TÜBİTAK

Research Article

Turk J Zool
34 (2010) 111-117
© TÜBİTAK
doi:10.3906/zoo-0807-33

Cytogenetic analysis of *Pseudophoxinus antalyae*, Bogustkaya, 1992 (Pisces: Cyprinidae) from the Eastern Mediterranean River Basin, Turkey

Serap ERGENE[1], Arzu KARAHAN[2], Mustafa KURU[3]

[1]University of Mersin, Faculty of Science & Arts, Department of Biology, Mersin - TURKEY

[2]Middle East Technical University, Institute of Marine Science, Erdemli, Mersin - TURKEY

[3]Başkent University, Faculty of Education, Department of Biology, Ankara - TURKEY

Received: 31.07.2008

Abstract: The karyotype of *Pseudophoxinus antalyae* was analyzed using G-banding, C-banding, Ag-NOR staining, and Q-banding. Chromosome analysis of this species, which lives in the Berdan River and Berdan Dam Lake, was performed with the air-drying technique, using gill and fin epithelium cells. The diploid chromosome number of *P. antalyae* was determined to be 2n = 50, comprising 16 metacentric, 14 submetacentric, 12 subtelocentric, and 8 acrocentric (NF = 92) chromosomes. The largest chromosome pair of the complements was characteristically a submetacentric. The nucleolar organizer regions (NORs) were apparently located in subtelocentric chromosome pairs. The karyotype of *P. antalyae* is reported for the first time.

Key words: *Pseudophoxinus antalyae,* karyotype, banding, Turkey

Türkiye'nin Doğu Akdeniz bölgesindeki nehirlerinde yaşayan *Pseudophoxinus antalyae*, Bogustkaya 1992 (Pisces: Cyprinidae)'nin sitogenetik analizi

Özet: Giemsa bant, C-bant, Ag-NOR bant ve Quinacrine bant teknikleri kullanılarak *Pseudophoxinus antalyae*'nin karyolojik analizi yapılmıştır. Kromozom analizi Berdan nehri ve Berdan barajında yaşayan örneklerin solungaç ve yüzgeç epitellerinden, havada kurutma tekniği kullanılarak yapılmıştır. *P. antalyae*'nin diploit kromozom sayısı 50, karyotipi ise 16 metasentrik, 14 submetasentrik, 12 subtelosentrik ve 8 akrosentrik (NF = 92) olarak belirlenmiştir. En büyük kromozomun submetasentrik olduğu görülmüştür. NOR bölgeleri subtelosentrik kromozom çiftleri üzerinde bulunmaktadır. *P. antalyae*'nin karyotipi bu çalışma ile ilk kez tanımlanmıştır.

Anahtar sözcükler: *Pseudophoxinus antalyae,* karyotip, bantlama, Türkiye

* E-mail: sr.ergene@gmail.com

Introduction

The study of fish chromosomes has received considerable attention in recent years because of their importance in classification, evolution, and heredity (Ozouf-Costaz and Foresti, 1992). Moreover, cytogenetic studies of fish have been used as biological indicators of water pollution (Al-Sabti, 1991; Klinkhardt, 1993).

Generally, a large number of small chromosomes characterizes fish karyotypes. This discourages many researchers from pursuing fish karyotype analysis, and therefore karyological data on fish are available for only a small percentage (about 10%) of the some 25,000 taxonomically known species (Ojima, 1985; Klinkhardt et al., 1995; Froese and Pauly, 2006; Nelson, 2006).

Cyprinid fishes comprise a major element of the ichthyofauna of Africa, Asia, Europe, and North America. The family Cyprinidae has about 220 genera and about 2420 species, and is the largest family of freshwater fishes (Nelson, 2006). More than 95 cyprinid species have been reported from Turkey (Kuru, 1980). Despite the diversity of Turkey's fishes, data on their chromosomes are scarce (Çolak et al., 1985; Ergene et al., 1998a, 1998b, 1999). The present study is the first to cytogenetically examine *P. antalyae*.

