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COMPARATIVE KARYOTYPE IN DIFFERENT LINEAGES OF CYPRINID FISH (TELEOSTEI: CYPRINIFORMES: CYPRINIDAE)

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ABSTRACT. The analysis of the karyotype was performed through the investigation of the number and structure of the cyprinid fish species chromosomes. The fish used in this study were caught in the Danube River. For the subsequent analysis of karyotypes, the fish were injected intra-peritoneal with doses of 0.02 ml/g body weight of 0.1% colchicine solution and left for 180 min before sacrificing. The karyotype conservation of the Romanian genera *Leuciscinae* and *Barbinae* subfamilies was determined by metaphase investigation. The study also emphasized that the *Abramis bjoerka* and *Scardinius erythrophtalmus* are phylogenetically close, belonging to the *Leuciscinae* lineage, while the *Barbus barbus* species belongs to a different group of cyprinids, the *Barbinae* lineage.

Keywords: karyotype, cytotaxonomy of cyprinids, *Scardinius erythrophthalmus, Abramis bjoerka, Barbus barbus*

INTRODUCTION

The Cyprinids are one of the most wide spread species of freshwater fish in Romania. The most complete characterization of the cyprinid species in Romania (21 genera) was undertaken by Banarescu in 1964 based on morphological data. Since morphological data classifications are no longer considered cutting edge, chromosomal analysis is used for genetic studies and for taxonomy and phylogeny research. The division stage best suited for the karyotype analysis is the mitotic metaphase, when chromosomes present the maximum point of condensation and colorability. Chromosomes were classified according to Levan, Fredga & Sandberg (1964).

The karyotypes of diploid cyprinids are characterized by relatively small chromosomes with centromere positions placed gradually from a median to a nearly terminal position. Studies undertaken so far regarding the karyotype of cyprinids have shown a very low variability. A typical karyotype for the cyprinids consists of 6-8 pairs of metacentric chromosomes (m), 12-17 pairs of submeta- and subtelocentric chromosomes (sm, st) and 3-4 pairs of acrocentric chromosomes (a). The largest chromosomes in the Eurasian *Leuciscinae* were included in this last category (Ràb, 1991; Ràb and Collares-Pereira, 1995).

This study reports on the karyotypes of Romanian fish species: *Scardinius erythrophthalmus, Abramis bjoerka* and *Barbus barbus.* We established that three Romanian genera of the subfamily *Leuciscinae* and the *Barbinae* are karyotypically conserved, nearly identical to that found in most other representatives of the Eurasian cyprinids (Muhammet Gaffaroglu et al., 2006). The phylogenetic relationships between these three species based on the karyotype analysis groups the *Scardinius* species with the *Abramis* species, while from the phylogenetic point of view *Barbus* species is more distant.

MATERIALS AND METHODS

Cyprinid fish species were caught in the Danube River in the Tulcea County (in the south-east of Romania). The fish were transported alive to the laboratory and kept in a well-aerated aquarium at 20-25^{0}C before analysis. Chromosomes were prepared directly from the head kidney. Thus, the first step was to inject the fish intra-peritoneal with doses of 0.02 ml/g body weight of 0.1% colchicine solution and allow the solution to penetrate the tissues for 180 min before sacrificing. The kidney tissues were removed and placed in hypotonic 0.75 M KCl solution for 20 min. Then they were fixed in fresh solution (3 part of 70% methanol: 1 part glacial acetic acid) for 40 min. Staining was performed with 4% Giemsa solution for 5 min. Observations and microphotographs were taken with a Olympus light microscope. Chromosomes were classified on the basis of the arm-length ratio.

RESULTS AND DISCUSSIONS

The karyotype of the *Leuciscus* species described in the literature (Ràb P., 1996) is very similar to that of other species analyzed, with few taxon-specific characteristics (Sofradzija, 1977; Mazik et al., 1986; Ràb & Collares-Pereira, 1995). The karyotypes in the cyprinid family have a relatively high conservative character (Rab and Collares-Pereira, 1995). Thus, regarding the observed interpopulation variability, *L. carolitertii* seems to be more homogeneous in terms of a karyological formula. However, the number of elements with terminal or almost terminal centromeres seems to be even more reduced in the Iberian chubs than in the known European *Leuciscus* taxa.

