestai* should be a more primitive scraper whereas *C. aydinensis* should be the most derived scraper among the four species.

From the other countries *C. capoeta* (Safar, 2000), *C. damascina* (Gorshkova et al., 2002) and *C. sevangi* (Krysanov, 1999) were reported hexaploidy as detected in four studied species. *C. sevangi* differs from *C. aydinensis*, *C. bergamae*, *C. erhani* and *C. pestai* by having 110 uniarmed chromosomes (with FN = 190) (Krysanov, 1999).

Moreover, *C. antalyensis*, *C. baliki* (Karasu-Ayata et al., 2017) and *C. damascina* (Unal and Gaffaroğlu, 2016) showed no sex chromosome differentiation like *C. aydinensis*, *C. bergamae*, *C. erhani* and *C. pestai*.

Cytogenetic studies were mainly limited to detect chromosome number and morphology in the genus *Capoeta* (Table 2). Notably, chromosomal banding data (C-banding and Ag-NORs) revealed in only two *Capoeta* species to date (Gaffaroğlu et al., 2012; Unal and Gaffaroğlu, 2016). C-bands were located mainly on the pericentromeres and terminal regions of some chromosomes in four studied *Capoeta* species. *C. aydinensis* has the least C-bands compared to the other three species. *C. bergamae* and *C. pestai* have more C-banded chro-

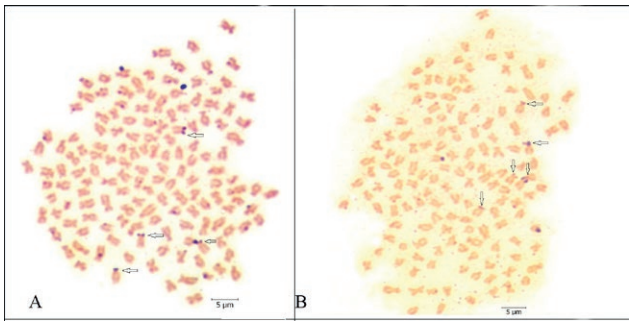

Figure 6. Ag-NOR polymorphisms of *Capoeta bergamae*. Four Ag-NORs (A) and, five Ag-NORs (B). Arrows indicate the Ag-NORs bearing chromosomes. Scale bars = 5 µm.

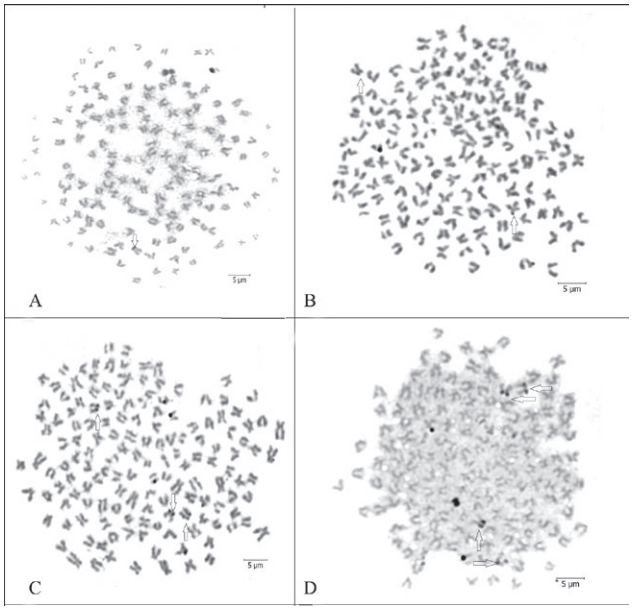

Figure 7. Ag-NOR polymorphisms of *Capoeta erhani*. One Ag-NOR (A), two Ag-NORs (B), three Ag-NORs (C) and, four Ag-NORs (D). Arrows indicate the Ag-NORs bearing chromosomes. Scale bars = 5 µm.

mosomes than *C. aydinensis* and *C. erhani*. Similarly, *C. damascina* (Unal and Gaffaroğlu, 2016) and *C. antalyensis* (Gaffaroğlu et al., 2012) had centromeric C-bands as this study. Heterochromatic blocks that were reported in *C. damascina* (Unal and Gaffaroğlu, 2016) are not observed in this study. Due to the lack of the chromosomal banding data for most of the species of the genus *Capoeta* from different countries, no comparison should be made. However, our results show the basal chromosomal banding information for the genus *Capoeta*.