Materials and methods

In all, 15 *P. antalyae* specimens were collected from Berdan Dam Lake and Berdan River (Figure 1). Chromosome preparation was performed as described by Ergene et al. (1999). The preparations were stained for 20 min with 5% Giemsa in pH 6.8 phosphate buffer. Nucleolar organizer regions (NORs) were identified following the silver ($AgNO_3$) staining method of Howell and Black (1980). C-bands were obtained according to Sumner (1972). Q-banding with quinacrine was performed according to Schimid (1980). Metaphase chromosomes were banded using the conventional Trypsin-Giemsa banding technique (Seabright, 1971). Giemsa-stained, G-banded, Ag-NOR-stained, Q-banded, and C-banded mitotic chromosomes were photographed using a digital camera and the images were digitally processed using Adobe Photoshop v.7.0 software. The karyogram was constructed with chromosomes organized in order of decreasing size and the chromosomes were classified according to Levan et al. (1964). The fundamental number (FN)—or arm number—was determined by considering meta-, submeta-, and subtelocentric chromosomes with 2 arms, and acrocentric chromosomes with only 1 arm. The arm ratio was determined using the Micro-Measure program. A haploid ideogram was prepared based on the measurements of C-banded, Ag-NOR stained, and G-banded chromosomes.

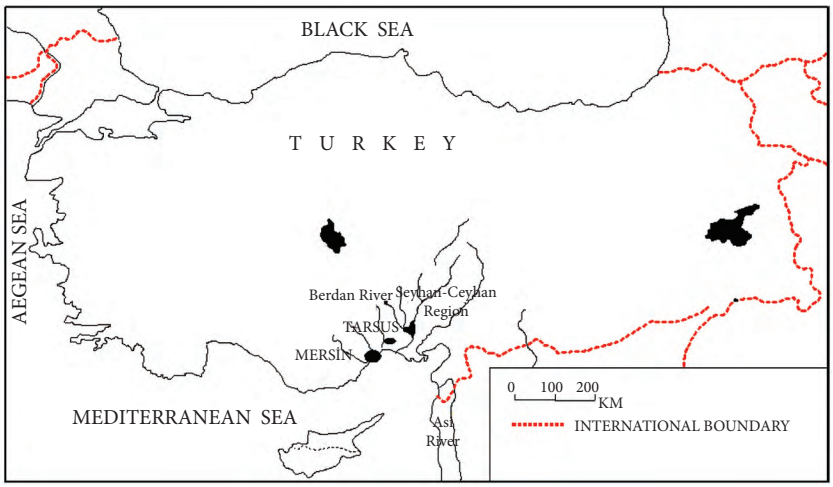

Figure 1. *P. antalyae* collection site.

Results

The diploid chromosome number of *P. antalyae* is 2n = 50, comprising 16 metacentric, 14 submetacentric, 12 subtelocentric, and 8 acrocentric chromosomes (16 m + 14 sm + 12 st + 8 a), and was observed in the great majority of cells (Table). Well-dispersed metaphase plates of *P. antalyae* after banding are presented in Figure 2a-d. The arm number is NF = 92.

Table. Chromosome number frequency distribution of in *P. antalyae*.

Examined specimens	Evaluated metaphase number	Chromosome number	Occurrence %	Standard error
	2	38	0.6	8.1
	8	42	2.2	5.3
15	14	45	3.9	3.2
	12	48	3.4	1.1
	320	50	89.9	0.4
	356		100	

The karyotype was determined according to the arm measurement, which is a consequence of C-banding (Figure 2b). G-banding showed the following: 5 band regions on chromosome arm No. 9, 4 band regions on chromosome arm No. 1, 2, 10, 16, and 17, 3 band regions on chromosome arm No. 5, 6, 12, 14, 15, and 22, 2 band regions on chromosome arm No. 3, 4, 8, 11, 19, and 21, and 1 band region on chromosome arm No. 7, 13, 18, 20, 23, and 25 (Figures 2d, 2e, and 3). A very large heterochromatin region was observed on metacentric chromosome arm No. 7. The clear band region schema is given in Figure 2e. Satellites were observed on the terminal region of the short arm in subtelocentric pair No. 17 (Figures 2c and 4). The chromosome arm ratio of *P. antalyae* was obtained using the Micro-Measure program and is shown in Figure 5. The largest chromosome pair of the complements was characteristically submetacentric. A clear Q-band region was not seen in any chromosomes. The C-band positive heterochromatic regions are distributed in centromeric positions in many chromosomes (Figure 2a).

Discussion

Bogustkaya (1992) revised the *Pseudophoxinus* species of Anatolia based on the skull and sensory canal. Accordingly, the *Rutilus tricolor* specimen was identified as a new species, *P. antalyae* (Bogustkaya, 1992).

