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Authors: Valić, Damir, Kapetanović, Damir, Zanella, Davor, Mrakovčić, Milorad, Teskeredžić, Emin, et al.

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The karyotype and NOR phenotype of *Telestes ukliva* (Cyprinidae)

Damir VALIĆ[1], Damir KAPETANOVIĆ[1], Davor ZANELLA[2], Milorad MRAKOVČIĆ[2], Emin TESKEREDŽIĆ[1], Višnja BESENDORFER[3], Marie RÁBOVÁ[4] and Petr RÁB[4]

[1] *Laboratory for Research and Development of Aquaculture, Division for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička 54, HR-10000 Zagreb, Croatia; e-mail: dvalic@irb.hr*
[2] *Department of Zoology, Division of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, HR-10000 Zagreb, Croatia*
[3] *Department of Molecular Biology, Division of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, HR-10000 Zagreb, Croatia*
[4] *Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, 277 21 Liběchov, Czech Republic*

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Abstract. The karyotype and major ribosomal sites as revealed by silver staining and CMA_3-fluorescence of Croatian leuciscine cyprinid endemic to Cetina River, *Telestes ukliva* were studied. The diploid chromosome number was invariably 2n = 50. Karyotype consisted of eight pairs of metacentric, 13 pairs of submetacentric and four pairs subtelocentric chromosomes. The largest chromosome pair of the complement was subtelocentric, which is a characteristic cytotaxonomic marker for all representatives of the cyprinid lineage Leuciscinae. The nucleolar organizer regions (NORs) were detected in the telomeres of two pairs of medium-sized submetacentric chromosomes. Staining with CMA_3 revealed four positive signals that corresponded to NOR sites. No heteromorphic sex chromosomes were found. The karyotype pattern of *T. ukliva* is nearly identical to that found in most other representatives of the Eurasian leuciscine cyprinids, while the multiple NOR phenotype appears to be more derived as opposed to a uniform one NOR-bearing chromosome pair, ubiquitous in this group.

Key words: fish cytogenetics and cytotaxonomy, chromosome banding, multiple nucleolar organizer region (NOR), leuciscine cyprinids

Introduction

Ten freshwater fish species locally endemic to the Adriatic basin in Croatia includes also the ukliva dace, *Telestes ukliva* (Heckel, 1843). This species is present throughout the Cetina River, with the exception of the river mouth. Most species of genus *Telestes* occur in the eastern Adriatic drainages and suffer from habitat alterations and loses. Many of them were earlier included in the genus *Leuciscus*, but present molecular and morphological data indicate that the genus *Telestes* is well defined monophyletic lineage being more closely related to *Chondrostoma*, *Achondrostoma*, *Pseudochondrostoma*, *Parachondrostoma*, *Protochondrostoma*, *Iberochondrostoma* and *Phoxinellus* (Freyhof et al. 2006) than to *Leuciscus*. Three species earlier referred to *Phoxinellus* have recently been transferred to *Telestes* (Freyhof et al. 2006). However, the systematics of *Telestes* and its actual diversity has not yet been well resolved (Kottelat & Freyhof 2007).

Extensive cytotaxonomic screening on the *´Leuciscus´*-like species from Bosnia and Herzegovina, namely *Squalius cephalus, S. albus, L. idus, L. leuciscus, T. souffia, T. turskyi* and *S. svallize* from the area of Adriatic basin was conducted by Sofradžija (1977). This and some other cytogenetic studies showed that all investigated species have an invariable diploid chromosome number 2n = 50 and very similar karyotype

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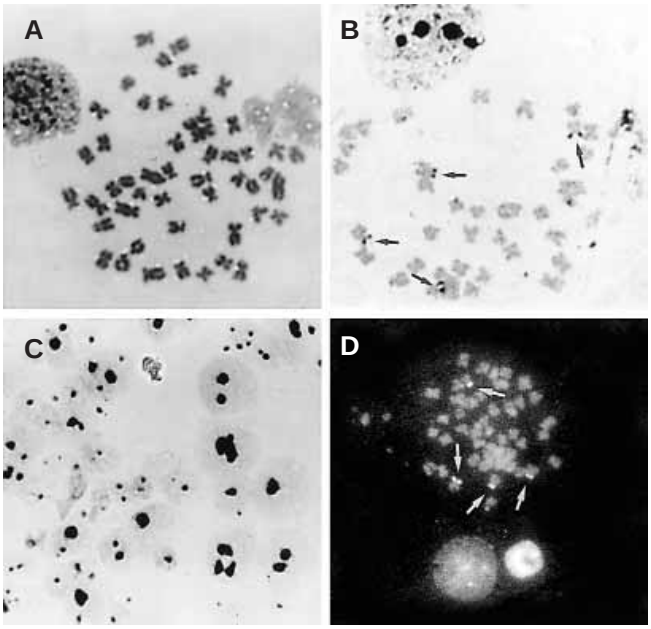

