



Short communication

Karyotype of mullet *Liza abu* Heckel, 1846 (Pisces: Mugilidae) from the Tigris River, Turkey

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Introduction

Cytogenetic studies have become an invaluable tool contributing to the solution of many systematic and evolutionary problems in fishes (Amemiya and Gold, 1988; Rab et al., 1991; Nirchio et al., 2003). Classical cytogenetic analyses made use of differential staining techniques to investigate numbers and chromosomal locations of the nucleolus organizer regions (NORs) and the chromosomal distribution and composition of the constitutive heterochromatin (i.e. Gold et al., 1990; Collares-Pereira, 1992; Boron, 1995; Rab et al., 1996; Rossi et al., 1996, 2005; Sola et al., 2007; Nirchio et al., 2009).

The family Mugilidae is very important as a food fish for humans. Its members are distributed throughout the world from temperate to tropical coastal waters, readily entering estuaries and even resident in freshwaters. This family includes about 17 genera with 72 species (Nelson, 2006; Sola et al., 2007; Coad, 2010). In Turkey, the Mugilidae family is represented by nine species: *Mugil cephalus*, *M. soiuy*, *Liza ramada*, *L. aurata*, *L. abu*, *L. saliens*, *L. carinata*, *Chelon labrosus*, and *Oedalechilus labeo* (Geldiay and Balik, 1988; Kuru, 2004; Turan et al., 2005). Among these, *L. abu* is known to inhabit and spawn in freshwater in southeastern Asia, in the Tigris-Euphrates and Orontes river basins (Coad, 2010).

Cytogenetic analyses are available for 16 species of Mugilidae mainly from the Mediterranean Sea and South America (reviewed in Sola et al., 2007 and Arai, 2011). However, the karyology of *L. abu* had not been studied. In the present survey, the cytogenetic analysis of *L. abu* is reported through Giemsa and Ag-NOR staining. The obtained karyological data was compared with those of closer species available in the literature.

Materials and methods

Samples (eight males and 12 females) were collected between 1995 and 1997 from the Devegeçidi reservoir (38°03'N; 39°

57'E; altitude: 747 m) and Tigris River near Diyarbakir city, Turkey (37°50'N; 40°14'E; alt.: 585 m). Morphological species identification was based on that of Coad (2010). Fish were transported alive to the laboratory and immediately karyotyped. Chromosome preparations were made from kidney cells using the air-drying technique described by Rab (1981). Ag-staining followed Howell and Black (1980). Chromosomes were classified using the nomenclatures proposed by Levan et al. (1964), and arranged in decreasing size on the basis of relative length and centromeric position (long/short arms ratio). Metaphases were analyzed under a Nikon ECLIPSE 80i microscope and microphotographs taken with a Nikon DS-Fi1-U2 camera, using NIS-Elements Documentation software. Images were processed using ADOBEPHOTOSHOP 8.0.

Results

Table 1 shows the number of specimens per sex and the chromosome number distribution. *Liza abu* showed a diploid number of $2n = 48$ chromosomes with a karyotype formula $2M$ and $46A$ (Fig. 1a,b) and $NF = 50$. Morphologically distinct sex chromosomes were not observed. Chromosomes uniformly decrease in size, making it difficult to clearly identify the homologues, with the exception of the metacentric chromosomes pair, classified as number 1, and of chromosome pair number 24, which is considerably smaller than the others. With the Ag-staining technique, the nucleolus organizer regions in *L. abu* were detected on the terminal region of one middle-sized acrocentric chromosomes pair (Fig. 1c).

Discussion

The diploid chromosome number reported here for *L. abu* is the same as previously described for mugilid species, except for *M. curema* (Le Grande and Fitzsimons, 1976; Nirchio and Cequea, 1998; Sola et al., 2007; Arai, 2011). These observations suggest that the karyotype with $2n = 48$ could be the more conservative condition for Mugilidae (Sola et al., 2007). It has been proposed that the reduced chromosome number observed in *M. curema* ($2n = 24$ and $2n = 28$) could be the result of extensive Robertsonian fusions, from an ancestral taxon with an all-acrocentric chromosome complement like that of *M. cephalus* (Rossi et al., 2005).

As previously described (Sola et al., 2007), four main cytotypes (A, B, C1, and C2) can be distinguished in mullet species. The chromosome complement of *L. abu* reported in this

Table 1
Number of specimens and chromosome number distribution of *Liza abu* from the Tigris River (Turkey)

Sexes	Specimens	Chromosome number distribution			Total cells
		46	47	48	
Male	8	4	8	52	64
Female	12	2	11	70	81