The majority of cyprinid species have between 2n = 50 and 2n = 100 chromosomes (Oelerman and Skelton, 1990). The *P. antalyae* diploid chromosome number is 2n = 50, which corresponds with cyprinid chromosomes.

Most chromosome banding studies of fish are related to C-bands, or silver- and chromomycin A3-bands for identifying NORs, while descriptions of the distinct structural Q-, G-, and replication-banding patterns are limited (Ueda and Naoi, 1999). Weak compartmentalization of genomes due to the base composition (AT- or GC-rich DNA) of cold-blooded vertebrates has been reported (Hudson et al., 1980; Medrano et al., 1988). Weak compartmentalization of fish chromosomes is thought to be a primary cause of the limited number of reports on the distinct structural Q- and G-banding patterns.

G-banding can be used to identify chromosomal abnormalities, such as translocations, because there is a unique pattern of light and dark bands for each chromosome (Ueda and Naoi, 1999). C- and Ag-banding analysis proved useful in the investigation of karyotype evolution in bitterlings. Heterochromatin has played an important role in the karyotypic diversification of fish (Ueda et al., 2001). In the present study a very large heterochromatin region was seen on metacentric chromosome arm No. 7 (Figures 2e and 3). This chromosome pair can be defined as a marker for the genus *Pseudophoxinus*.

The low rate of polymorphism in the rDNA transcription unit allows characterization of the rDNA of each species using only a few specimens, making this DNA useful for interspecific comparisons. In addition, the different coding regions of the rDNA repeats usually show distinct evolution rates. As a result, this DNA can provide information about almost any systematic level (Hillis and Dixon, 1991). Satellited chromosomes are known to be useful as markers in fish karyotypes (Denton, 1973). Although NORs are seen on the short arms of

