Note on the karyotype and NOR phenotype of the cyprinid fish *Parachondrostoma arrigonis*

by

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ABSTRACT. - Karyotype and cytogenetic characteristics of critically endangered Iberian endemic cyprinid fish *Parachrondrostoma arrigonis* (Steindachner, 1866) were investigated using sequential C-banding, Ag- and CMA3-stainings in two specimens identified on morphological criteria. The diploid chromosome number was 2n = 50 with 8 pairs of metacentrics, 14 pairs of submetacentrics to subtelocentrics and 3 pairs of subtelo-acrocentrics. The largest chromosome pair of the complement was characteristically subtelo- to acrocentric. In the single analysed male, the nucleolar organizer regions (NORs) detected by Ag- and CMA3-stainings were in the telomeres of a single, middle-sized subtelocentric chromosome pair which is a pattern common in a number of other Leuciscinae. In the single analysed female, in which we could not confirm or reject interspecific hybridization, the NOR phenotype detected by these stainings showed at least seven positive sites on various chromosomes including characteristic large subtelo- to acrocentric pair of chromosomes. The CMA3-fluorescence revealed also in this female an entirely CMA3-positive arm, nature of such phenomenon (variation) is unknown at present. The complicated NOR phenotype of this specimen might suggest either hybrid situation or possible involvement of an association between mobile elements and rDNA loci. Karyotype structure homogeneity found for male of *P. arrigonis* with those in the most other representatives of the European leuciscine fishes may confirm karyotypic conservation within this cyprinid lineage.

RÉSUMÉ. - Note sur le caryotype en bandes du poisson cyprinidé *Parachondrostoma arrigonis*.

Le caryotype et les caractéristiques cytogénétiques de *Parachondrostoma arrigonis* (Steindachner, 1866), espèce endémique gravement menacée de la Péninsule Ibérique, ont été étudiés par marquage séquentiel des bandes C, positives au nitrate d'argent et à la chromomycine A3 (CMA3) chez deux spécimens identifiés sur des critères morphologiques. Le nombre diploïde est 2n = 50 (8 paires de chromosomes métacentriques, 14 paires de subméta-subtélocentriques, et 3 paires de subtélo-acrocentriques). La paire la plus grande est typiquement subtélo-acrocentrique. Chez le mâle examiné, les régions porteuses des organisateurs nucléolaires (NORs) révélées par le nitrate d'argent et la CMA3 sont situées sur les télomères d'une paire de chromosomes subtélocentriques de taille intermédiaire, comme souvent chez les Leuciscinae. Chez la femelle examinée, les NORs sont détectés sur au moins sept régions situées sur différentes paires, parmi lesquelles une paire caractéristique constituée de grands chromosomes subtélocentriques à acrocentriques. Curieusement, chez cette femelle la CMA3 colore la totalité d'un bras chromosomique. La complexité du phénotype NORs de cet individu peut résulter d'une situation d'hybridation interspécifique ou d'une association des gènes ribosomiques avec des éléments transposables mobiles. L'homogénéité entre le caryotype du spécimen mâle de *P. arrigonis* et ceux de la plupart des autres Leuciscinae d'Europe confirmerait la conservation du caryotype au sein de cette lignée de Cyprinidae.

Key words. - Cyprinidae - Leuciscinae - *Parachondrostoma* - *Chondrostoma* - Fish cytogenetics - Cytotaxonomy - NOR phenotype - Chromosome banding.

The cyprinid group knew formerly as genus *Chondrostoma* Agassiz, 1835 contains some 30 species distributed from Iberian Peninsula to the Ural Mountains as well as southward to the Middle East from Anatolia to Iran (Elvira, 1997). Although the actual species account remains unknown (*e.g.* Robalo *et al*., 2007), the highest species and lineage diversity exists in southern peninsulas (Doadrio and Carmona, 2004). Two main evolutionary lineages were identified within the genus: Iberian and Euro-Asian (Doadrio and Carmona, 2004). Recently Robalo *et al*. (2007) defined five new genera 1. *Achondrostoma*; 2. *Iberochondrostoma*; 3. *Pseudochondrostoma*; 4. *Parachondrostoma* and 5. *Protochondrostoma* represented by highly diverged lineage of '*Genei*', from southern and western slopes of the Alps.