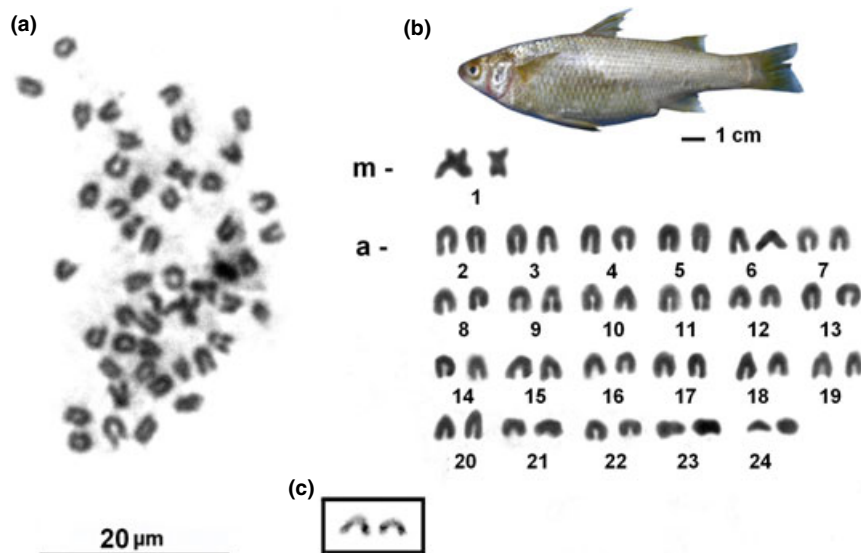


Fig. 1. Giemsa-stained metaphase plate (a), karyotype (b) silver-stained chromosomes (c) of *Liza abu* from the Tigris River (Turkey)

study conforms to cytotype B, with 46 acrocentric chromosomes which are shared by *L. aurata*, *L. ramada*, *L. saliens*, *C. labrosus* and *O. labeo* species (Gornung et al., 2004; Sola et al., 2007). However, *L. abu* differs from these species due to the presence of two metacentric chromosomes, which is an unusual feature in mugilid fish. The fundamental numbers of this species ($NF = 50$) are shown in some mugilid species such as *C. labrosus*, *L. aurata*, *L. ramada* and *L. saliens* (Sola et al., 2007). The high NF values observed in *L. abu* (50) suggest the occurrence of rearrangements in the chain acting upon the karyotypes of the species, presumably derived from a pericentric inversion. Pericentric inversions and the gain or loss of heterochromatic arms change the arm number, but not the chromosome number (Gibson, 1984).

Karyological information about the Mugilidae shows that NORs generally occur on a single chromosome pair, and two groups of species can be identified according to the location of their NORs (Nirchio et al., 2007; Sola et al., 2007). In the first group, NORs are located in a terminal position on the long arm of the largest chromosome pair (including *M. cephalus*, *M. platanus*, *M. liza*, and both *M. incilis* and *M. curema* $2n = 24$ from Venezuela); in the second group, NORs are located on the short arm of a subtelocentric chromosome pair (which includes *Liza aurata*, *L. ramada*, *L. saliens*, *Chelon labrosus* and *Oedalechilus labeo*, *A. monticola* and *M. curema* $2n = 28$ from Brazil). Finally, in *M. rubrioculus* and *M. trichodon*, the single pair of NORs is interstitially located (Nirchio et al., 2005, 2007). Compared to these species, *L. abu* from the Tigris River shows a completely different localization of NORs, i.e. these chromosomal regions are indeed in terminal positions on a mid-sized acrocentric chromosome pair. Mugilidae have a conservative chromosome macrostructure, reinforcing the hypothesis that small structural chromosome rearrangements involving active NOR sites are the main cause of the karyotypic diversification seen in this group (Nirchio et al., 2005).

In conclusion, the present study offers basic and important karyotype information for a mugilid species, *L. abu*, for which data were unknown and from a geographic area where other, not yet investigated, *Liza* species are present. Data obtained add a new cytotype to those already described in

this family, characterized by the presence of two large metacentric chromosomes. More detailed chromosomal data from other species of the Mugilidae family will be necessary in order to understand the patterns of the karyotypic relationship among species within this taxon.

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References

- Amemiya, C. T.; Gold, J. R., 1988: Chromosomal NORs phenotypes as taxonomic and systematic characters in North American cyprinid fish. *Genetica* **76**, 81–90.
- Arai, R., 2011: Fish karyotypes: a check list. Springer, Tokyo-Berlin-Heidelberg-New York, 340 pp.
- Boron, A., 1995: Chromosome banding studies of *Noemacheilus barbatus* (Linnaeus, 1758) from Poland. *Caryologia* **43**, 239–246.
- Coad, B. W., 2010: Freshwater fishes of Iraq. Pensoft Publishers, Sofia-Moscow, 294 pp.
- Collares-Pereira, M. J., 1992: *In vivo* direct chromosome preparation (protocol for air drying technique). In: First International Workshop on Fish Cytogenetic Techniques. C. Ozouf-Costaz, F. Foresti, L. Almeida Toledo (Eds). Museum Nat. Histoire Naturelle, Paris, France, pp. 15–19.
- Geldiay, R.; Balik, S., 1988: Freshwater fish species of Turkey. Ege University, Izmir, 519 pp. [In Turkish].
- Gibson, L. J., 1984: Chromosomal changes in mammalian speciation: a literature review. *Origins* **11**, 67–89.
- Gold, J. R.; Li Y., ; Shipley, N. S.; Powers, P. K., 1990: Improved methods for working with fish chromosomes with a review of metaphase chromosome banding. *J. Fish Biol.* **37**, 563–575.
- Gornung, E.; Mannarelli, M. E.; Rossi, A. R.; Sola, L., 2004: Chromosomal evolution in Mugilidae (Pisces, Mugiliformes): FISH mapping of the (TTAGGG) n telomeric repeat in the six Mediterranean mullets. *Hereditas* **140**, 158–159.
- 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**, 1014–1015.
- Kuru, M., 2004: Recent systematic status of inland water fishes of Turkey. *Gazi Univ. J. Educ. Fac.* **24**, 1–21. [In Turkish]