Fig. 1. *Metaphase plates (A, B, D) and inter-phase nuclei (C) of* Telestes ukliva *from Cetina River. Metaphase chromosomes after Giemsa staining (A), or staining with silver nitrate (B). Arrows indicate nucleolar organizer regions (NORs) on short arms of two submetacentric chromosome pairs. C) Interphase nuclei with different number and size of nucleoli after staining with silver nitrate. D) Metaphase chromosomes and interphase nuclei after CMA_3 staining. CMA_3-positive signals are terminally located on short arms of two submetacentric chromosome pairs (arrows). Interphase nuclei with four CMA_3-positive signals.*

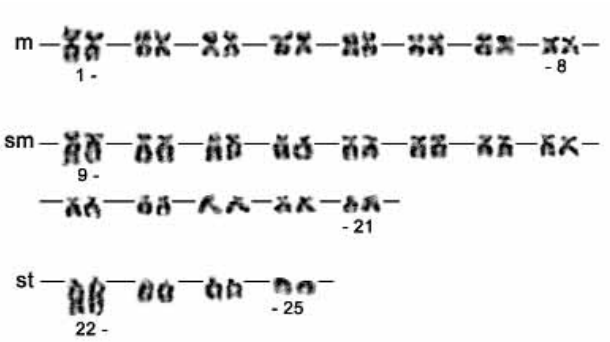

Fig. 2. *Karyotype of* Telestes ukliva *arranged from Giemsa-stained chromosomes.*

(Lieder 1954, Wolf et al. 1969, Fontana et al. 1970, Berberović & Sofradžija 1972, Sofradžija 1977, Collares-Pereira et al. 1998).

Giemsa, C-banding, fluorochrome and silver staining are the most widely used techniques for differential chromosome banding in fishes (e.g. Boroń 1999, Boroń 2001, Ocalewicz et al. 2004, Kirtiklis et al. 2005). Nucleolar organizer regions (NORs) are widely used chromosomal markers to infer inter- and intra-specific relationships in various fish taxa (e.g. Collares-Pereira & Ráb 1999, Boroń 2001). NORs in neotelosteans are associated with GC-rich heterochromatin and therefore easily detected by fluorescent staining with GC-specific antibiotic CMA_3 (Ráb et al. 1999). Silver staining of NORs is not only used as a chromosomal marker to characterize the karyotype, but also to detect transcriptionally active NORs (e.g. Birstein & Vasil'ev 1987, Gromicho & Collares-Pereira 2004). However, recent molecular studies show that not all sites of rDNA are detected with CMA_3 or silver staining (Pendás et al. 1993, Reed & Philips 1995, Ráb et al. 1996).

The aim of this study was to describe karyotype and some other chromosomal characteristics of the ukliva dace, including number and location of NORs using Ag-staining and CMA_3-fluorescence.

Material and Methods

Specimens sampled by electrofishing performed every two months in the Cetina River from 1997 to 2000 were analysed. A total of 10 unsexed juvenile specimens used for cytogenetic analysis were transported alive to the laboratory and kept in well-aerated aquarium until analysis. The specimens analyzed are deposited as vouchers in the Department of Zoology, Division of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia.

Mitotic chromosome preparations were obtained from fish kidneys and gills following modified method by Vitturi et al. (1986). Slides were examined under light microscope Opton III (Zeiss) and microphotographs were used for morphometric analysis. Chromosomes were classified according to Levan et al. (1964).

After destaining of Giemsa-stained slides with fixative and series of alcohol, those were stained with chromomycin A_3 (CMA_3) (Sigma) and counter-stained with 4,6-diamidino-2-phenylindole (DAPI) (Sigma) according to the modified method of Schweizer & Ambros (1994). Fluorescence was observed under the fluorescent microscope Opton Axioplan (Zeiss). Modified method described by Hizume et al. (1980) was used for the silver staining of chromosomes and nucleoli. For nucleoli, a total of 735 cells from 4 specimens were analyzed.

Results

The chromosome complement in all investigated specimens of *T. ukliva* possessed diploid chromosomes

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number 2n = 50 (Fig. 1A and 2). The karyotype consisted eight pairs of metacentric (m), 13 pairs of submetacentric (sm) and four pairs of subtelocentric (st) chromosomes (Fig. 2). Total number of chromosome arms in the complement was NF = 92. The largest chromosome pair of the complement was subtelocentric, which is a characteristic cytotaxonomic marker for all representatives of the cyprinid lineage Leuciscinae.

Staining with CMA_3 revealed CMA_3-positive signals at the terminal position of the short arms of two sm chromosome pairs (Fig. 1D). Differences in size of CMA_3-positive signals between homologous chromosomes were not detected. Four CMA_3-positive signals were also recorded in interphase nuclei.