Genus *Parachondrostoma* is represented by species *P. arrigonis* (Steindachner, 1866) together with closely related *P. miegii, P. turiense* and *P. toxostoma*. The species is endemic in the Júcar River basin in southeastern Spain,

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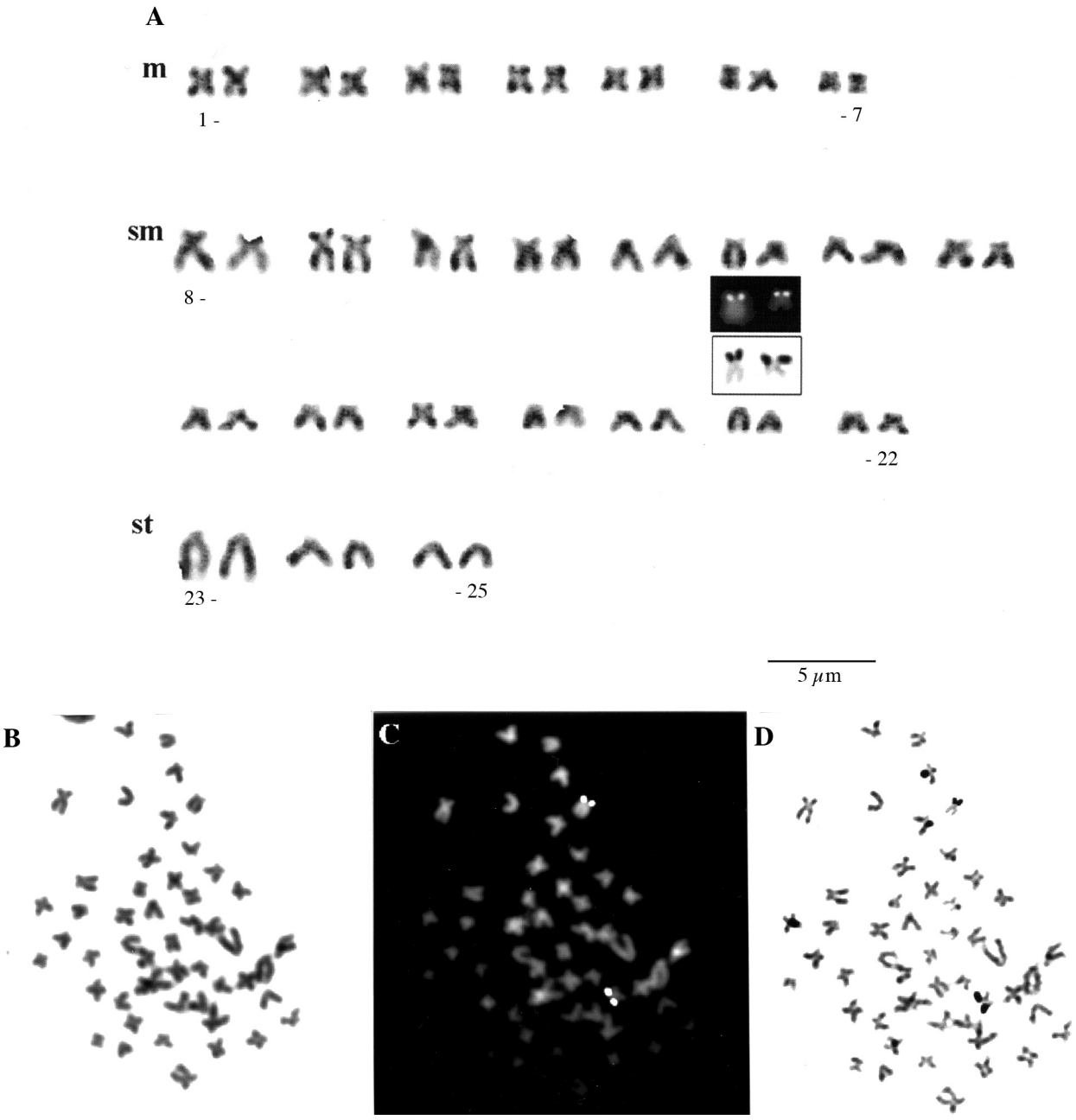