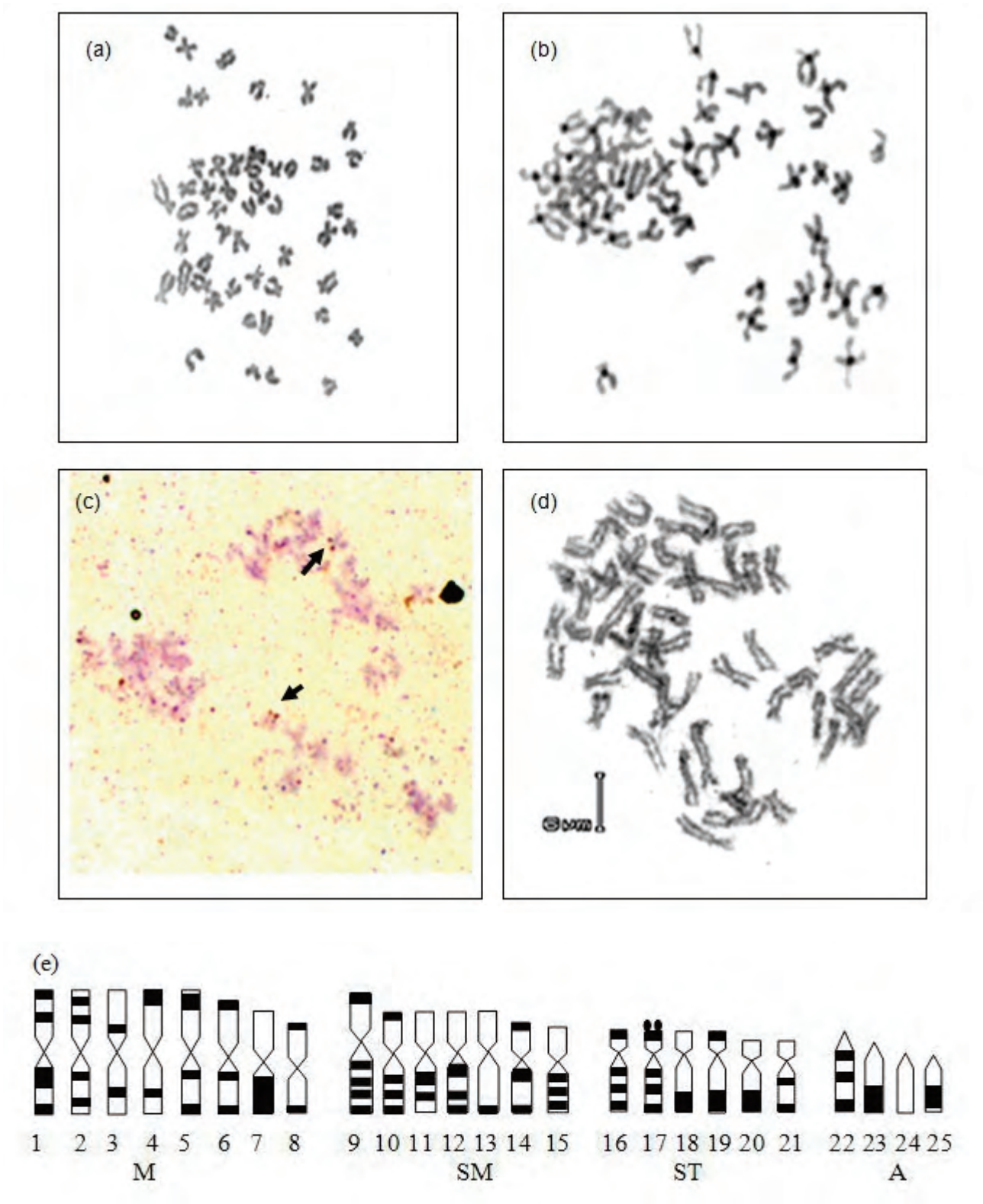

Figure 2. The metaphase plate (a), C-banding (b), Ag-NOR-banding (c), G-banding (d), and ideogram of *P. antalyae* (G-banding) (e).

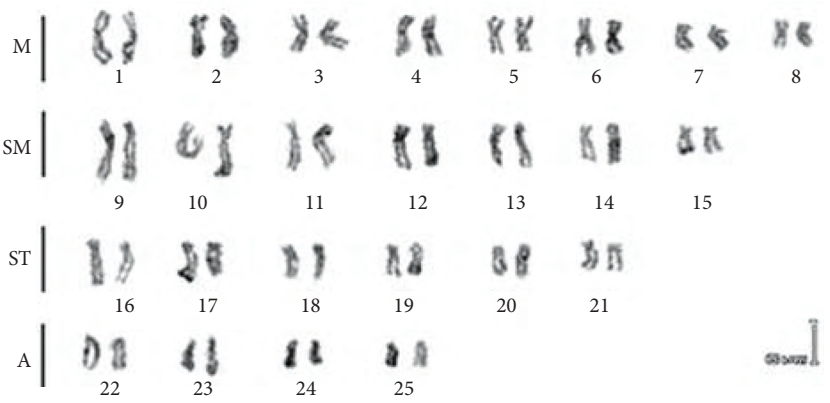

Figure 3. Giemsa banded karyotype of *P. antalyae* from Turkey.

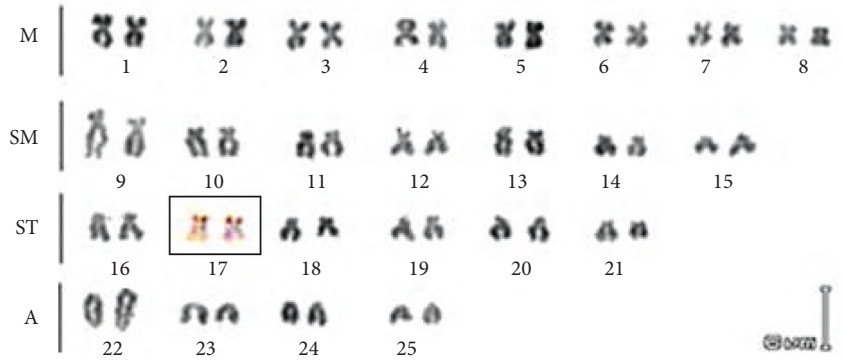

Figure 4. Giemsa stained and Ag-NOR banded karyotype of *P. antalyae* from Turkey.

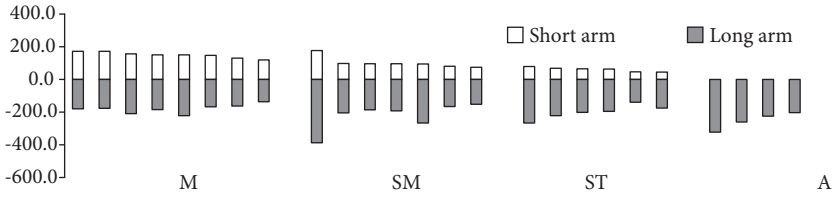

Figure 5. Arm ratio of *P. antalyae* from Turkey.