DAPI staining did not reveal any DAPI-positive signals on *T. ukliva* chromosomes (picture not shown). Silver staining revealed four positive NOR sites located terminally on the short arms of two sm pairs of chromosomes, which corresponded to four CMA_3-positive signals. In most cells, all four NORs were active and of similar size (Fig. 1B). After silver staining, the interphase cells with one to five nucleoli were recorded (Fig. 1C). Cells with one (31.97%) and two (31.16%) nucleoli were the most frequent, indicating the association of NORs, while the number of cells with five nucleoli was very low (2.04%). Cells with three and four nucleoli were somewhat less frequent, 23.40% and 11.43% respectively.

Discussion

The chromosome number 2n = 50 and the karyotype of species under study corresponds entirely to that found to be characteristic for representatives of the subfamily Leuciscinae examined as yet, including easily recognizable largest pair of chromosomes – cytotaxonomic leuciscine marker (Ráb et al. 2008 and references therein). The largest m and smallest m pairs of chromosomes are also easily recognizable in all species investigated. Results of extensive cytogenetic study by Sofradžija (1977) correspond with findings in our paper. Morphologically differentiated sex chromosomes were not observed, what could be explained by the fact that the assignment of particular chromosome into chromosome categories in cyprinids is sometimes very difficult due to their small size and hence identification of possible sex chromosomes is problematical without other genetic tools (Ráb & Collares-Pereira 1995).

Differential staining of chromosomes with CMA_3 indicated four positive CMA_3-signals located terminally on the short arm of two pairs of sm chromosomes. It should be mentioned that not all CMA_3 positive sites represent rDNA cistrons, but indicate either transcriptionally inactived rRNA loci or just GC-rich heterochromatic blocks (Collares-Pereira & Ráb 1999), as well as not all rDNA sites are detected with CMA_3 or silver staining, what some authors interpreted as a polymorphism (Boroń 2001).

Silver staining revealed four AgNORs corresponding to CMA_3-positive signals. In the most of the interphase cells, one to four nucleoli were observed, indicating various levels of rRNA activity. The association of NORs, which results from the formation of less than the maximum number of nucleoli, is also characteristic of NOR behaviour in the genomes of other fish species (e.g. Woznicki et al. 2000). Silver staining also showed that there was no size difference of positive Ag-NOR signals between homologous and nonhomologous NOR-bearing chromosomes. In a small number of interphase nuclei, five nucleoli were detected. This finding points to the possibility that an additional rDNA locus exists, which was not detected either by CMA_3 or silver staining of metaphase chromosomes.

Presently, the number and location of NORs is frequently used in fish descriptive cytotaxonomy as a useful marker (e.g. Boroń 2001, Rábová et al. 2001, Rábová et al. 2003, Gromicho & Collares-Pereira 2004). In most analyzed European leuciscins, one NOR-bearing sm to st chromosome pair has been detected (e.g. Ráb & Collares-Pereira 1995, Rábová et al. 2003, Bianco et al. 2004). This character is considered as ancestral. Multiple NORs have been considered as a derived character and has been reported in several leuciscine and phoxinine European species (Rodriges & Collares-Pereira 1996, Boroń et al. 1997, Collares-Pereira & Ráb 1999, Boroń 2001, Bianco et al. 2004, Gromicho & Collares-Pereira 2004, Gaffaroglu et al. 2006).

To reliably establish the exact number of rDNA loci, fluorescence *in situ* hybridisation (FISH) with the appropriate probe for rDNA detection on chromosomes has to be applied. This technique was successfully applied in determination of number and position of rDNA loci in a various fish species, including those from Cyprinidae family (e.g. Collares-Pereira & Ráb 1999, Rábová et al. 2003, Ocalewicz et al. 2004).

Staining with DAPI did not show any positive signals (AT-rich heterochromatin regions) along *T. ukliva* chromosomes. The same results were found for chromosomes of other cyprinid species (Mayr et al.

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1986, Ocalewicz et al. 2004) and together with other findings it could be concluded that this is another common feature for genomes of cyprinids. The minor differences in the karyotype between *T. ukliva* from the Cetina River and those of other species from Bosnia and Herzegovina indicate that speciation of these species, isolated from the last glaciation period was accompanied by certain, but at present level of detection nearly invisible chromosomal rearrangements. In addition, FISH experiments have to be included in future studies in order to determine the exact number and position of NORs, since this chromosome marker seems to be the most informative in the reconstruction of evolutionary events and relationships between closely related fish species at the chromosome level (Phillips & Reed 1996, Collares-Pereira & Ráb 1999, Rábová et al. 2001, Fontana et al. 2003, Rábová et al. 2003).

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