Cytologia **84**(4): 331–334

Chromosomal Studies of *Luciobarbus kottelati* (Teleostei, Cyprinidae)

Muradiye Karasu Ayata[1]* and Muhammet Gaffaroğlu[2]

[1] Faculty of Health Sciences, Kirsehir Ahi Evran University, Kirsehir, Turkey
[2] Department of Molecular Biology and Genetics, Faculty of Science, Kirsehir Ahi Evran University, Kirsehir, Turkey

Received October 4, 2018; accepted July 17, 2019

Summary The chromosomal data (chromosome number and morphology, Ag-NOR and C-banding patterns) of endemic *Luciobarbus kottelati* was presented from Turkey. The diploid chromosome number was found to be $2n=100$. The karyotype consisted of nine pairs of metacentric, 17 pairs of submetacentric and 24 pairs of subtelo-acrocentric chromosomes (Fundamental number, Fn=152). Evident C-bands were on the pericentromeric regions of three chromosome pairs. The number of Ag-NOR bearing chromosomes varied from two to six. This study is a contribution to barbels chromosomal knowledge.

Key words Chromosome, Karyotype, Ag-NOR, C-band, Chromosome banding, *Luciobarbus kottelati*.

Cyprininae "the largest subfamily of the family Cyprinidae" contains more polyploid species than any other fish groups. Most polyploids in this subfamily are either tetraploids ($2n=100$) or hexaploids ($2n=150$) (Yang *et al.* 2015). The genus *Luciobarbus* belongs to this subfamily. The species of this genus are distributed in northwestern Africa (Morocco, Algeria), the Iberian and Balkan peninsulas and western Asia (Turan *et al.* 2008). There are 14 species of this genus in the inland waters of Turkey (Kuru *et al.* 2014). From these species, *L. kottelati* has been described from the Büyük Menderes River in recent years.

Chromosomal studies have been conducted in only four species of the genus *Luciobarbus* from Turkey (Kılıç-Demirok 2000, Kaya 2009, Gaffaroğlu *et al.* 2013, Unal and Gaffaroğlu 2016). However, there is no chromosomal study about *L. kottelati*. The aim of this study is to reveal chromosomal properties of endemic *L. kottelati* with conventional cytogenetic techniques (Giemsa staining, C-banding, and Ag-staining) for the first time.

Materials and methods

Fish sampling and chromosome preparation

One male and one female specimen of *L. kottelati* were collected from Küfi Creek, Çivril, Denizli, Turkey (38°21′N, 29°50′E). These specimens were carried alive to the laboratory and maintained in aquaria until processing. Species identification followed Geldiay and Balık (2007) and Turan *et al.* (2008). This study was carried out after permission from the Ahi Evran University Local Ethics Committee for Animal Experiments (permit number: 68429034/19). The air-drying technique of Collares-Pereira (1992) was performed on the head kidney for chromosome analyses.

Chromosome staining

At least ten slides were prepared from each specimen. Some slides were stained by 5% Giemsa. The C-banding technique of Sumner (1972) was used for determining constitutive heterochromatin regions and Ag-staining of Howell and Black (1980) was used for determining active NORs. After analysis, the specimens were deposited in 70% ethanol at the Cytogenetics Laboratory of the Department of Molecular Biology and Genetics, Faculty of Arts and Sciences of the Kırşehir Ahi Evran University, Kirşehir, Turkey (MKA 94-95).

Microscopy, image processing and karyotyping

The preparations were scanned with a Leica DM 3000 microscope (Leica Microsystems GmbH, Germany) and photographs of metaphases were taken with AKAS software (Argenit Microsystem, Turkey). Chromosomes were measured with digital caliper and karyotype was arranged manually. Chromosomes were classified according to the criterion of the centromeric index introduced by Levan *et al.* (1964). For calculating the Fn, meta-submetacentric chromosomes were taken as biarmed whereas subtelo-acroentric chromosomes were taken as uniarmed. Figure mounting and image brightness and contrast adjustments were performed in Adobe Photoshop CS6.

