



## Chromosomal Studies of Two Different Populations (Turkey) of *Luciobarbus escherichii* (Steindachner, 1897)

Muhammet Gaffaroğlu<sup>1,\*</sup>, Muradiye Karasu Ayata<sup>1</sup>, Sevgi Ünal<sup>2</sup>, Atilla Arslan<sup>3</sup>

<sup>1</sup>Ahi Evran University, Science and Art Faculty, Department of Biology, Kırşehir, Turkey.

<sup>2</sup>Gazi University, Science Faculty, Department of Biology, Ankara, Turkey.

<sup>3</sup>Selçuk University, Science Faculty, Department of Biology, Konya, Turkey.

\* Corresponding Author: Tel.: +90 3862804544; Fax: +90 3862804525;  
E-mail: mgaffaroglu@yahoo.com

Received 15 July 2013  
Accepted 15 December 2013

### Abstract

This study was carried out on the chromosomal features of two populations (Kızılırmak and Sakarya Rivers) belonging to *Luciobarbus escherichii* (Steindachner, 1897). Metaphase chromosomes were obtained from kidney cells. The diploid chromosome number was  $2n=100$ , consisting of 7 pairs of metacentric, 22 pairs of submetacentric and 21 pairs of subtelocentric chromosomes. The arm number (NF) was 158. Constitutive heterochromatin regions with C-banding were determined on the centromeres of chromosomes. NOR was observed on the short arms of 2 pairs of submetacentric chromosomes. This study is a contribution to cytogenetics of Anatolian cyprinids.

**Keywords:** Cyprinidae, karyotype, C-banding, NOR.

### *Luciobarbus escherichii* (Steindachner, 1897)'nin İki Farklı Populasyonunda (Türkiye) Kromozomal Araştırmalar

#### Özet

*Luciobarbus escherichii* (Steindachner, 1897)'nin iki farklı populasyonunda (Kızılırmak ve Sakarya Nehri) kromozomal araştırmalar yapıldı. Metafaz kromozomları böbrek hücrelerinden elde edildi. *L. escherichii*'nin diploit kromozom sayısı  $2n=100$ ; kromozom morfolojisi ise 7 çift metasentrik, 22 çift submetasentrik ve 21 çift subtelo-akrosentrik olarak belirlendi. Kol sayısı (NF) 158 olarak hesaplandı. C-bantlama ile kromozomların sentromerinde konstitüif heterokromatin bölge gözlemlendi. Ayrıca 2 çift submetasentrik kromozomun kısa kollarının ucunda NOR tespit edildi. Bu çalışmanın Anadolu cyprinidlerinin sitogenetiğine katkı sağlayacağı düşünülmektedir.

**Anahtar Kelimeler:** Cyprinidae, karyotip, C-bantlama, NOR.

#### Introduction

It was reported that there were 1226 species belonging to subfamily Barbinae (Fam: Cyprinidae) (Eschmeyer and Fong, 2013). The genus *Luciobarbus*, which belongs to this subfamily, has 12 species (*Luciobarbus kersin*, *L. mursa*, *L. brachycephalus*, *L. esocinus*, *L. xanthopterus*, *L. caspius*, *L. mystaceus*, *L. capito*, *L. lydianus*, *L. kottelati*, *L. escherichii*, *L. pectoralis*) in Anatolia. Four of these species are endemic. Among aforementioned species, *Luciobarbus escherichii* (Steindachner, 1897) is reported to exist in Sakarya River and its tributaries, Porsuk Creek and Dam, İznik Lake, small creeks and streams in the Black Sea, Çoruh River Basin, streams of Afyon Province (Aksu Creek, Karadirek Stream) and streams of Muğla Province (Yuvarlakçay,

Dalaman Creek, Eşen Creek, Tersakan and Namnam Creeks) (Güçlü *et al.*, 2011).

Fish chromosome studies have been carried out for many years. It was noted that from the subfamily Barbinae, diploid chromosome numbers of 154 species was determined. It was also defined that these species have diploid, tetraploid and, hexaploid forms and the number of chromosomes varies between 48-150 (Arai, 2011).

Although chromosomal studies have been done in the species of *Luciobarbus* which exist in Anatolia (Kılıç-Demirok, 2000; Kaya, 2009), there is no study of *L. escherichii*. The purpose of this study is to reveal the chromosomal features (with Giemsa, Ag-NOR staining and C-banding) of two populations belonging to *L. escherichii*.