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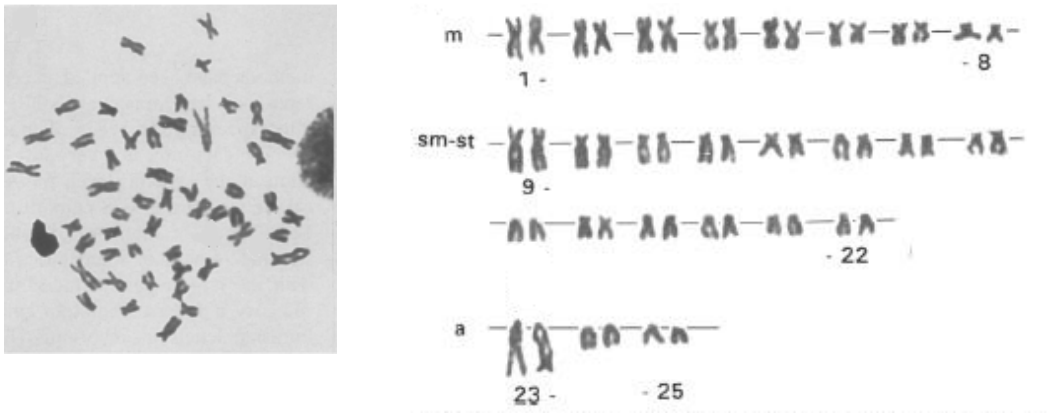
A.



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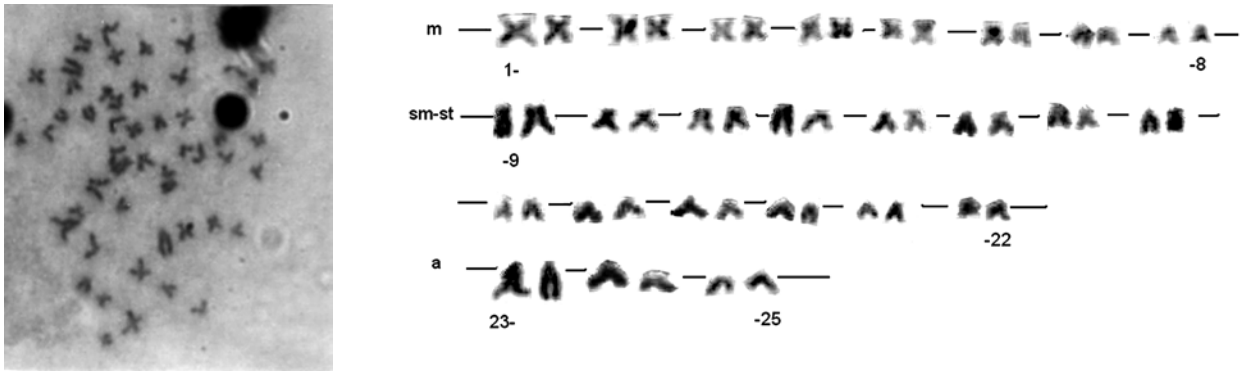

Fig. 1 Karyotypes of two cyprinid species arranged from Giemsa-stained chromosomes: m – metacentric, sm – submetacentric, st – subtelocentric and a – acrocentric chromosomes. **A**. *Leuciscus borysthenicus* (Ràb, P, 1996), **B**. *Scardinius erythrophthalmus (our investigated species).*

A.

B.

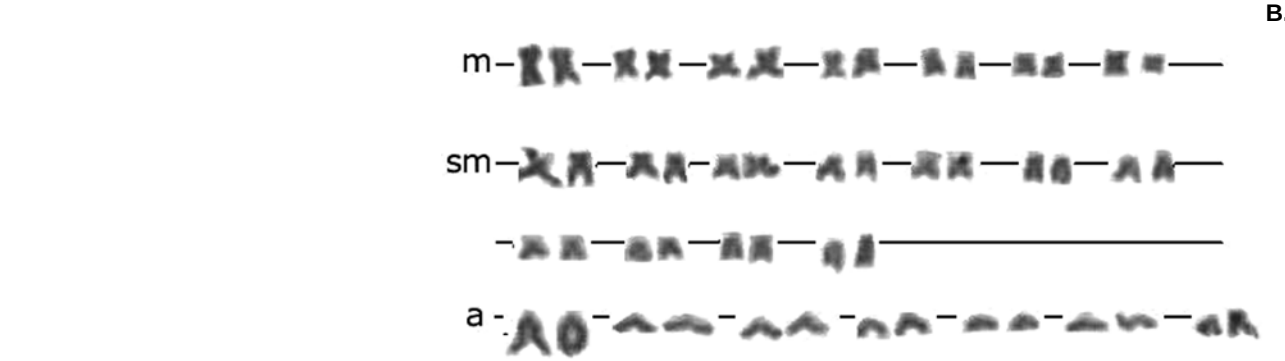

Fig. 2. Karyotypes of two cyprinid species arranged from Giemsa-stained chromosomes: m – metacentric, sm – submetacentric, st – subtelocentric and a – acrocentric chromosomes. **A**. *Abramis brama* (Ràb, P, 1996), **B**. *Abramis bjoerka (our investigated species).*

