

KARYOTYPE ANALYSIS OF *Barbus barbus* L. AND *Barbus peloponnensis* V. (Cyprinidae)
AND FREQUENCIES OF BREAKS AND GAP-TYPE STRUCTURAL CHROMOSOME
CHANGES IN FISHES FROM THE RIVER VAPA

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The chromosomes of *B. barbus* L. and *B. peloponnensis* V. from the river Vapa were cytogenetically studied. *B. barbus* has a diploid chromosome number $2n=100$. The karyotype consists of 30 metacentrics, 18 submetacentrics and subacrocentrics and 52 acrocentric chromosomes. *B. peloponnensis* V. also has the same diploid chromosome number $2n=100$, but the karyotype contains 10 metacentrics, 44 submetacentrics subacrocentrics and 46 acrocentric chromosomes.

The frequencies of the breaks and gap-type structural chromosome aberrations were analysed. Relatively low frequencies were detected (lower than 1%) and they were at the level that might be found in the laboratory animal controls and in the tissue culture controls. The data obtained indicate that in the environmental conditions of the fishes studied, there were no genotoxic agents which could express their effects on the chromosomes of these living organisms.

Key words: karyotype, chromosomes, pisces, genotoxicity, chromosomal aberration, *Barbus barbus* L., *Barbus peloponnensis* V.

INTRODUCTION

It is well known that the fish species *Barbus barbus* L. and *Barbus peloponnensis* V. (Cyprinidae) are of tetraploid origin. The literature contains data with regard to duplicate content of nuclear DNA and obviously duplicated chromosome number in respect to these fish species (Ohno et al., 1967), as well as duplicated gene expressions (Klose et al., 1969; Enel et al., 1971; Trintaphyllidis et al., 1971). Polyploidisation of the whole genome seems to have occurred independently in the genera *Cyprinus*, *Barbus* and *Carassius*, in the family Cyprinidae (Kirpichnikov, 1973). The tetraploidisation was not autopolyploidisation of ancestral genomes (Allendorf and Thorgaard, 1984). Many investigators have worked on phylogenetic relationships among the

species of *Barbus* and their migrations (Karakousis et al., 1995; Berrebi, 1995; Machordomm et al., 1995). Berrebi (1995) reported the existence of an inter-continental gradient frequently having different levels of ploidy that include diploid, tetraploid and hexaploid species. He suggested an integrated study of genetics, karyology, paleontology, morphology and parasitology as the means of assessing the value of various evolutionary hypotheses proposed in the literature.

One of the aims of this work was to detect eventual structural changes on the chromosomes in fishes from the river Vapa.

Various chemicals can initiate damage to chromosome structures and detection of these effects of their actions point to their presence in the environment. Industrial chemical products can spread in nature together with various toxic materials disposed of without control in the neighbourhood of large towns and industrial plants. Changes on chromosomes in fishes from contaminated waters indicate their possible presence at some localities of free waters in Serbia (Fišter, 1992; Fišter et al., 1994; Fišter et al., 1997; Fišter et al., 1998).

The objective of this work was to detect the frequencies of breaks and gaps in fishes from relatively clean waters in the river Vapa on the Pešter Plateau as such data could be useful for comparison with those obtained for fishes from other waters contaminated with toxic and genotoxic agents.

MATERIAL AND METHODS

The chromosomes of two fish species *Barbus barbus* L. and *Barbus peloponnensius* V. (Cyprinidae) were analysed in specimens caught from the river Vapa from the Pešter Plateau. Mitotic metaphase chromosomes were obtained from kidney tissue by preparation according to Fontana et al. (1970). About 30-35 metaphase figures of chromosomes were examined in every individual fish and the mean values of the mitotic indexes were determined.

The characteristic property of the karyotype of these fish species was determined (number and morphology of chromosomes) and the frequencies of structural chromosome changes, breaks and gaps calculated.

RESULTS

The cytogenetical analysis of five specimens of the fish species *Barbus barbus* L. (Cyprinidae) is given in Table 1. The diploid chromosome number in all individuals examined was $2n=100$ (Figure 1).

On the basis of their morphology, the chromosomes were arranged in the following groups: 15 pairs of metacentrics, i. e. 30 metacentric (M) chromosomes; 9 pairs of submetacentric and subacrocentrics (SM, SA), i. e. 18 submetacentrics and subacrocentrics and 26 pairs of acrocentrics (A), i. e. 52 acrocentric chromosomes (Figure 1). Hence, the fundamental chromosome arm number (NF) equals 148.

