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

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**Abstract.** The karyotype and major ribosomal sites as revealed by silver staining and CMA<sub>3</sub>-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 subtelo-centric chromosomes. The largest chromosome pair of the complement was subtelo-centric, 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<sub>3</sub> 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.

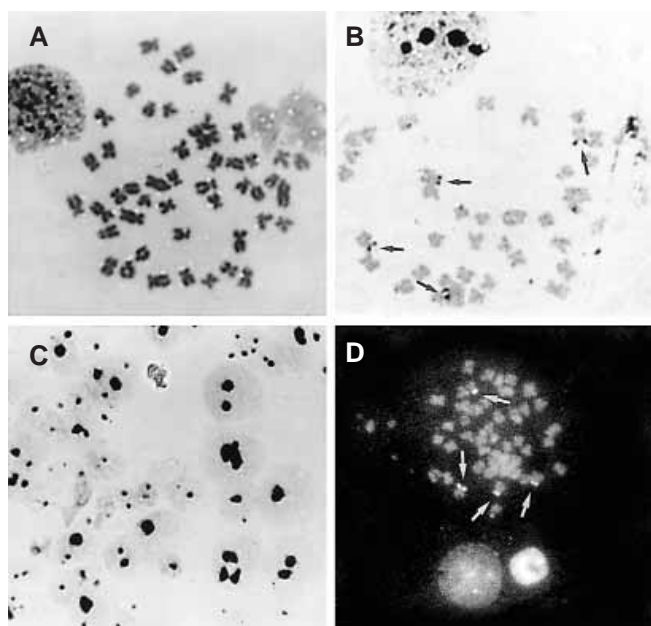
**Key words:** fish cytogenetics and cytotaxonomy, chromosome banding, multiple nucleolar organizer region (NOR), leuciscine cyprinids

## Introduction

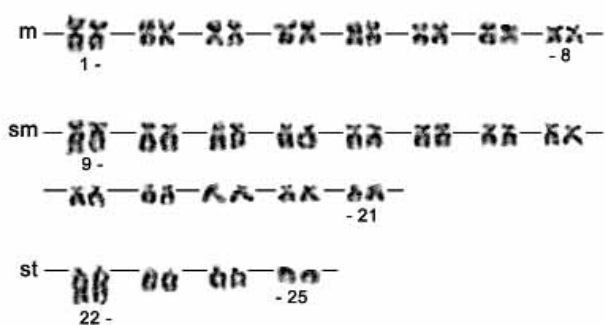
Ten freshwater fish species locally endemic to the Adriatic basin in Croatia includes also the ukliva dace, *Telestes ukliva* (Heckel, 1843). This species is present throughout the Cetina River, with the exception of the river mouth. Most species of genus *Telestes* occur in the eastern Adriatic drainages and suffer from habitat alterations and losses. 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



**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<sub>3</sub> staining. CMA<sub>3</sub>-positive signals are terminally located on short arms of two submetacentric chromosome pairs (arrows). Interphase nuclei with four CMA<sub>3</sub>-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 neoteloosteans are associated with GC-rich heterochromatin and therefore easily detected by fluorescent staining with GC-specific antibiotic CMA<sub>3</sub> (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<sub>3</sub> 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<sub>3</sub>-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<sub>3</sub> (CMA<sub>3</sub>) (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

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 inactivated 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 (Rodrigues & 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.

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. ukliwa* 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).