Scardinius erythropthalmus (Fig.1*)* obtained karyotype shows the same chromosome groups as the karyotype presented by Rab, (1996) for *Leuciscus borysthenicus* species, 2n=50, 16m (metacentric chromosomes), 28sm-st (submetacentric-subtelomeric chromosomes), 6a (acrocentric chromosomes), NF=86 (NF-number of chromosomial arms) respectively. Similar karyotypes were also discovered by Hellmer et al. (1991) among the *Leuciscinae* species: *Rutilus rutilus* and *Scardinius erythrophthalmus* through the same Giemsa-stained technique.

The karyotype described in the literature (Konrad Ocalewicz, 2004) for the bream species (*Abramis brama*) shows 7 pairs of metacentric chromosomes, 11 pairs of submetracentric chromosomes and 7 pairs of acrocentric chromosomes. The karyotype (Fig. 2) obtained for the silver bream (*Abramis bjoerka*) presents the same groups of chromosomes, 2n=50, 14m, 22sm, 14a, NF=86 respectively, as expected.

The detection of the heteromorphic sexual system in cyprinids is still a problem and it requires careful confirmation, especially for the groups with small chromosomes (Rab & Collares-Pereira, 1995). Females with heterogametes were discovered in the *Leuciscinae* from the Iberic peninsula. Vujosevic et al. (1983) identified sexual chromosomes (ZW/ZZ) in *Leuciscus cephalus* in the Danube River; the W chromosome in the Iberic species seems to be the largest Sm chromosome (Fig. 3).

According to the data in the literature (Rab & Collares-Pereira, 1995), the first pair of submetacentric chromosomes in the karyotypes analyzed for the silver bream and bream may be sexual chromosomes (Fig.3), especially since the rudd and white bream individuals analyzed were females.

Fig. 3 Karyotypes of *Abramis bjoerka* (a) şi *Scardinius erythrophtalmus* (b) species obtained in this study. Sexual chromosomes are indicated by arrowheads (ZW/ZZ).

The species in the *Barbus* genus are generally identified as a polyphyletic group (Myers 1960, Howes 1987, Berrebi et al. 1996), including species with three levels of polyploidy: diploid, tetraploid (Wolf et al. 1969) and hexaploid (Oellermann & Skelton 1990, Golubstov & Krysanov 1993, Guegan et al. 1995). Polyploidy is generally considered an important diversification and speciation mechanism in eukaryotes (Ohno, 1970; Soltis & Soltis, 1999). The karyotypes of three *Barbus* species belonging to a small group of *Barbus senso lato* have been analyzed by Rab P. et al., (1995): *B. bigoniei* with the 2n=50, NF=96 karyotype, *B.ablabes* with the 2n=50, NF=98 karyotype and *B.macrops* with the 2n=50, NF=92 karyotype (Fig.4). The first pair of metacentric chromosomes was remarkably higher.

The *Barbus barbus* species analyzed by us showed a tetraploid karyotype (Fig.5) 4n=96 chromosomes distributed in the following groups: 12m, 38sm, 46a, NF=146. The origin of European tetraploid *Barbinae* has not been elucidated yet as is the case of the *Barbinae* in the southern area of Africa (Tsigenopoulos C.S. et al., 2002).

The majority of cyprinids present a karyotype rich in *m* and *sm* chromosomes (Lee G.Y. et al., 1986, Ueda T. et al., 2001), while the *Abramis bjoerka* and *Scardinius erythrophtalmus* species presented such a karyotype. The karyotypes of the two species are very similar:

Abramis: 2n=50, 14m, 22sm,14a, NF=86
Scardinius: 2n=50, 16m, 28m, 6a, NF=86

Based on the karyotype analysis, these species are phylogenetically close, although karyotypes comparative analysis studies are generally based on chromosome differential staining techniques (sequential C-banding, Ag-staining; Ueda & Kobayashi, 1990, Takai & Ojima, 1999, Inafuku et al., 2000; Kikuma et al., 2000, Ueda T. et al., 2001). The karyotype of the *Barbus* species is very different in composition and in NF: 4n=96, 12m, 38sm, 46a, NF=146 – from the evolutionary point of view this species is further away from the other two.