## Materials and Methods

Eight (4 male, 4 female) samples of *Luciobarbus escherichii* were collected from Kızılırmak (Kırşehir, Kesikköprü, Turkey) (38°57'N, 34°11'E), whereas 8 (5 male, 3 female) samples were collected from Sakarya River (Eskişehir, Eminekin, Turkey) (39°22'N, 31°06'E) (Figure 1). Metaphase preparations were prepared according to Collares-Pereira (1992). Technique of Sumner (1972) was used for C-banding of sample preparations, whereas technique of Howell and Black (1980) was used for silver staining. At least 10 metaphases were examined from each sample. Chromosomes were classified according to Levan *et al.* (1964).

## Results

The diploid chromosome number of *Luciobarbus escherichii* was determined as  $2n=100$  for both populations (Figure 2 and 3). It was found out that the karyotype prepared from the samples of Sakarya River consists of 7 pairs of metacentric (M), 22 pairs of submetacentric (SM) and 21 pairs of subtelocentric (ST-A) chromosomes (Figure 4). NF was 158. The differentiations of sex chromosomes were not observed.

NOR was observed on the short arms of 2 pairs of submetacentric chromosomes in the Sakarya River population (Figure 4).

By using C-banding, constitutive heterochromatin regions were observed on the centromeres of several chromosomes in both populations (Figure 5 and 6).

## Discussion

As a result of this study, no difference in the diploid chromosome numbers of the two populations

of *Luciobarbus escherichii* is detected.

It was reported that polyploidy plays an important role in the evolution of fish (Comber and Smith, 2004). According to Rab and Collares-Pereira (1995), polyploidy in cyprinids is a complicated event that occurs from various origins. Chromosomal studies have been realized in some species of the subfamily Barbinae from Anatolia and it has been observed that the majority of them are polyploid (Table 1). Some of these species are in tetraploid forms (*Luciobarbus mystaceus*) whereas some of them are in hexaploids (*Carasobarbus luteus*, *Kosswigobarbus kosswigi*). It is thought that *L. escherichii* belongs to tetraploid forms.

While *L. escherichii* and *L. capito* differ from each other regarding their chromosome number, *L. escherichii* and *L. mystaceus*'s chromosome numbers resemble (Kaya, 2009; Kılıç-Demirok, 2000). However, their chromosome morphologies are different from each other. *L. escherichii*'s metacentric and subtelocentric chromosome number is less than *L. mystaceus* whereas its submetacentric chromosome number is more.

*L. escherichii* has the same diploid chromosome number with other *Luciobarbus* species (*L. bocagei*, *L. brachycephalus*, *L. comizo*, *L. microcephalus*, *L. sclateri*, *L. steindachneri*) that previously studied (Arai, 2011). Chromosome morphologies of these species were reported as: for *L. bocagei* 64 M/SM and 36 A; for *L. brachycephalus* 24 M, 46 SM/ST and 30 A; for *L. comizo* 12 M, 60 SM, 28 ST/A; for *L. microcephalus* 18 M, 50 SM, 32 ST/A; for *L. sclateri* 10 M, 44 SM, 46 ST/A and for *L. steindachneri* 10 M, 48 SM, 42 ST/A (Arai, 2011). *L. escherichii* has different chromosome morphology (14 M, 44 SM and 42 ST/A) from these species.

On the other hand *L. escherichii* has the same diploid chromosome number with other species which are from the same subfamily. These species are; *Barbus capito* and *B. mursa* (Darestani *et al.*, 2006);



Figure 1. Collecting localities of *Luciobarbus escherichii* samples.

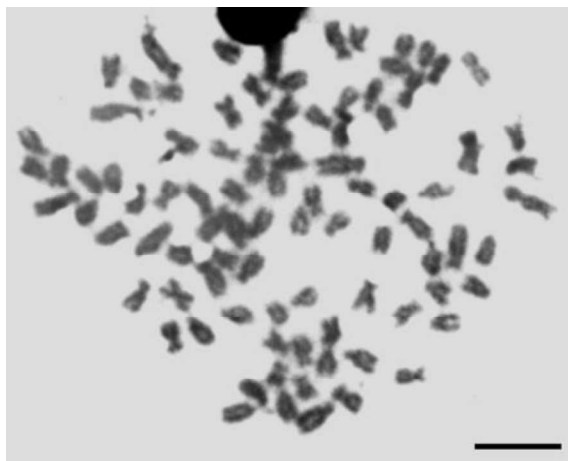


Figure 2. Standard giemsa stained metaphase of Kızılırmak population of *Luciobarbus escherichii*. Scale bar: 5µm.