chromosomes in particular, sometimes they can be seen on the long arms of M and A chromosomes (Sola et al., 1993; Rab and Collares-Pereira, 1995; Rab et al., 1996). Furthermore, they can be seen between telomeres and centromeres (Galetti et al., 1984; Amemiya and Gold, 1988; Jankun et al., 1998). In addition, NORs can be observed in sex chromosomes, though rarely (Bertollo and Cavallaro, 1992). Generally, the NOR-phenotype is observed at the terminal on short arms of mid-sized acro-subtelocentric chromosomes (Gold and Amemiya, 1986; Takai and Ojima, 1986; Amemiya and Gold,

1988; Takai and Ojima, 1992), rarely at the terminal on short arms of mid-sized submetacentric chromosomes (Gold et al., 1988; Magtoon and Arai, 1993), and rarely at the terminal on 1 arm of a mid-sized metacentric chromosome (Gold and Li, 1994) in Cyprinidae. The number of NORs in Cyprinidae varies between 1 and 4. Ag-NOR's in the present study were observed on the terminal region of the short arm of No. 17 subtelocentric chromosomes (Figures 2c and 3).

The chromosome number and morphology obtained with chromosomal analysis are used to identify species and define the relationships and differences between species. The chromosome number and morphology can vary between fish species. This variation can be used in the search for the evolutionary relationship between interpopulation and intrapopulation (Thorgard and Disney, 1990). The present study is the first to determine the karyotype of *P. antalyae*. This data on the karyological structure of this Turkish freshwater species will be a useful addition to the fish cytogenetic database.

Acknowledgments

This study was supported by the University of Mersin.

References

Al-Sabti, K. 1991. Handbook of genotoxic effects and fish chromosomes, Joseph Stephan Institute Press, Ljubjiana. Yugoslavia.

Amemiya, C.T. and Gold, J.R. 1988. Chromosomal NORs as taxonomic and systematic characters in North American Cyprinid fishes. Genetica. 76: 1-90.

Bertollo, L.A.C. and Cavallaro, Z.L. 1992. A highly differentiated ZZ/ZW sex chromosome system in a Characidae fish *Tniportheus guentheri*. Cytogenetics and Cell Genetics. 60: 60-63.

Bogutskaya, N.G. 1992. A revision of species of the genus *Pseudophoxinus* (Leuciscinae, Cyprinidae) from Asia Minor. Mitt. Hamb. Zool. Mus. Inst. 61: 261-290.

Çolak, A., Sezgin, I. and Süngü, Y.S. 1985. Sazangiller familyasına ait beni balığında (*Cyprinion macrostomus*, Heckel, 1843) kromozomal araştırmalar. Doğa Bilim Dergisi. A2: 9-12.

Denton, T.E. 1973. Fish chromosome methodology. Illinois, USA. Charles C. Thomas. Springfield, 166 p.

Ergene, S., Kaya, F., Pekcan, I. and Oral, A. 1998a. Karyological analysis of *Orechromis niloticus* (L., 1758) (Pisces, Cichlidae) used in Aquaculture. First Intern. Sypm. on Fisheries & Ecology. Proc. 2-4 September Trabzon, Turkey.

Ergene, S., Kuru, M. and Çavaş, T. 1998b. Karyological analysis of *Barbus plebejus* (Heckel, 1843). 2nd International Kızılırmak Congress of Applied Sciences 20-22 May Kırıkkale, Turkey 426-433.

Ergene, S., Portakal, E. and Karahan, A. 1999. Karyological analysis and body proportion of Catfish (Clariidae, *Clarias lazera*, Valeciennes. 1840) in Göksu Delta Turkey. Turkish Journal of Zoology. 23: 423-426.

Froese, R. and Pauly, D. 2006. FishBase http://www.fishbase.org. Accessed January.

Galetti, P.M.Jr., Foresti, F., Bertollo, L.A.C. and Moreira Filho, O. 1984. Characterization of eight species of Anostomidae (Cypriniformes) fish on the basis of the nucleolar organizing region. Caryologia. 37: 401-406.

Gold, J.R. and Amemiya, C.T. 1986. Cytogenetic studies in North American minnows (Cyprinidae). XII. Patterns of chromosomal nucleolus organizer region variation among 14 species. Can. J. Zool. 64: 1869-1877.

Gold, J.R., Zoch, P.K. and Amemiya, C.T. 1988. Cytogenetic studies in North American minnows (Cyprinidae). XIV. Chromosomal NOR phenotypes of eight species from the genus Notropis Cytobios. 54(218-219): 137-147.