Results and discussion

The diploid chromosome number of *L. kottelati* was

* Corresponding author, e-mail: mkarasu@ahievran.edu.tr
DOI: 10.1508/cytologia.84.331

2*n*=100 in 84% out of 100 analyzed metaphase plates (Fig. 1A). The karyotype consisted of nine pairs of metacentric, 17 pairs of submetacentric and 24 pairs of subtelo-acrocentric chromosomes (Fig. 1B). The Fn was calculated as 152. The diploid chromosome number (2*n*=100) of *L. kottelati* is in concordance with previous karyological studies in the Anatolian representatives of the genus *Luciobarbus* (except *L. capito*) (Table 1). However, the karyotype of *L. kottelati* shows some differences with these species. The number of subtelo-acrocentric chromosomes of *L. kottelati* is the same as *L. mystaceus*, so they share the same Fn. But the number of metacentric chromosomes show differences between them. This situation may be the result of pericentromeric inversions (and/or translocations involving centromeres) as reported by Ayata *et al.* (2016). According to the chromosome number (2*n*=100), *L. kottelati* is tetraploids as reported for *L. pectoralis* (Unal and Gaffaroğlu 2016) and for several cyprinine barbines (Rab and Collares-Pereira 1995). Finally, *L. kottelati* shows similarity to *L. mursa* (Vasilyan *et al.* 2009), *L. capito* (Geng *et al.* 2013) and five Iberian *Luciobarbus* species (Collares-Pereira and Madeira 1990) in diploid chromosome number (2*n*=100). Also, the majority of *Luciobarbus* species possess a relatively high number of subtelo-acrocentric chromosomes (Vasilyan *et al.* 2009, Geng *et al.* 2013).

The longest chromosome pair in the karyotype was subtelo-acrocenric similar to *L. pectoralis* (Unal and Gaffaroğlu 2016). Morphologically differentiated sex chromosomes were not observed in *L. kottelati* as were not in *L. capito* (Kaya 2009), *L. mursa* (Vasilyan *et al.* 2009), *L. escherichii* (Gaffaroğlu *et al.* 2013) and *L. pectoralis* (Unal and Gaffaroğlu 2016).

Evident C-bands were on the pericentromeric regions of three subtelo-acrocentric chromosome pairs (Fig. 1C, D). C-band pattern of *L. kottelati* is fairly similar to the results that have been obtained in Anatolian cyprinines like *L. escherichii* (Gaffaroğlu *et al.* 2013) and *L. pectoralis* (Unal and Gaffaroğlu 2016).