Figure 3. Standard giemsa stained metaphase of Sakarya River population of *Luciobarbus escherichii*.

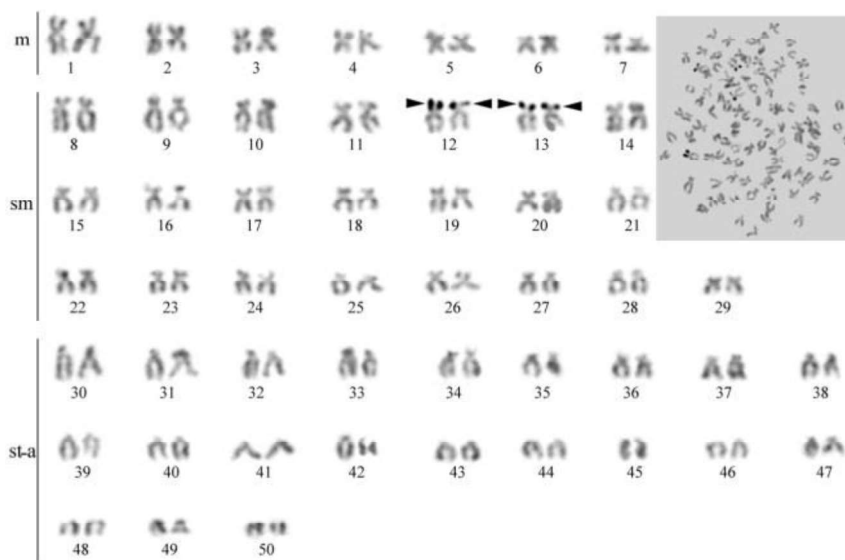
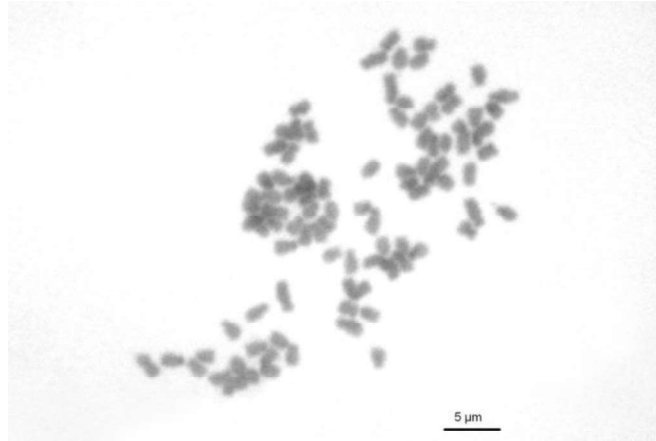
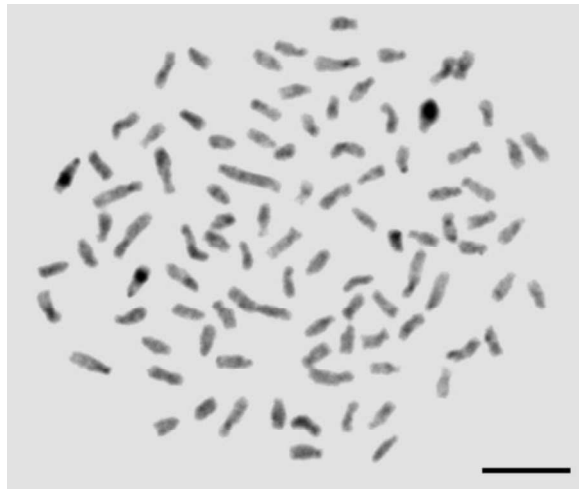


Figure 4. Silver-stained metaphase spread and karyotype of *Luciobarbus escherichii*. Arrows indicate the position of active Ag-NORs.