Ag-NOR numbers have a stable distribution pattern among the four species newly analysed. It is assumed

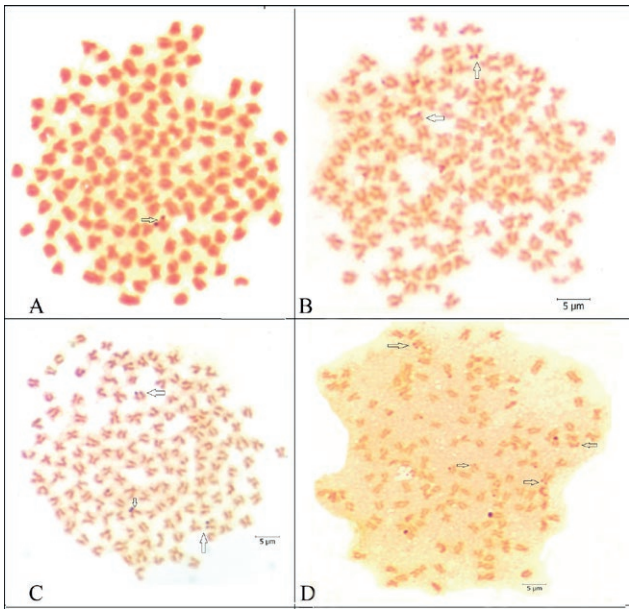

Figure 8. Ag-NOR polymorphisms of *Capoeta pestai*. One Ag-NOR (A), two Ag-NORs (B), three Ag-NORs (C) and, four Ag-NORs (D). Arrows indicate the Ag-NORs bearing chromosomes. Scale bars = 5 µm.

that two Ag-NORs in diploid barbins (Yüksel and Gaffaroğlu, 2006), four Ag-NORs in tetraploid barbins (Karasu-Ayata and Gaffaroğlu, 2019) and six Ag-NORs in hexaploid barbins (Unal and Gaffaroğlu, 2016) are common features. The Ag-NORs observed in the species studied here followed the similar feature observed in the other *Capoeta* species. *C. aydinensis*, *C. bergamae*, *C. erhani* and *C. pestai* are similar to *C. damascina* (Unal and Gaffaroğlu, 2016) and *C. antalyensis* (Gaffaroğlu et al., 2012) in terms of Ag-NOR numbers. Otherwise, *C. bergamae*, *C. erhani* and *C. pestai* are similar to *C. damascina* (Unal and Gaffaroğlu, 2016) in terms of locations of Ag-NORs on the submetacentric chromosomes. *C. antalyensis* (Gaffaroğlu et al., 2012) has Ag-NORs on submeta-subtelocentric chromosomes like *C. aydinensis*. Moreover, Ag-NOR number polymorphism has not been reported in *C. damascina* and *C. antalyensis* (Gaffaroğlu et al., 2012; Unal and Gaffaroğlu, 2016) as observed in *C. bergamae*, *C. erhani* and *C. pestai*. Ribosomal DNA sites are considered as hot spots for chromosomal rearrangements such as duplications, fusions, fissions and inversions. Also, these sites should be correlated with transposable elements or repetitive DNAs (Araya-Jaime et al., 2022). In this context, Ag-NOR number polymorphisms that were detected in the three species in this study should be derived after the above mentioned chromosomal rearrangements.

Table 1. Collection data of the studied species.

Species	Locality	Coordinate
C. aydinensis (2 individuals)	Suçıkan Spring, Dinar, Afyon (Büyük Menderes River)	38°04'N, 30°10'N
C. bergamae (8 individuals)	Dibekdere Stream, Ahmetli, Manisa (Gediz River)	38°33'N, 27°57'E
C. erhani (11 individuals)	Çakıt Stream, Şekerpınarı, Pozantı, Adana (Seyhan River)	37°27'N, 34°52'E
C. pestai (2 individuals)	Kayabaşı Stream, Beyşehir, Konya (South of Beyşehir Lake)	37°29'N, 31°30'E

Table 2. Karyological data for the genus *Capoeta* from Türkiye.

Species	2n	Karyotype formular	FN	References
C. trutta	150	70m-sm+80st-a	220	Kılıç-Demirok and Ünlü, 2001
C. umbla	150	86m-sm+64st-a	236	Kılıç-Demirok and Ünlü, 2001
C. capoeta	150	34m+66sm+12st+38a	250	Kaya, 2003
C. barroisi	150	26m+54sm+26st+38a	230	Kaya, 2003
C. damascina	150	46m+42sm+62st-a	238	Unal and Gaffaroğlu, 2016
C. antalyensis	150	84m-sm+66st-a	234	Karasu-Ayata et al. 2017
C. baliki	150	88m-sm+62st-a	238	Karasu-Ayata et al. 2017
C. aydinensis	150	54m+42sm+54st-a	246	This study
C. bergamae	150	56m+30sm+64st-a	236	This study
C. erhani	150	50m+42sm+58st-a	242	This study
C. pestai	150	44m+40sm+66st-a	234	This study

2n: diploid chromosome number, FN: fundamental number, m: metacentric, sm: submetacentric, st-a: subtelo-acrocentric.

In conclusion, our results provide new data on the cytogenetic features of four *Capoeta* species. The endemic *C. aydinensis*, *C. bergamae*, *C. erhani* and *C. pestai* were analysed for the first time. Karyotype differences that were observed in this study highlight cytogenetics as an important tool for cytotaxonomy. The chromosomal features with classical and molecular cytogenetic techniques of the other *Capoeta* species need to be studied to reveal detailed cytotaxonomy of the genus.

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