Ag-NORs were observed on the short arms of sub-

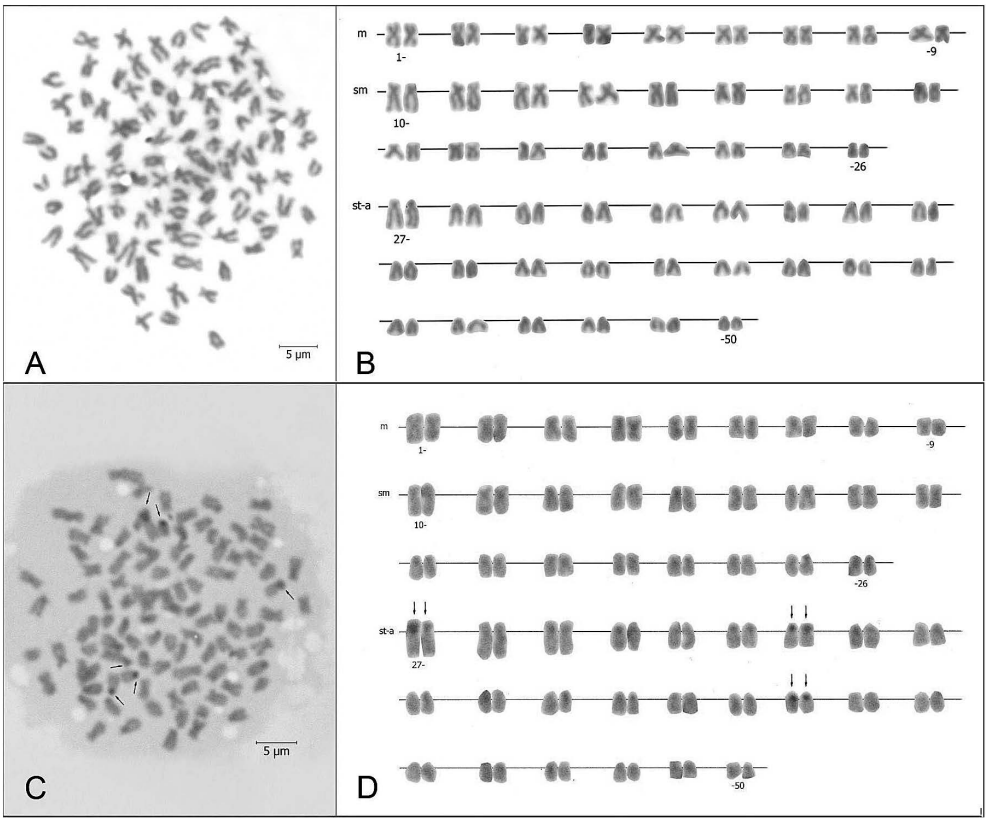

Fig. 1. Giemsa stained metaphase (A), the arranged karyotype (B), C-banded metaphase (C), the arranged karyotype (D) of *L. kottelati*. m: metacentric, sm: submetacentric, st-a: subtelo-acrocentric. Arrows indicate major C-bands.

Table 1. Karyological studies in the genus *Luciobarbus* from Turkey.

Species	2*n*	Karyotype	Fn	Cytogenetic features	References
L. mystaceus	100	22 m+30sm+48st-a	152	—	Kılıç-Demirok (2000)
L. capito	120	32 m+42sm+8st+38a	194	—	Kaya (2009)
L. escherichii	100	14 m+44sm+42st-a	158	C-bands, Ag-NORs	Gaffaroğlu *et al.* (2013)
L. pectoralis	100	20 m+42sm+38st-a	162	C-bands, Ag-NORs	Unal and Gaffaroğlu (2016)
L. kottelati	100	18 m+34sm+48st-a	152	C-bands, Ag-NORs	This study

metacentric chromosomes. The number of Ag-NOR signals varied from two to six in the 72 scored Ag-NOR stained metaphase plates (Table 2, Fig. 2A–E). Six Ag-NORs were located in two pairs of submetacentric chromosomes and in two non-homologous submetacentric chromosomes (Fig. 2F). The majority of diploid cyprinids (2*n*=50) have two Ag-NORs whereas tetraploid cyprinids (2*n*=100) have two (*Cyprinus carpio*) to six (*Barbus meridionalis*) Ag-NORs (Rab *et al.* 1993, Rab and Collares-Pereira 1995). On the other hand, *L. kottelati* shows two to six Ag-NORs in submetacentric chromosome pairs, and the most common number is four. This feature has also been reported in *L. escherichii* (Gaffaroğlu *et al.* 2013) from Anatolia, whereas the Ag-NOR number of *L. kottelati* is different from *L. pectoralis* (two Ag-NORs) (Unal and Gaffaroğlu 2016), and from other Anatolian cyprinines like *Capoeta antalyensis* (six Ag-NORs) (Gaffaroğlu *et al.* 2012), *Cyprinus carpio* (two Ag-NORs) and *Capoeta damascina* (six Ag-NORs) (Unal and Gaffaroğlu 2016). Moreover, the observed Ag-NOR number in this study is common in many tetraploid cyprinine species like *Barbus cyclolepis* (Rab *et al.* 1996) and *Carassius carassius* (Spoz *et al.* 2014). Nevertheless, the Ag-NOR location

Table 2. Ag-NOR number polymorphism of *L. kottelati*.

Number of Ag-NORs	Ag-stained metaphase plates (%)
2	13 (18)
3	10 (14)
4	31 (43)
5	16 (22)
6	2 (3)
Total	72 (100)

