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Karyomorphology of Two Cyprinid Barbels (Teleostei: Cyprinidae) from Gediz River, Turkey

Muhammet Gaffaroğlu[a], Muradiye Karasu-Ayata[b, *] (ORCID: 0000-0001-8890-8547), and Sevgi Unal-Karakus[c]

[a] *Department of Molecular Biology and Genetics, Faculty of Science, Kırşehir Ahi Evran University, Kırşehir, Türkiye*

[b] *Faculty of Health Sciences, Kirsehir Ahi Evran University, Kirsehir, Türkiye*

[c] *Department of Molecular Biology and Genetics, Faculty of Science, Bartin University, Bartin, Türkiye*

**e-mail: mkarasu@ahievran.edu.tr*

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Abstract—*Barbus pergamonensis* Karaman, 1971 and *Luciobarbus lydianus* (Boulenger, 1896) were studied karyologically. Karyotypes and chromosomal banding techniques with C-banding and silver staining were determined. Diploid chromosome numbers (2*n*) were invariably 100; karyotypes were composed of 26 metasentric (m), 20 submetacentric (sm) and 54 subtelo-acrocentric (st-a) chromosomes in *B. pergamonensis* and 24 m, 22 sm and 54 st-a chromosomes in *L. lydianus*. No heteromorphic sex chromosomes were determined. C-bands were observed on the pericentromeric regions of some of the chromosomes in the studied species. Multiple nucleolus organizer regions were detected in both species. This study shall contribute to barbels cytotaxonomy.

Keywords: cytogenetics, cytotaxonomy, karyotype, chromosomal bandings, Gediz River

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INTRODUCTION

The genera *Barbus* Daudin, 1805 and Luciobarbus Heckel, 1843 belong to subfamily Barbinae (Çiçek et al., 2020). The members of the genus *Barbus* distribute in Europe, the Caucasus, Turkey, and the Aral and Caspian basin and the members of the genus *Luciobarbus* distribute in north western Africa (Morocco, Algeria), the Iberian and Balkan peninsulas and western Asia (Turan et al., 2008). The genus *Barbus* comprises 12 species in the inland waters of Turkey (Çiçek et al., 2020). From this species, *B. pergamonensis* distributes in the streams and rivers of the Aegean Sea basin (Güçlü et al., 2020). It was concluded that this species was restricted in Gediz, Bakır and Madra Rivers in Turkey (Güçlü et al., 2020). Otherwise, the genus *Luciobarbus* has 14 species in the inland waters of Turkey (Çiçek et al., 2015). Endemic *L. lydianus* distributes in Gediz River that drains to Aegean Sea and Aşağiçavuşlu Stream that drains to Sea of Marmara (Turan et al., 2008).

Advanced techniques have been widely applied in cytogenetic studies of many fish species. This data provides useful determinations into their karyotype differentiations. However, chromosomal analysis of several fish species is still not reported. Difficulty in obtaining good metaphase spreads both in quality and quantity is the reason for this purpose (Sassi et al., 2020).

Polyploid cyprinids are a large group and widely distributed in Eurasia and Africa. The fresh water fishes of the genera *Barbus* and *Luciobarbus* are in tetraploid lineage (2*n* = 100) of Eurasian barbels (Levin et al., 2019). Two Anatolian *Barbus* species have been studied karyologically (Gaffaroğlu et al., 2013; Sahin, 2015) whereas four Anatolian *Luciobarbus* species have been studied karyologically to date (Kılıç-Demirok, 2000; Kaya, 2009; Unal and Gaffaroğlu, 2016; Ayata and Gaffaroğlu, 2019). There is no karyological study in *B. pergamonensis* and *L. lydianus*. Therefore, the aim of this study is to determine karyological properties of two barbels with conventional cytogenetic techniques for the first time.