Figure 1. Karyotype of the fish species *Barbus barbus* L.Table 1. Frequencies of chromosome breaks and gaps and mitotic Index values in fishes (*Barbus barbus* L. and *Barbus peloponnensis* V.) from the river Vapa

<i>Barbus barbus</i>				<i>Barbus peloponnensis</i>			
No.	Mitoses examined	Breaks and gaps	Mitotic Index	No.	Mitoses examined	Breaks and gaps	Mitotic Index
1.	30	—	4.26	1.	30	—	3.42
2.	35	—	4.31	2.	30	—	4.32
3.	35	—	3.97	3.	30	1	3.92
4.	30	1	3.81	4.	30	—	3.78
5.	30	—	4.12	5.	30	—	4.14
				6.	30	—	3.62
Total	160	1	20.47	Total	180	1	23.20
		0.62%	$\bar{X}=4.09$			0.55%	$\bar{X}=3.68$

A total of 160 metaphase figures of chromosomes was examined for this fish species and 0.62% breaks and gap-type changes were detected (Table 1; Figure 3). The mean value of the mitotic index was 4.09 (Table 1; Figure 4).

The cytogenetical analysis of six individuals of the fish species *Barbus peloponnensis* V. is also shown in Table 1. In all of them the diploid chromosome number was $2n = 100$ (Figure 2).

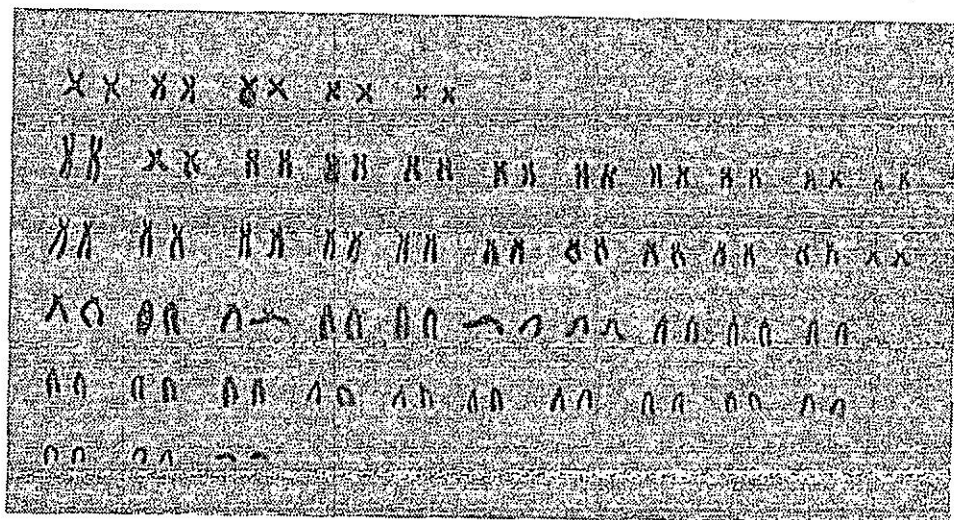
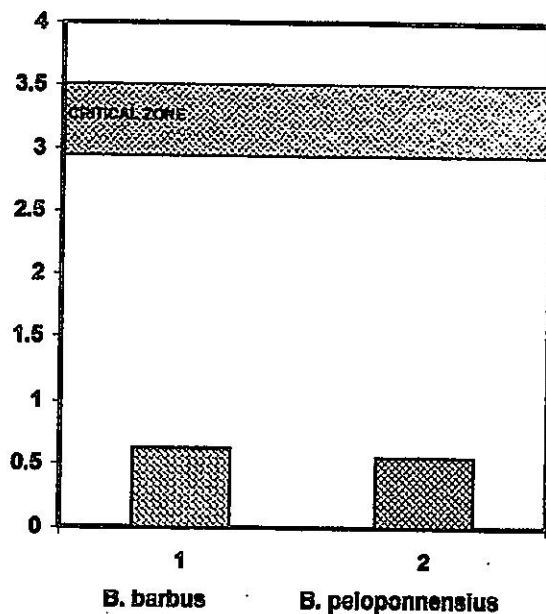
Figure 2. Karyotype of the fish species *Barbus peloponnensis* V.

Figure 3. Frequencies of chromosome breaks and gaps in fishes from the river Vapa

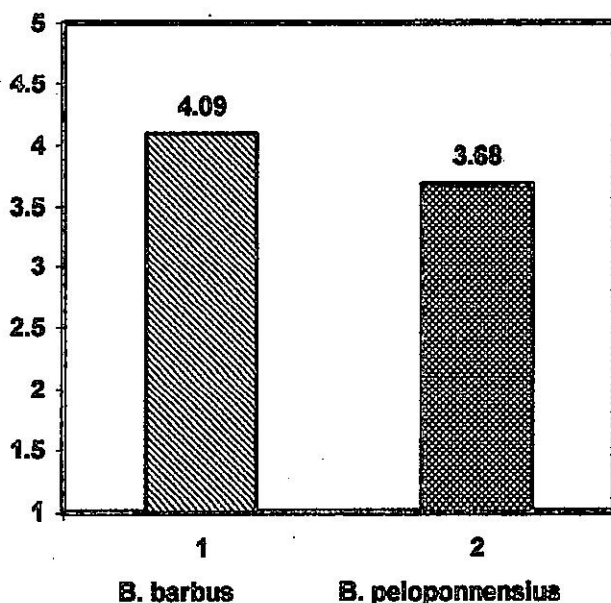


Figure 4. Mean values of mitotic index in fishes from the river Vapa

According to their morphology, the chromosomes were grouped in the following classes: five pairs of metacentric (M), i. e. 10 metacentric chromosomes; 22 pairs of submetacentric and subacrocentric (SM, SA) chromosomes, i. e. 44 submetacentrics and subacrocentrics and 23 pairs of acrocentric (A) chromosomes, i. e. 46 acrocentrics. The fundamental arm number (NF) equaled 154 (Figure 2).