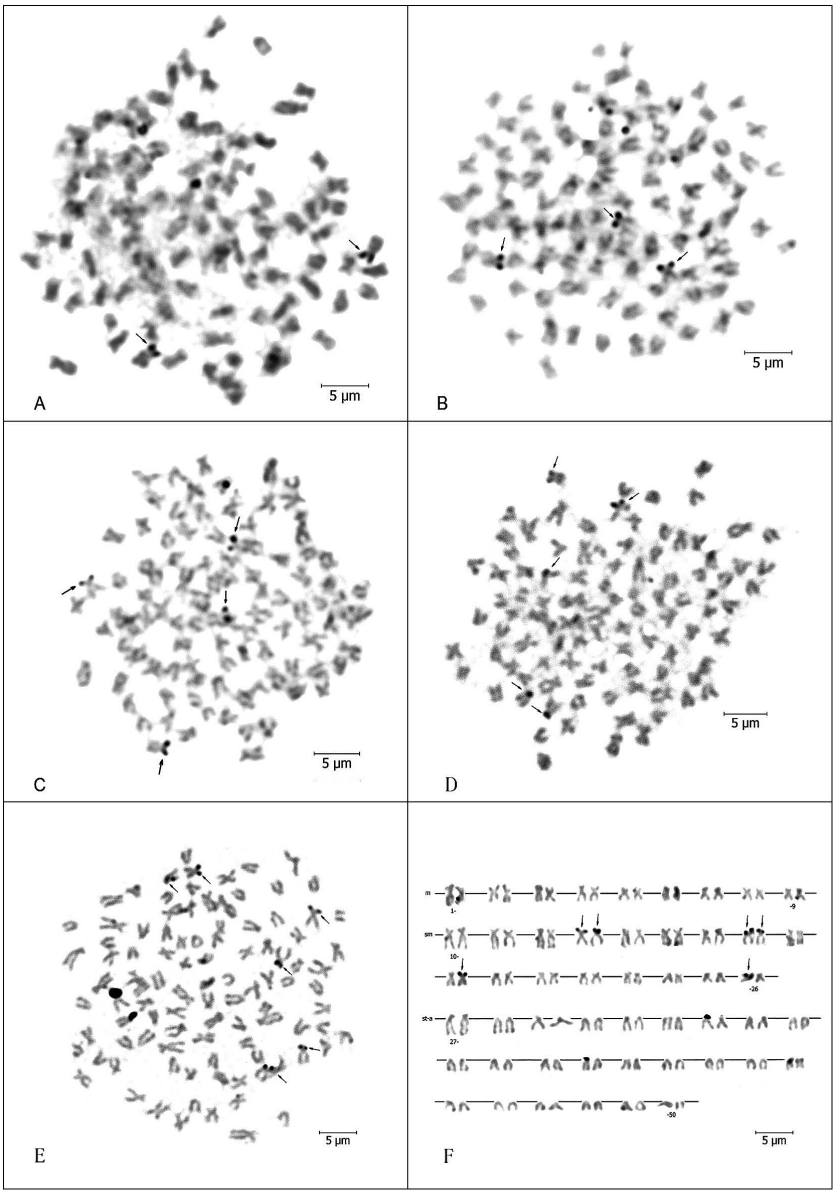

Fig. 2. Ag-stained metaphase of *L. kottelati* with two (A), three (B), four (C), five (D), and six Ag-NORs (E). F, arranged karyotype of E. Arrows indicate Ag-NOR signal.

appears to be more variable in these species than *L. kottelati*. The Ag-NOR number polymorphism that is observed in this study has not been reported in other Anatolian *Luciobarbus* species (Gaffaroğlu *et al.* 2013, Unal and Gaffaroğlu 2016) and other Anatolian cyprinines (Gaffaroğlu *et al.* 2012, Rab *et al.* 1996, Unal and Gaffaroğlu 2016) but similar results have been reported in several Anatolian leuciscine cyprinids (Ayata *et al.* 2016).

Acknowledgements

The authors are thankful to Mr. Ali Ayata for helping in the field and to Dr. S. Serkan Güçlü for identifying the specimens and to Dr. Sevgi Ünal for her laboratory support. This study was supported by the Ahi Evran University Scientific Research Projects Coordination Unit. Project Number: FEF.A3.16.013.

References

Ayata, M. K., Yüksel, E. and Gaffaroğlu, M. 2016. Cytogenetic studies on six species of the leuciscine genus *Pseudophoxinus* Bleeker, 1860 (Teleostei, Cyprinidae). Caryologia **69**: 215–222.