- Le Grande, W. H.; Fitzsimons, J. M., 1976: Karyology of the mullets *Mugil curema* and *Mugil cephalus* (Perciformes: Mugilidae) from Louisiana. *Copeia* **2**, 388–391.
- Levan, A.; Fredga, K.; Sandberg, A. A., 1964: Nomenclature for centromeric position on chromosomes. *Hereditas* **52**, 201–220.
- Nelson, J. S., 2006: *Fishes of the world*, 4th edn. John Wiley and Sons, Hoboken, New Jersey, 601 pp.
- Nirchio, M.; Cequea, H., 1998: Karyology of *Mugil liza* and *M. curema* from Venezuela. *Bol. Inv. Mar. Cost.* **27**, 45–50.
- Nirchio, M.; Cervigon, F.; Porto, J. I. R.; Perez, J. E.; Gomez, J. A.; Villalaz, J., 2003: Karyotype supporting *Mugil curema* Valenciennes, 1836 and *Mugil gaimardianus* Desmarest, 1831 (Mugilidae: Teleostei) as two valid nominal species. *Sci. Mar.* **67**, 113–115.
- Nirchio, M.; Ron, E.; Rossi, A. R., 2005: Karyological characterization of *Mugil trichodon* Poey, 1876 (Pisces: Mugilidae). *Sci. Mar.* **69**, 525–530.
- Nirchio, M.; Oliveira, C.; Ferreira, I. A.; Perez, J. E.; Gaviria, J. I.; Harrison, I.; Rossi, A. R.; Sola, L., 2007: Comparative cytogenetic and allozyme analysis of *Mugil rubrioculus* and *Mugil curema* (Teleostei: Mugilidae) from Venezuela. *Interciencia* **32**, 757–762.
- Nirchio, M.; Oliveira, C.; Ferreira, I. A.; Martins, C.; Rossi, A. R.; Sola, L., 2009: Classical and molecular cytogenetic characterization of *Agonostomus monticola*, a primitive species of Mugilidae (Mugiliformes). *Genetica* **135**, 1–5.
- Rab, P., 1981: Karyotype of European catfish *Silurus glanis* (Siluridae, Pisces), with remarks on cytogenetics of siluroid fishes. *Folia Zool.* **30**, 271–286.
- Rab, P.; Roth, P.; Vasiljeva, E. D., 1991: Chromosome banding study of the golden loach *Sabanejewia aurata* from Slovakia. Japan (Cobitidae). *Jpn. J. Ichthyol.* **38**, 141–146.
- Rab, P.; Karakousis, Y.; Rabova, M.; Economidis, P. S., 1996: Banded karyotype of cyprinid fish *Leuciscus borysthenicus*. *Ichthyol. Res.* **43**, 463–468.
- Rossi, A. R.; Crosetti, D.; Gornung, E.; Sola, L., 1996: Cytogenetic analysis of global populations of *Mugil cephalus* (striped mullet) by different staining techniques and fluorescent *in situ* hybridization. *Heredity* **76**, 77–82.
- Rossi, A. R.; Gornung, E.; Sola, L.; Nirchio, M., 2005: Comparative molecular cytogenetic analysis of two congeneric species, *Mugil curema* and *M. liza* (Pisces, Mugiliformes), characterized by significant karyotype diversity. *Genetica* **125**, 27–32.
- Sola, L.; Gornung, E.; Mannarelli, M. E.; Rossi, A. R., 2007: Chromosomal evolution in Mugilidae, Mugilomorpha: an overview. In: *Fish cytogenetics*. E. Pisano, C. Ozouf-Costaz, F. Foresti, B. G. Kapoor (Eds). Science Publishers, Enfield, NH, USA, pp. 165–194.
- Turan, C.; Çalışkan, M.; Küçüktaş, H., 2005: Phylogenetic relationships of nine mullet species (Mugilidae) in the Mediterranean Sea. *Hydrobiologia* **532**, 45–51.
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