MATERIALS AND METHODS

Thirteen specimens of *B. pergamonensis* were collected from Tabakdere, Salihli, Manisa, Turkey (38°28′ N, 28°03′ E) and four specimens of *L. lydianus* were collected from Demirköprü Dam Lake, Salihli, Manisa, Turkey (38°40′ N, 28°23′ E). The specimens were carried alive to the laboratory. They were kept in well aerated aquarium until analysis. The chromosomal study was carried out after permission from the Kirsehir Ahi Evran University Local Ethics Committee for Animal Experiments (permit no. 68429034/08). The air-drying technique of Bertollo et al. (2015) was applied from the head kidney for chromosome preparations.

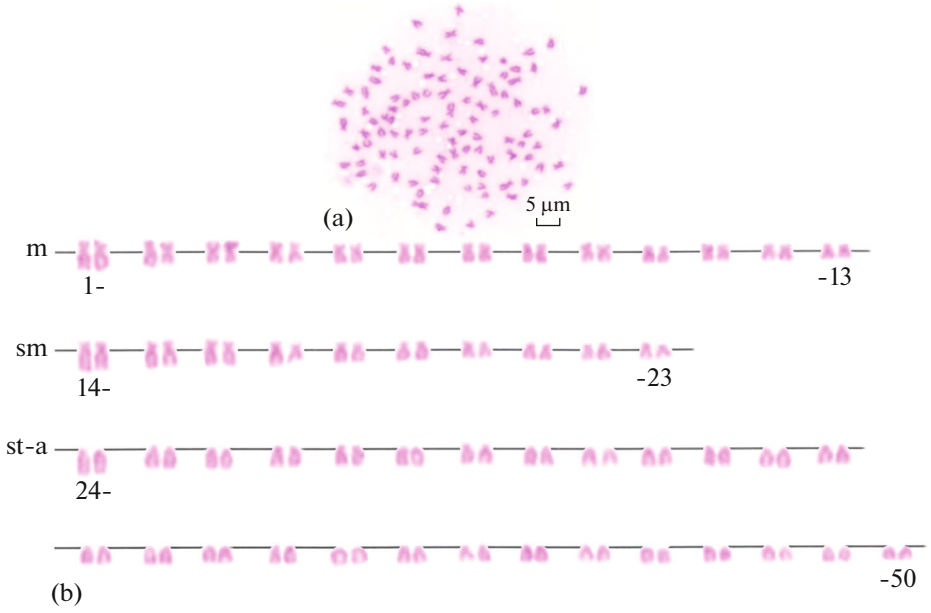

Fig. 1. Giemsa stained metaphase (a) and the corresponding karyotype (b) of *Barbus pergamonensis*. Scale bar = 5 μm.

At least 10 slides were prepared from each specimen. Some of them were stained by 5% Giemsa. After analysis, the specimens were deposited as vouchers in 70% ethanol at the Cytogenetics Laboratory of the Faculty of Arts and Sciences of the Kırşehir Ahi Evran University, Kırşehir, Turkey under the collection numbers MKA 120-137. The C-banding technique of Sumner (1972) was used for determining of constitutive heterochromatin regions whereas the silver-staining technique of Howell and Black (1980) was applied for determining nucleolus organizer regions (NORs). The chromosome slides were scanned under a Leica DM 3000 microscope (Leica Microsystems GmbH, Germany) and metaphases were photographed with AKAS software (Argenit Mikrosistem, Turkey). Chromosomes were measured with digital calliper. Karyotypes were arranged manually. Chromosomes were classified according to Levan et al. (1964). For calculating the NF (fundamental number), m- and sm chromosomes were taken as biarmed whereas st-a chromosomes were taken as uniarmed. Image processings were performed in Adobe Photoshop CS6.