## Literature

- Berberović Lj. & Sofradžija A. 1972: Pregled podataka o hromosomskim garniturama slatkovodnih riba Jugoslavije [A review of the karyological data regarding freshwater fishes of Jugoslavia]. *Acta Biol. Jug.-Ichthyologica* 4 (3): 1–21. (in Serbo-Croatian with English summary)
- Bianco P.G., Aprea G., Balletto E., Capriglione T., Fulgione D. & Odierna G. 2004: The karyology of the cyprinid genera *Scardinius* and *Rutilus* in southern Europe. *Ichthyol. Res.* 51: 274–278.
- Birstein V.J. & Vasil'ev V.P. 1987: Nucleolar organizer regions, their function and polyploidy in fishes. *J. Obshchey Biol.* 6: 729–747.
- Boroń A. 1999: Banded karyotype of spined loach *Cobitis taenia* and triploid *Cobitis* from Poland. *Genetica* 105: 293–300.
- Boroń A. 2001: Comparative chromosomal studies on two minnow fish, *Phoxinus phoxinus* (Linnaeus, 1758) and *Eupallasella perenurus* (Pallas, 1814); an associated cytogenetic-taxonomic considerations. *Genetica* 111: 387–395.
- Boroń A., Jankun M. & Kuszniarz J. 1997: Chromosome study of swamp minnow *Eupallasella perenurus* (Dybowski, 1916) from Poland. *Caryologia* 50: 85–90.
- Collares-Pereira M.J., Próspero M.I., Biléu R.I. & Rodrigues E.M. 1998: *Leuciscus* (Pisces, Cyprinidae) karyotypes: Transect of Portuguese populations. *Genetics and Molecular Biology* 21 (1): 63–69.
- Collares-Pereira M.J. & Ráb P. 1999: NOR polymorphism in the Iberian species *Chondrostoma lusitanicum* (Pisces: Cyprinidae) – re-examination by FISH. *Genetica* 105: 301–303.
- Fontana F., Chiarelli B. & Rossi A.C. 1970: Il cariotipo di alcune specie Cyprinidae, Centrarchidae, Characidae studiate mediante colture “in vitro”. *Caryologia* 23: 549–564.
- Fontana F., Lanfredi M., Congiu L., Leis M., Chicca M. & Rossi R. 2003: Chromosomal mapping of 18S-28S and 5S rRNA genes by two-colour fluorescence in situ hybridization in six sturgeon species. *Genome* 46: 473–477.
- Freyhof J., Lieckfeldt D., Bogutskaya N.G., Pitra C. & Ludwig A. 2006: Phylogenetic position of the Dalmatian genus *Phoxinellus* and description of the newly proposed genus *Delminichthys* (Teleostei: Cyprinidae). *Mol. Phylogenet. Evol.* 38: 416–425.
- Gaffaroglu M., Yuksel E. & Ráb P. 2006: Note on the karyotype and NOR phenotype of leuciscine fish *Acanthobrama marmid* (Osteichthyes, Cyprinidae). *Biologia* 61: 207–209.
- Gold J.R. & Avise J.C. 1977: Cytogenetic studies of North American minnows (Cyprinidae). I. Karyology of nine California genera. *Copeia* 1977: 541–549.
- Gromicho M. & Collares-Pereira M.J. 2004: Polymorphism of major ribosomal gene chromosomal sites (NOR-phenotypes) in the hybridogenetic fish *Squalius alburnoides* complex (Cyprinidae) assessed through crossing experiments. *Genetica* 122: 291–302.
- Hizume M., Sato S. & Tanaka A. 1980: A highly reproducible method of nucleolus organizing regions staining in plants. *Stain Technol.* 55: 87–90.
- Kirtiklis L., Boroń A. & Porycka K. 2005: Chromosome banding patterns of the gudgeon, *Gobio gobio* (Actinopterygii, Cyprinidae). *Acta Ichthyologica et Piscatoria* 35 (2): 119–123.
- Kottelat M. & Freyhof J. 2007: Handbook of European freshwater fishes. *Kottelat, Cornol, Switzerland and Freyhof, Berlin, Germany.*
- Levan A., Fredga K. & Sandberg A.A. 1964: Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201–220.