In this fish species a total 180 mitotic metaphase figures of the chromosomes was examined and 0,55% breaks and gap-type changed were found (Table 1; Figure 3). The mean value of the mitotic index was 3.68 (Table 1; Figure 4).

DISCUSSION

Barbus barbus L. (Cyprinidae) in Serbia has been found in free waters of Vojvodina e. g. in the Danube, but *Barbus peloponnesius* V. has been found only in higher regions and in mountain free waters such as the river Vapa on the Pešter Plateau.

The results obtained for the karyotype analysis in the fish species *Barbus peloponnensis* V. were in accordance with the data presented by Berberović and Sofradžija (1972), Sofradžija and Berberović (1973), Cataudella et al. (1977), Vujošević et al. (1983) and Fišter (1992), but in these papers the species was named as *Barbus meridionalis petenyi* H. It is obvious now that the name earlier used, was wrong, because the species *Barbus meridionalis* does not occur in the areas of our country.

The total chromosome number was $2n=100$, the same in both species and about half of the chromosomes were acrocentrics; 52 in *Barbus barbus* L. (NF=148) and 46 in *Barbus peloponnensis* V. (NF=154).

The analysis of the structural changes, breaks and gaps showed that the frequencies of those changes were lower than the proposed level of the critical area (3,0-3,5%); the criteria being presented in our previous papers (Fišter, 1992; Fišter et al., 1994; Fišter et al., 1996; Fišter et al., 1997; Fišter et al. 1998).

The values were in the area of spontaneously occurring changes, that might be found in the controls of untreated laboratory animals and the controls of tissue cultures.

The mean values of mitotic indexes in both species were higher than the values obtained in fishes from contaminated waters, caught at some localities on the rivers Sava and Danube (Fišter, 1992; Fišter et al., 1994; Fišter et al., 1996; Fišter et al., 1997; Fišter et al., 1998). A decrease in mitotic activity might be a useful indicator of the presence of toxic materials, i. e. contaminated waters.

It may be possible to confirm that in the water environment of the fishes studied, i. e. in the river Vapa on the Pešter Plate there were no genotoxic agents which could express their effects in the chromosomes of these living organisms.

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**ANALIZA KARIOTIPA *Barbus barbus* L. i *Barbus peloponnensius* V. (Cyprinidae) i
UČESTALOST STRUKTURNIH HROOZOMSKIH PROMENA TIPa GAPA i PREKIDA KOD
RIBA IZ REKE VAPE**

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SADRŽAJ

Analiziran je kariotip dve vrste riba familije Cyprinidae, *Barbus barbus* L. (rečne mreene) i *Barbus peloponnensius* V. (potočne mreene), kod jedinki uhvaćenih u planinskoj reci Vapi na Peštorskoj Visoravni. Kariotip obe ispitivane vrste čini diploidan broj $2n=100$ hromozoma. Vrsta *Barbus barbus* L. ima 15 pari metacentrika, tj. 30 metacentričnih hromozoma; 9 pari submetacentrika i subakrocentrika, tj. 18, submetacentričnih i subakrocentričnih hromozoma i 26 pari akrocentrika, tj. 52 akrocentrična hromozoma. Vrednost broja kraka (NF) iznosi 148. Vrsta *Barbus peloponnensius* V., ima pet pari metacentrika, tj. 10 metacentričnih hromozoma; 22 parosubmetacentrika i subakrocentrika, tj. 44 submetacentrična i subakrocentrična hromozoma i 23 para akrocentrika, tj. 46 akrocentričnih hromozoma. Vrednost broja kraka (NF) iznosi 154.

Analizirana je i učestalost promena tipa gapa i prekida na metafaznim hromozomima obe vrste. Ustanovljeno je da je učestalost ovih promena kod riba iz relativno čiste, nezagađene planinske reke Vape bila vrlo niska, niža od 1%. Vrednosti učestalosti ovih promena ni u jednom slučaju nisu prešle nivo njihovog - spontanog dešavanja, tj. nivo koji se normalno očekuje u kontrolama kod laboratorijskih životinja ili u kontrolama kultura tkiva. Ove vrednosti su bile daleko ispod predpostavljene kritične zone (3,0-3,5% promena), pa se zaključuje da je sredina u kojoj ovi organizmi žive bez povišenog genetičkog rizika u odnosu na moguće prisustvo zagađenja genotoksičnim agensima. Vrednosti mitotskog indeksa više su od onih ustanovljenih kod riba iz vrlo zagađenih voda, pa bi mogle delimično da ukažu da u reci Vapi na Peštorskoj Visoravni, nisu prisutne ni druge toksične materije koje bi mogle ugroziti žive organizme.