RESULTS

The 2*n* of *B. pergamonensis* and *L. lydianus* was invariably 100 (Figs. 1a, 2a); their karyotypes were composed of 26 m, 20 sm and 54 st-a chromosomes in *B. pergamonensis* (Fig. 1b) and 24 m, 22 sm and 54 st-a chromosomes in *L. lydianus* (Fig. 2b). NF was calculated as 146 in *B. pergamonensis* and *L. lydianus*. The largest chromosome pair is a sm in *B. pergamonensis* whereas a st-a in *L. lydianus*. No heteromorphic sex chromosomes were observed in the karyotypes of the two species. C-positive heterochromatins were observed on the pericentromeric regions of some of the chromosomes in the studied species (Figs. 3a, 3b). The most common Ag-NORs were determined terminally on the short (p) arms of two sm chromosome pairs in *B. pergamonensis* (Fig. 4d) and *L. lydianus* (Fig. 5b). Also, Ag-NOR number variability was observed in *B. pergamonensis* (Table 1, Figs. 4a–4c, 4e, 4f) and in *L. lydianus* (Table 1, Figs. 5a, 5c, 5d).

DISCUSSION

Karyological properties are useful markers in fish cytotaxonomy. Cytogenetic markers in the karyotypes may be used for the identification of the species (Kumar et al., 2019). Polyploid cyprinids have very large genomes and numbers of chromosomes. Within Cyprinidae many species have ploidies of tetraploid, hexaploid and octaploid (Geng et al., 2013). The two species studied here have $2n = 100$, the tetraploid level. Geng et al. (2013) stated out that tetraploid species demonstrate stronger adaptability to the environment. Most *Barbus* and *Luciobarbus* species from Anatolia also have $2n = 100$ as this study (Table 2). Only one species *L. capito* (Kaya, 2009)'s 2*n* is different from Anatolian barbels (Table 2). The species investigated in our study have $2n = 100$, thereby giving additional evidence that this should be the basic diploid number in the genera *Barbus* and *Luciobarbus*. The classification of the karyotypes showed that the karyotypes of *B. pergamonensis* and *L. lydianus* are very similar. These species have 46 biarmed and 54 uniarmed chromosomes. Only one biarmed chromosome pair is different among them. The two species studied in this

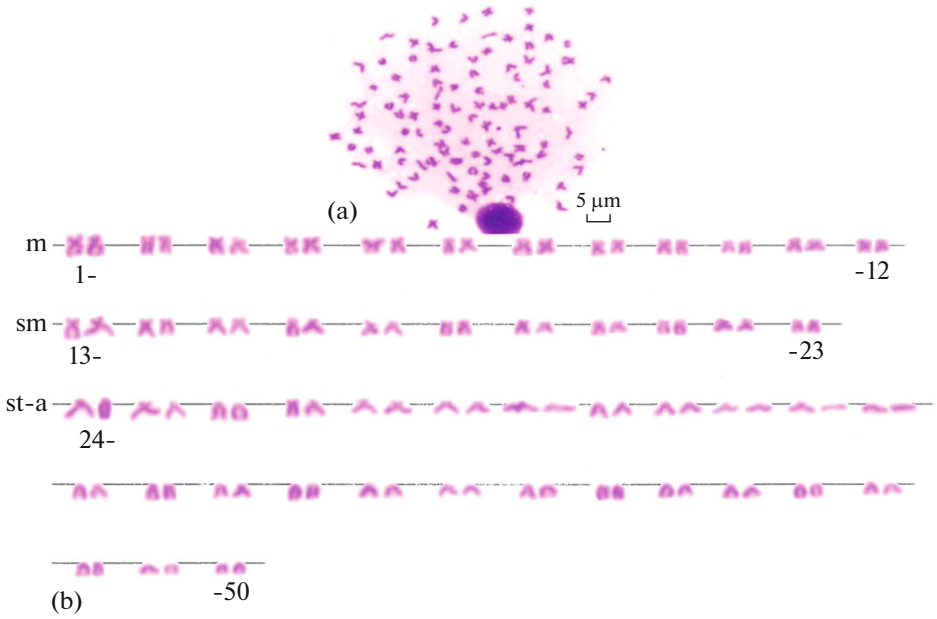

Fig. 2. Giemsa stained metaphase (a) and the corresponding karyotype (b) of *Luciobarbus lydianus.* Scale bar = 5 μm.