Collares-Pereira, M. J. 1992. *In vivo* Direct Chromosome Preparation (Air Drying Technique). First International Workshop on Fish Cytogenetic Techniques, Concarneau. pp. 15–20.

Collares-Pereira, M. J. and Madeira, J. M. 1990. Cytotaxonomic studies in Iberian cyprinids. III. karyology of Portuguese populations of *Barbus* Cuvier, 1817, with some reconsiderations on the karyological evolution of Cyprinidae. Caryologia **43**: 17–26.

Gaffaroğlu, M., Ayata, M. K., Yüksel, E. and Ünal, S. 2012. NOR and C-banding of *Capoeta antalyensis* (Battalgil, 1944) (Actinopterygii, Cyprinidae). Ecology 2012 Symposium. Kilis, Turkey. p. 455. (*in Turkish*)

Gaffaroğlu, M., Ayata, M. K., Ünal, S. and Arslan, A. 2013. Chromosomal studies of two different populations (Turkey) of *Luciobarbus escherichii* (Steindachner, 1897). Turk. J. Fish Aquat. Sci. **13**: 875–879.

Geldiay, R. and Balık, S. 2007. Freshwater Fishes of Turkey. 2nd Edn. Ege University Faculty of Fisheries Publications, İzmir. (*in Turkish*)

Geng, L. W., Xu, W., Jiang, H. F. and Tong, G. X. 2013. Karyotype analysis of *Barbus capito* (Güldentstadt, 1773) using curve measurement software. J. Appl. Ichthyology **29**: 922–924.

Howell, W. M. and Black, D. A. 1980. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: A 1 step method. Experientia **36**: 1014–1015.

Kaya, F. 2009. Karyological investigation of some economically important fishes living in the Göksu river. Ph.D. Thesis, Mersin University, Mersin. (*in Turkish*)

Kılıç-Demirok, N. 2000. The studies on the chromosomes of some cyprinid species and subspecion of the Tigris River Systems. Ph.D. Thesis, Dicle University, Diyarbakır. (*in Turkish*).

Kuru, M., Yerli, S. V., Mangıt, F., Ünlü, E. and Alp, P. 2014. Fish biodiversity in inland waters of Turkey. J. Acad. Doc. Fish Aquac. **3**: 93–120.

Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201–220.

Rab, P. and Collares-Pereira, M. J. 1995. Chromosomes of European cyprinid fishes (Cyprinidae, Cypriniformes): A review. Folia Zool. **44**: 193–214.

Rab, P., Karakousis, Y. and Rabova, M. 1996. Karyotype, NOR phenotype and C-banding study of *Barbus cyclolepis* from Greece. Folia Zool. **45**: 77–83.

Rab, P., Ozouf-Costaz, C. and 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**: 195–198.

Spoz, A., Boron, A., Porycka, K., Karolewska, M., Ito, D., Abe, S., Kirtiklis, L. and Juchno, D. 2014. Molecular cytogenetic analysis of the crucian carp, *Carassius carassius* (Linnaeus, 1758) (Teleostei, Cyprinidae), using chromosome staining and fluorescence *in situ* hybridisation with rDNA probes. Comp. Cytogenet. **8**: 233–248.

Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. **75**: 304–306.

Turan, D., Ekmekçi, F. G., İlhan, A. and Engin, S. 2008. *Luciobarbus kottelati*, a new species of barbel (Teleostei: Cyprinidae) from the Büyük Menderes river, Turkey, with rediagnose of *L. lydianus*. Zootaxa **1824**: 35–44.

Unal, S. and Gaffaroğlu, M. 2016. Karyology of six cyprinid fishes from Seyhan and Ceyhan rivers in Anatolia. Caryologia **69**: 362–369.

Vasilyan, D. Z., Stepanyan, I. E. and Pipoyan, S. K. 2009. Karyotypes of some cypriniform fishes from the water bodies of Armenia. J. Ichthyol. **49**: 627–634.

Yang, L., Sado, T., Hirt, M. V., Pasco-Viel, E., Arunachalam, M., Li, J., Wang, X., Freyhof, J., Saitoh, K., Simons, A. M., Miya, M., He, S. and Mayden, R. L. 2015. Phylogeny and polyploidy: Resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). Mol. Phylogenet. Evol. **85**: 97–116.