Gold, J.R. and Li, Y. 1994. Chromosomal NOR karyotypes and genome size variation among squawfishes of the genus *Ptychocheilus* (Teleostei: Cyprinidae). Copeia: 60-65.

Hillis, D.M. and Dixon, M.T. 1991. Ribosomal DNA molecular evolution and phylogenetic inference. Quart Rev Biol. 66: 411-453.

Howell, W.M. and Black, D.A. 1980. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer. 1 Step Method Experiential. 36: 1014-1015.

Hudson, A.P., Cuny, G., Cortadas, J., Haschemeyer, A.E.V. and Bernardi, G. 1980. An analysis of fish genomes by density gradient centrifugation. Eur. J. Biochem. 112: 203-210.

Jankun, M., Woznicki, P., Dajnowicz, G., Demska-Zakes, K., Luczynski, M.J. and Luczynski, M. 1998. Heterochromatin and NOR location in northern Pike (*Esox Lucius*). Acquit Sci. 60: 17-21.

Klinkhardt, M.B. 1993. Fish chromosomes as sensitive toxicity indicators–possibilities and limits fish Ecotoxicology Ecophysiology. Braunbeck W Hanke W Segner H (VCH, Weinheim). 45-54.

Klinkhardt, M., Tesche, M. and Greven, H. 1995. Database of fish chromosomes, Westarp Wissenschaften, Germany.

Kuru, M. 1980. Freshwater fishes of Turkey, Hacettepe University Science. Fac. Press. 12, 1.

Levan, A., Fredga, L. and Sandberg, A.A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas. 52: 201-220.

Magtoon, W. and Arai, R. 1993. Karyotypes and distribution of nucleolus organizer regions in cyprinid fishes from Thailand. Jap. J. Ichthyol. 40(1): 77-85.

Medrano, L., Bernardi, G., Couturier, J., Dutrillaux, B. and Bernardi, G. 1988. Chromosome banding and genome compartmentalization in fishes. Chromosoma, 96: 178-183.

Nelson, J.S. 2006. Fishes of the world, John Wiley and Sons Inc, New York.

Oelerman, L.K. and Skelton, P.H. 1990. Hexaploidy in yelowfish species (Barbus, Pisces, Cyprinidae) from Southern Africa. J. of Fish Biology. 37: 105-115.

Ojima, Y. 1985. Fish chromosome data retrieval list, Kwansei Gaukin. University Nishinomiya.

Ozouf-Costaz, C. and Foresti, F. 1992. Fish cytogenetic research. Advances Applications and Perspectives Netherlands. J. Zool. 42: 277-290.

Rab, P. and Collares-Pereira, M. 1995. Chromosomes of European Cyprinid fishes (Cyprinidae, Cyprinformes). A Review Folia Zoo. 4: 3193-214.

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.

Seabright, M.R., 1971. A rapid banding technique for human chromosomes. Lancet 2: 971-972.

Schimid, M. 1980. Chromosoma banding an Amphibia IV. Differentiation of GC and AT rich regions in Anura. Chromosoma 77: 83-103.

Sola, L., Bressanello, S., Rossi, A.R., Iaselli, V., Crosetti, D. and Cataudella, S. 1993. A karyotype analysis of the genus *Dicentrarchus* by different staining techniques. Journal of Fish Biology 43: 329-337.

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

Takai, A. and Ojima, Y. 1986. Some features on nucleolus organizer regions in fish chromosomes. In: T. Uyeno, R. Arai, T. Taniuchi, and K. Matsuura (eds), Indo Pacific Fish Biology, Tokyo, Ichthyol. Soc. of Japan. p. 899-909.

Takai, A. and Ojima, Y. 1992. Chromosomal distribution of nucleolus organizer regions in Japanese Cyprinid fish. Cytobios. 71: 7-17.

Thorgard, G.H. and Dısney, J.E. 1990. Chromosome preparation and analysis. Methods for fish biology. American Fisheries Society, Bethesda, Maryland, USA.

Ueda, T. and Naoi, H. 1999. BrdU-4Na-EDTA-Giemsa band karyotypes of 3 small freshwater fish, *Danio rerio, Oryzias latipes,* and *Rhodeus ocellatus* Genome 42: 531-535.

Ueda, T., Naoi, H. and Arai, R. 2001. Flexibility on the karyotype evolution in bitterlings (Pisces, Cyprinidae). Genetica. 111: 423-432.