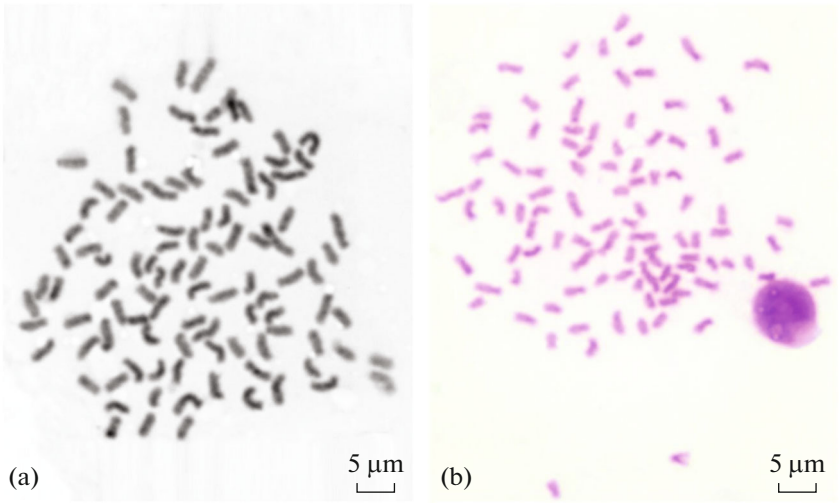

Fig. 3. C-banded metaphases of *Barbus pergamonensis* (a) and *Luciobarbus lydianus* (b). Scale bar = 5 μm.

research have more uniarmed chromosomes than the other Anatolian barbels except *B. tauricus* (Table 2). The karyotype evolution should be concerned pericentric inversions and/or translocations involving centromeres among the members of the genera *Barbus* and *Luciobarbus*. Ganai et al. (2011) reported that karyotypes with more biarmed chromosomes are regarded to represent a derived condition. Also, karyotypes with more uniarmed chromosomes are regarded to represent a primitive condition (Ganai et al., 2011). According to the number of uniarmed chromosomes *L. lydianus* should be considered as a primitive fishes of the genus *Luciobarbus* (Table 2). Other Anatolian *Luciobarbus* species have more derived karyotypes (Table 2). Otherwise, *B. pergamonensis* has more derived karyotype like *B. escherichii* (Gaffaroğlu et al., 2013) whereas *B. tauricus* (Şahin, 2015) should be considered as a primitive fish of the genus *Barbus* (Table 2). To compare with the previous studies, the NF of *B. pergamonensis* and *L. lydianus* is different from other Anatolian barbels (Table 2). The NF of *B. pergamonensis* is higher than *B. tauricus* (Şahin, 2015) whereas lower than *B. escherichii* (Gaffaroğlu et al., 2013). Moreover, the NF of *L. lydianus* is lower than all Anatolian *Luciobarbus* species (Table 2).