**Figure 5.** C-banded metaphase of Kızılırmak population of *Luciobarbus escherichii*. Scale bar: 5μm.



**Figure 6.** C-banded metaphase of Sakarya River population of *Luciobarbus escherichii*. Scale bar: 5μm.

*B. barbuis* and *B. peloponnensius* (Fister *et al.*, 1999) and *Pseudobarbus afer*, *P. asper*, *P. burchelli*, *P. burgi*, *P. phlegethon* and *P. tenuis* from the genus *Pseudobarbus* (Naran *et al.*, 2006). It was reported that karyotype of *B. barbuis* consists of 30 M, 18 SM and 52 A chromosomes and *B. peloponnensius* consists of 10 M, 44 SM-Subacrocentric and 46 A chromosomes (Fister *et al.*, 1999). Karyotype of *L. escherichii* is different from these species. While *L. escherichii*'s chromosome morphology is similar to *P. asper* which consist of 14 M, 46 SM, 32 ST and 8 A chromosomes, but it differs from the other *Pseudobarbus* species (Naran *et al.*, 2006).

Fishes usually breed bisexual. Though, sex chromosome systems determined on karyotypes are known only in a restricted group of species (Arai, 2011). The differentiation of sex chromosomes was not observed in *L. escherichii* as it was reported in many cyprinid species too (Gaffaroğlu *et al.*, 2006).

Regarding the C-band blocks, there is no differentiation on the samples of *L. escherichii* living in Kızılırmak and Sakarya Rivers. Such C-band blocks were reported for some previously studied

Anatolian Cyprinids too (Gaffaroğlu and Yüksel, 2009).

It was reported that *Barbus meridionalis* has a small amount of C-positive heterochromatin (Rab *et al.*, 1993) while *B. cyclolepis* has less C-positive heterochromatin than the other tetraploid barbels (Rab *et al.*, 1996). *L. escherichii* is different from these species with respect to inclusion of C-band in majority of chromosomes.

With regard to number and location of NOR, no discrepancy has been observed between Kızılırmak and Sakarya River populations of *L. escherichii*.

*L. escherichii* and *B. cyclolepis* (2n=100) were similar in terms of the number of NOR but they have different localization of NOR (Rab *et al.*, 1996). As *B. meridionalis* (2n=100) has 4-6 NOR regions (Rab *et al.*, 1993), there is no polymorphism about the number of NOR in *L. escherichii*.

Diploid *Barbus bigornei*, *B. ablabes* and *B. macrops* (2n=48-50) have NOR in one pair of chromosome (Rab *et al.*, 1995) whereas tetraploid *L. escherichii* has NOR in two pairs of chromosomes.

Rab (1981) indicated that polyploidization is

**Table 1.** Chromosome studies in the subfamily Barbinae from Turkey

Species	Diploid chromosome number (2n)	Chromosome morphology	NF	References
<i>Barbus plebejus lacerta</i>	48	32M+16A	-	Ergene et al., 1993
<i>Luciobarbus mystaceus</i> (Reported in karyotype paper as <i>Barbus rajanorum mystaceus</i> )	100	22M+30SM+48ST	152	Kılıç-Demirok, 2000
<i>L. capito</i> (Reported in karyotype paper as <i>Barbus capito</i> )	120	32M+42SM+8ST+38A	194	Kaya, 2009
<i>Carasobarbus luteus</i>	150	34M+54SM+14ST+48A	238	Kaya, 2009
<i>C. luteus</i>	150	84M-SM+66ST-A	234	Değer et al., 2011a
<i>Kosswigobarbus kosswigi</i>	148	86M-SM+62ST-A	234	Değer et al., 2011b
<i>Luciobarbus escherichii</i>	100	14M+44SM+42ST-A	158	In this study

associated with the evolution of the subfamily Barbinae. Hence, further cytogenetic investigations should be made to fully understand the *Luciobarbus* species which are living in Anatolia.