- Lieder U. 1954: Chromosomenstudien an Knochenfischen. II. Über die Chromosomenzahl und Morphologie der Plötze (*Leuciscus rutilus* L.) und einiger ihrer Bastarde mit anderen Cypriniden. *Z. Fischerei Hilfswiss.* 3: 479–488.
- Mayr B., Ráb P. & Kalat M. 1986: NORs and counterstain-enhanced fluorescence studies in Cyprinidae of different ploidy level. *Genetica* 69: 111–118.
- Ocalewicz K., Jankun M. & Boroń A. 2004: Karyotypic characterization of bream, *Abramis brama* (Pisces, Cyprinidae). *Folia Zool.* 53: 329–334.
- Pendás A.M., Morán P. & Garcia-Vázquez E. 1993: Multi-chromosomal location of ribosomal RNA genes and heterochromatin association in brown trout. *Chromosome Res.* 1: 63–67.
- Phillips R.B. & Reed K.M. 1996: Application of fluorescence in situ hybridisation (FISH) techniques to fish genetics: a review. *Aquaculture* 140: 197–216.
- Ráb P. & Collares-Pereira M.J. 1995: Chromosomes of European cyprinid fishes (Cyprinidae: Cypriniformes): a review. *Folia Zool.* 44: 193–214.
- Ráb P., Karakousis Y., Rábová M. & Economidis P.S. 1996: Banded karyotype of cyprinid fish *Leuciscus borysthenicus*. *Ichthyol. Res.* 43: 463–468.
- Ráb P., Rábová M., Pereira C.S., Collares-Pereira M.J. & Pelikánová Š. 2008: Chromosome studies of European cyprinid fishes: interspecific homology of leuciscine cytotoxic marker – the largest subtelocentric chromosome pair as revealed by cross-species painting. *Chromosome Res.* 16: 863–873.
- Ráb P., Rábová M., Reed K.M. & Phillips R.B. 1999: Chromosomal characteristics of ribosomal DNA in the primitive semionotiform fish, longnose gar *Lepisosteus osseus*. *Chromosome Res.* 7: 475–480.
- Rábová M., Ráb P. & Ozouf-Costaz C. 2001: Extensive polymorphism and chromosomal characteristics of ribosomal DNA in a loach fish, *Cobitis vardarensis* (Ostariophysi Cobitidae) detected by different banding techniques and fluorescent *in situ* hybridisation (FISH). *Genetica* 111: 413–422.
- Rábová M., Ráb P., Ozouf-Costaz C., Ene C. & Wanzenböck J. 2003: Comparative cytogenetics and chromosomal characteristics of ribosomal DNA in the fish genus *Vimba* (Cyprinidae). *Genetica* 118: 83–91.
- Reed K.M. & Phillips R.B. 1995: Molecular cytogenetic analysis of the double-CMA<sub>3</sub> chromosome of lake trout, *Salvelinus namaycush*. *Cytogenet. Cell. Genet.* 70: 104–107.
- Rodrigues E.M. & Collares-Pereira M.J. 1996: NOR polymorphism in the Iberian species *Chondrostoma lusitanicum* (Pisces, Cyprinidae). *Genetica* 98: 59–63.
- Schweizer D. & Ambros P.F. 1994: Chromosome banding. In: Gosden J.R. (ed.), *Methods in molecular biology*. Vol 29: Chromosome analysis protocols. *Humana Press Inc., Totowa, New York*: 97–112.
- Sofradžija A. 1977: Kariologija i citotaksonomija vrsta roda *Leuciscus* iz voda Bosne i Hercegovine [Karyology and cytotaxonomy of the *Leuciscus* species from the waters in Bosnia and Herzegovina]. *Godišnjak Biol. Inst. Univ. u Sarajevu* 30: 113–211. (in Serbo-Croatian with English summary)
- Vitturi R., Catalano E., Macaluso M. & Zava B. 1986: Karyotypes of nine species of the family Labridae (Pisces, Perciformes). *Biol. Zbl.* 108: 223–226.
- Wolf U., Ritter H., Atkin N.B. & Ohno S. 1969: Polyploidisation in the fish family Cyprinidae, order Cypriniformes. I. DNA content and chromosome sets in various Cyprinidae. *Humangenetik* 7: 240–244.
- Woznicki P., Sanchez L., Martinez P., Pardo B.G. & Jankun M. 2000: A population analysis of the structure and variability of NOR in *Salmo trutta* by Ag, CMA<sub>3</sub> and ISH. *Genetica* 108: 113–118.