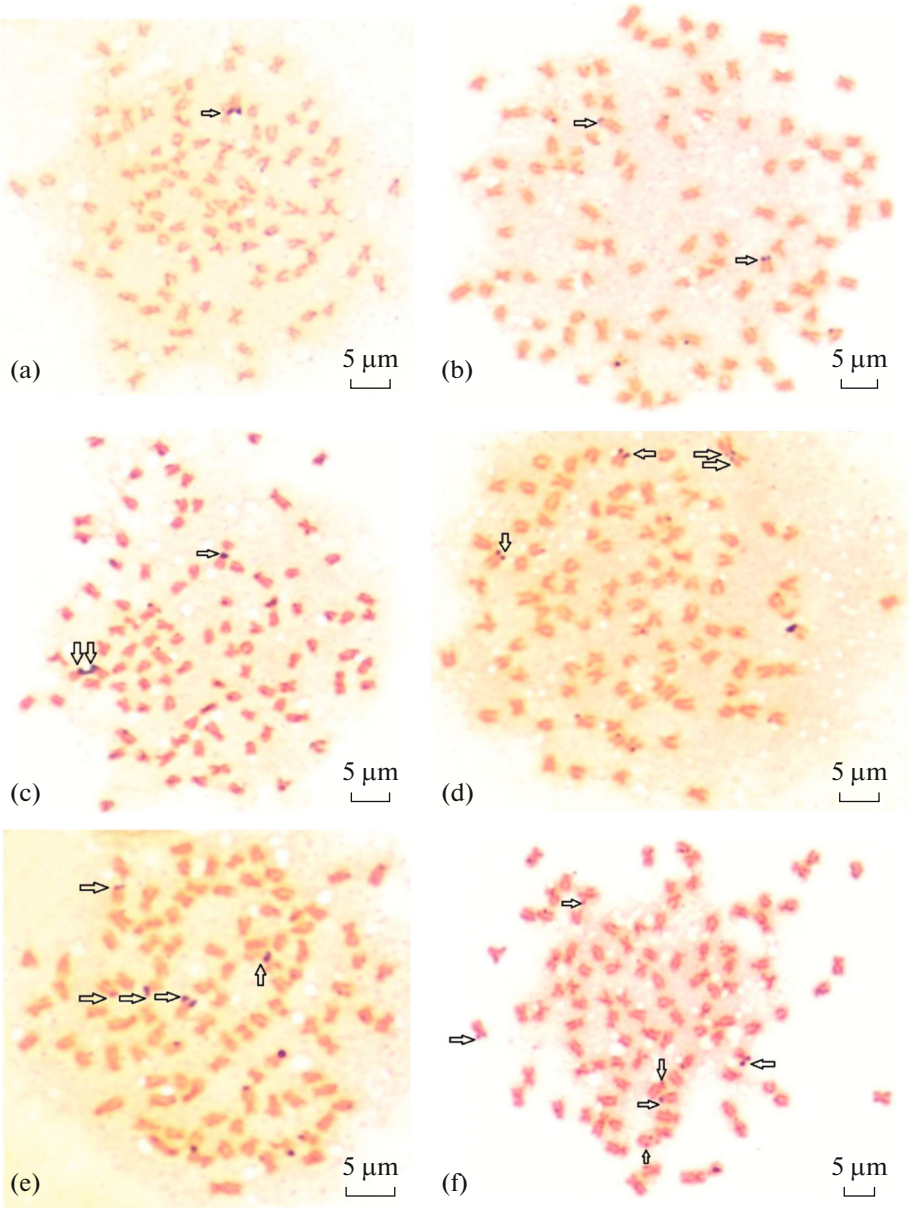

Fig. 4. Ag-stained metaphases of *Barbus pergamonensis* with one (a), two (b), three (c), four (d), five (e) and six Ag-NORs (f). Arrows indicate the Ag-NORs. Scale bar = 5 μm.

The other Barbus species from Europe *B. meridionalis* (Ráb et al., 1993, locality France) and *B. cyclolepis* (Ráb et al., 1996, locality Greece) which also has $2n = 100$ like *B. pergamonensis*. Compared with the above mentioned species chromosome morphologies have some differences. The number of metacentric chromosomes are the same in the three species, however the number of submetacentric chromosomes and the number of uniarmed chromosomes are different among them. The karyotype of *B. pergamonensis* which also distributes in Greece is more similar to karyotype of *B. cyclolepis* (Ráb et al., 1996). The number of biarmed chromosomes and uniarmed are in order: 46 and 54 in *B. pergamonensis* whereas 42 and 58 in *B. cyclolepis* (Ráb et al., 1996). Otherwise, *B. barbus* has the tetraploid level ($2n = 96$) (Luca et al., 2010) like *B. pergamonensis* and also their NF's are the same. However, some differences on the chromosome morphologies are available among them. The number of biarmed chromosomes of *B. barbus* (Luca et al., 2010) is higher than *B. pergamonensis*.