In conclusion, *Abramis bjoerka*, *Scardinius erythrophtalmus and Barbus barbus Romanian species* are karyotypically conserved, nearly identical to most other representatives of the Eurasian cyprinids. Based on the karyotype characteristics the *Abramis* and *Scardinius* species are close phylogenetically both belonging to the *Leuciscinae* lineage, while the *Barbus barbus* species is included in a different cyprinid group, the *Barbinae* lineage.

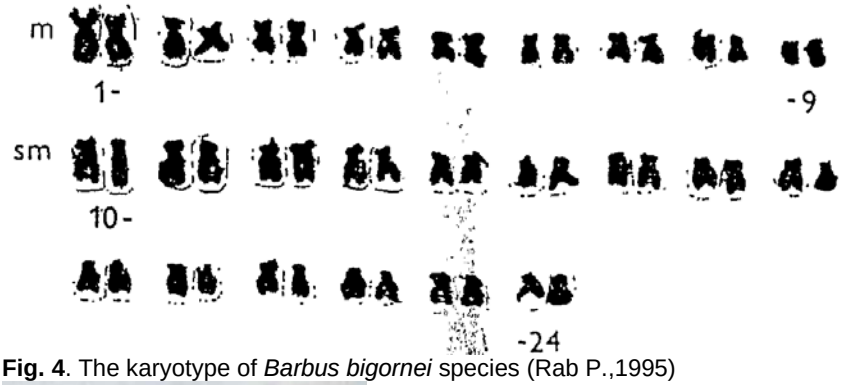

Fig. 4. The karyotype of *Barbus bigornei* species (Rab P.,1995)

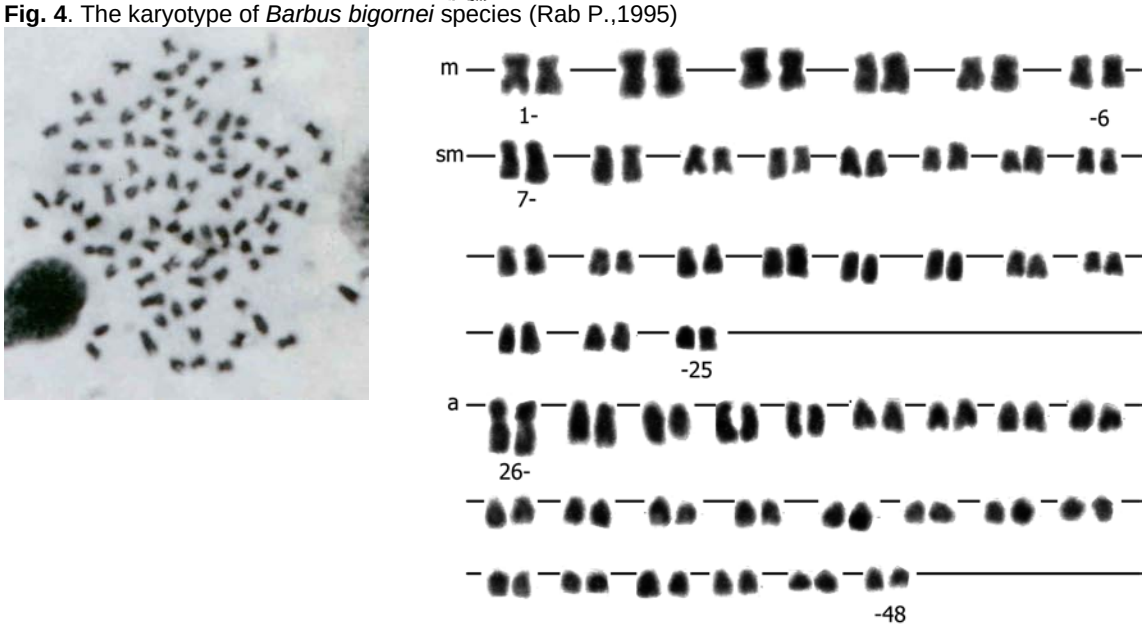

Fig. 5. Karyotypes of *Barbus barbus* obtained in this study, arranged by Giemsa-stained chromosomes: m – metacentric, sm – submetacentric, st – subtelocentric and a – acrocentric chromosomes.

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