## References

- Arai, R. 2011. Fish karyotypes A Check List. Springer, Japan, 340 pp.
- Collares-Pereira, M.J. 1992. In vivo direct chromosome preparation (Air Drying Technique), 1<sup>st</sup> Int. Workshop on fish cytogenetic techniques, Concarneau-France, 14-24 September pp: 15-9.
- Comber, S.C.L., Smith, C. 2004. Polyploidy in fishes: patterns and processes. Biological Journal of the Linnean Society, 82: 431-442.
- Darestani, S.P., Lakeh, A.A.B., Kiabi, B.H.A. 2006. Karyological study of *Barbus capito*, *Barbus mursa* and two populations of *Capoeta capoeta* from Northern Iran. Iranian Journal of Natural Resources, 58(4): 831-842.
- Değer, D., Ünlü, E., Gaffaroğlu, M. 2011a. Dicle Nehri'nde Yaşayan *C. luteus* (Heckel, 1843) Türünün Karyolojik Özellikleri. X. Ekoloji ve Çevre Kongresi, 4-7 Ekim, Çanakkale.
- Değer, D., Ünlü, E., Gaffaroğlu, M. 2011b. *Kosswigobarbus kosswigi* (Ladiges, 1960) türünün karyotip analizi. FABA, 7-9 Eylül, Samsun.
- Eschmeyer, W.N., Fong, J.D. 2013. Catalog of Fishes Online. <http://research.calacademy.org/redirect?url=http://research.archive.calacademy.org/research/Ichthyology/catalog/SpeciesByFamily.asp>. (Accessed February 22, 2013).
- Ergene, S., Kuru, M., Çavaş, T. 1993. Karyological analysis of *Barbus plebejus lacerta* (Heckel, 1843). II. Uluslararası Kızılırmak Fen Bilimleri Kongresi, 426-433s.
- Fister, S. Cakic, P, and Kataranovski, D. 1999. Karyotype analysis of *Barbus barbus* L. and *Barbus peloponnensius* V. (Cyprinidae) and frequencies of breaks and gap-type structural chromosome changes in fishes from the river Vapa. Acta Veterinaria-Beograd. 49(5-6): 385-392.
- Gaffaroğlu, M., Yüksel, E. 2009. Constitutive heterochromatin in *Acanthobrama marmid* and *Cyprinion macrostomus* (Osteichthyes, Cyprinidae). Kafkas University Veterinarian Faculty, 15 (2): 169-72.
- Gaffaroğlu, M., Yüksel, E. and Rab, P. 2006. Note on the karyotype and NOR phenotype of leuciscine fish *Acanthobrama marmid* (Osteichthyes, Cyprinidae). Biologia, 61 (2): 207-209. doi:10.2478/s11756-006-0031-y.
- Güçlü, S. S., Ertan, Ö. O., Küçük, F. 2011. Anadolu'daki *Luciobarbus* (Pisces: Cyprinidae) Türlerinin Taksonomik ve Zoocoğrafik Özellikleri. II. Türkiye Sulak Alanlar Kongresi, Kırşehir, 44. Sayfa.
- Howell, W. M., Black, D. A. 1980. Controlled Silver Staining of Nucleolus Organizer Regions with a Protective Colloidal Developer: a 1 Step Method. Experientia, 36: 1014-5.
- Kaya, F. 2009. Göksu Nehri'nde yaşayan bazı ekonomik balıkların karyolojilerinin incelenmesi. PhD Thesis.
- Kılıç-Demirok, N. 2000. Dicle Su Sisteminde yaşayan bazı cyprinid tür ve alttürlerinin kromozomları üzerine çalışmalar. PhD Thesis.
- Levan, A., Fredga, K., Sandberg, A.A. 1964. Nomenclature For Centromeric Position On Chromosomes. Hereditas, 52: 201-20.
- Naran, D., Skelton, P. H., Villet, M. H. 2006. Karyology of the redfin minnows, genus *Pseudobarbus* Smith, 1841 (Teleostei: Cyprinidae): one of the evolutionarily tetraploid lineages of South African barbines. African Zoology, 41(2): 178-182.
- Rab, P. 1981. Karyotypes of two African Barbels *Barbus barilooides* and *Barbus holotaenia*. Folia Zoologica, 30 (2): 181-190.
- Rab, P., Collares-Pereira M. J. 1995. Chromosomes of European Cyprinid fishes (Cyprinidae, Cypriniformes): A review. Folia Zoologica, 44 (3): 193-214.
- Rab, P., Karakousis, Y., Rabova, M. 1996. Karyotype, NOR phenotype and C-banding study of *Barbus cyclolepis* from Greece. Folia Zoologica, 45 (1): 77-83.
- Rab, P., Machordom, A., Perdices, A., Guegan, J. F. 1995. Karyotypes of three <<small></small> *Barbus* species (Cyprinidae) from Republic of Guinea (Western Africa) with a review on karyology of African small *Barbus*. Caryologia, 48(3-4): 299-307.
- Rab, P., Ozouf-Costaz, C., Berrebi, P. 1993. Karyotypes, distribution of centromeric heterochromatin and polymorphism of NORs in *Barbus meridionalis* from Southern France and Eastern Slovakia: preliminary results. Chaiers d' Ethologie, 13 (2): 195-198.
- Sumner, A.T. 1972. A simple Technique for Demonstrating Centromeric Heterochromatin. Experimental Cell Research, 75: 304-6.