Otherwise, the $2n$ of other *Luciobarbus* species from different countries, respectively *L. mursa* (Vasilyan et al., 2009), *L. capito* (Geng et al., 2013) and five Iberian *Luciobarbus* species (Collares-Pereira and Madeira, 1990) are the same as *L. lydianus*. $2n$ of

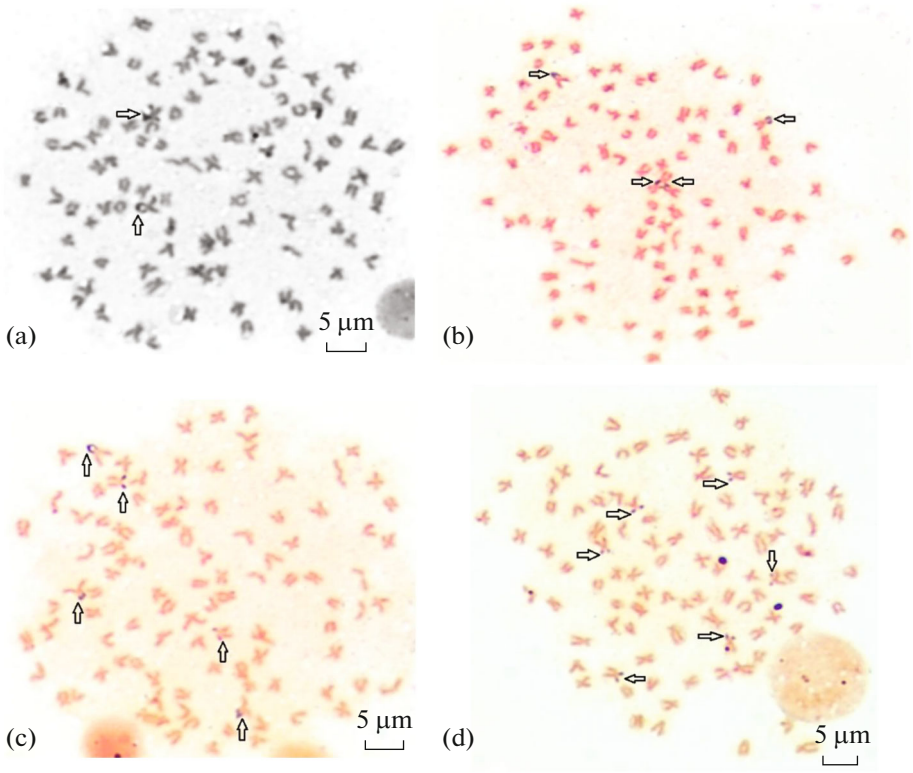

Fig. 5. Ag-stained metaphases of *Luciobarbus lydianus* with two (a), four (b), five (c) and six Ag-NORs (d). Arrows indicate the Ag-NORs. Scale bar = 5 μm.

L. capito was reported as 120 by Kaya (2009). In this sense, the karyotype of Turkey population of *L. capito* should be studied again. Some differences about the chromosome morphologies of *L. mursa* (Vasilyan et al., 2009), *L. capito* (Geng et al., 2013) and five Iberian *Luciobarbus* species (Collares-Pereira and Madeira, 1990) are available contrary to *L. lydianus.* The number of uniarmed chromosomes of *L. mursa* (Vasilyan et al., 2009) is higher than *L. lydianus.* The number of uniarmed chromosomes of *L. capito* (Geng et al., 2013) is lower than *L. lydianus.* According to this, the NF of *L. lydianus* is higher than *L. mursa* (Vasilyan et al., 2009) whereas is lower than *L. capito* (Geng et al., 2013). The number of uniarmed chromosomes of five Iberian *Luciobarbus* species ranges between 28 to 48 (Collares-Pereira and Madeira, 1990) whereas 54 in *L. lydianus.* According to this, the NF's of these species (Collares-Pereira and Madeira, 1990) are higher than this study.

The heteromorphic sex chromosomes were not observed in the studied two species. This phenomenon is same as all Anatolian barbels (Kılıç-Demirok, 2000; Kaya, 2009; Gaffaroğlu et al., 2013; Şahin, 2015; Unal and Gaffaroğlu, 2016; Ayata and Gaffaroğlu, 2019). These chromosomes were not reported in other barbels from different countries (Collares-Pereira and Madeira, 1990; Ráb et al., 1993; Ráb et al., 1996; Vasi-

Table 1. Ag-NOR number variations in *Barbus pergamonensis* and *Luciobarbus lydianus*

Number of Ag-NOR sites	Ag-stained metaphase plate, % of *B. pergamonensis*	Ag-stained metaphase plate, % of *L. lydianus*
1	9.09	–
2	19.69	1.56
3	7.57	–
4	59.11	53.13
5	3.03	20.31
6	1.51	25

Table 2. Karyological data for the genera *Barbus* and *Lucibarbus* from Turkey

Species	2*n*	Chromosome morphology	NF	References
B. escherichii	100	14 m + 44 sm + 42 st-a	158	Gaffaroğlu et al. (2013)
B. tauricus	100	6 m + 24 sm + 38 st + 32a	130	Şahin (2015)
B. pergamonensis	100	26 m + 20 sm + 54 st-a	146	This study
L. mystaceus	100	22 m + 30 sm + 48 st-a	152	Kılıç-Demirok (2000)
L. capito	120	32 m + 42 sm + 8 st + 38a	194	Kaya (2009)
L. pectoralis	100	20 m + 42 sm + 38 st-a	162	Unal and Gaffaroğlu (2016)
L. kottelati	100	18 m + 34 sm + 48 st-a	152	Ayata and Gaffaroğlu (2019)
L. lydianus	100	24 m + 22 sm + 54 st-a	146	This study

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

lyan et al., 2009; Luca et al., 2010; Geng et al., 2013) as this study.

The amount of C-positive heterochromatins as revealed by C-banding in *B. pergamonensis* and *L. lydianus* were low as usual in tetraploid barbels, also reported in *B. meridionalis* (Ráb et al., 1993) and *B. cyclolepis* (Ráb et al., 1996). Only, three barbel species—*B. escherichii* (Gaffaroğlu et al., 2013), *L. pectoralis* (Unal and Gaffaroğlu, 2016) and *L. kottelati* (Ayata and Gaffaroğlu, 2019)—from Anatolia have been studied in terms of C-banding. The location of C-positive heterochromatins in these species are similar to *B. pergamonensis* and *L. lydianus*. However, heterochromatic blocks that observed in *L. pectoralis* (Unal and Gaffaroğlu, 2016) were not observed in this study.

The location and numbers of the Ag-NORs are valuable cytogenetic markers in fish cytotaxonomy (Kumar et al., 2019). The most common Ag-NOR number were four in sm chromosomes of *B. pergamonensis* and *L. lydianus* as reported in *B. escherichii* (Gaffaroğlu et al., 2013) and *L. kottelati* (Ayata and Gaffaroğlu, 2019). However, with two Ag-NORs in sm chromosomes of *L. pectoralis* (Unal and Gaffaroğlu, 2016) is different from *B. pergamonensis* and *L. lydianus*. Ag-NOR variability that is observed in this study was reported only in *L. kottelati* (Ayata and Gaffaroğlu, 2019) from Anatolian barbels. The highest Ag-NOR number in *L. kottelati* was six (Ayata and Gaffaroğlu, 2019) as in *B. pergamonensis* and *L. lydianus*. The number of Ag-NORs of *B. cyclolepis* (Ráb et al., 1996) is similar to *B. pergamonensis*. However, the locations of Ag-NORs on st chromosomes of *B. cyclolepis* (Ráb et al., 1996) is different from *B. pergamonensis*. Otherwise, Ráb et al. (1993) reported multiple Ag-NORs (four to six) in *B. meridionalis*. This Ag-NOR variability was observed in this study too. The observed Ag-NOR variability should be related to transcriptionally inactive NORs (Kumar et al., 2019).

In conclusion, Geng et al. (2013) reported that karyotypes only provide a basis for fish cytotaxonomy. Fish karyotypes show conservative convergence and polymorphism in their karyotype evolutions. Karyotypes should not use as only index of cytotaxonomy, other molecular markers should be studied to a comprehensive determination (Geng et al., 2013).

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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