Figure 1. - Karyotype of male *Parachondrostoma arrigonis* arranged from Giemsa-stained chromosomes. Sequentially CMA_3-stained chromosomes with positive signals are shown in the second row and Ag-stained chromosomes with positive signals are shown in the third row. Sequentially Giemsa- (**B**), CMA_3- (**C**) and Ag- (**D**) stained metaphase from which the karyotype (**A**) was arranged. m: metacentric, sm: submetacentric, st: subtelocentric chromosomes. *[Caryotype d'un mâle* P. arrigonis *réalisé à partir des chromosomes colorés au Giemsa. Les chromosomes colorés positivement au CMA_3 sont présentés au deuxième rang, les chromosomes colorés positivement par l'Ag sont présentés au troisième rang. Le caryotype (**A**) a été réalisé à partir de métaphases colorées par le Giemsa (**B**), CMA_3 (**C**) et l'AG (**D**). m : métacentrique ; sm : submétacentrique ; st : subtélocentrique.]*

where its populations experienced dramatic decline in recent years. Although the precise data regarding actual extent of such decline of *P. arrigonis* are lacking, the field sampling in 2001 documented its presence in three localities, all in Cabriel River, a tributary of Júcar, out of 30 investigated and revealed its extremely low abundance (altogether 21 specimens from 1733 other fishes) (Jimenez and Lacomba, 2002). At present it thus occurs in a small fragment of its original 300 km^2 distribution in the Júcar River basin, Valencia and Castilla La Mancha provinces. The reasons for its disappearance were identified as drastic and large-scale alterations of rheophilic riverine habitat and competition with alien fish

species especially with *Pseudochondrostoma polylepis,* autochthonous originally in Tajo River basin. The species is therefore considered as critically endangered in Spain (Doadrio, 2001).

The chromosomes and other cytotaxonomic markers of these cyprinid fishes have been little studied. Ráb and Collares-Pereira (1995) reviewed all available older data based exclusively on conventionally Giemsa-stained chromosomes for 10 species of the genus. Revealed polymorphism regarding multiple NOR sites in *Iberochondrostoma lusitanicum* was confirmed as a fixed structural rearrangements of translocation type in the rDNA region from one ancestral NOR-bearing chromosome pair ubiquitous among leuciscine cyprinids to another pair of chromosomes (Collares-Pereira and Ráb, 1999).

This study reports on the karyotype of *P. arrigonis* analysed by means of sequential Giemsa-, CMA3 fluorescence and silver (Ag)-staining banding.

MATERIALS AND METHODS

Material examined

One male and one female were collected October 22nd 2003 during the ichthyological survey of the River Cabriel at the locality Casa del Pino (Valencia). After morphological determination made according to Doadrio (2001) and cytological analyses the specimens were deposited in the collection of the Museo Nacional de Ciencias Naturales, Madrid, under MNCN 246697 and MNCN 246698, respectively.

Chromosome preparation and banding

Standard direct procedure from head kidney and fibroblast culture from fin clips were used for chromosome preparation. CMA3-fluorescence and Ag-staining for detection of NORs detailed in Rábová *et al*. (2001) were employed.

At least 25 sequentially banded metaphase plates were examined per specimen. Chromosomes were classified according to Levan *et al*. (1964). 'Valid Animal Use Protocols' were in force at IAPG during this study.

RESULTS AND DISCUSSION

The diploid chromosome number in both specimens was 2n = 50. The karyotype consisted of 8 pairs of metacentrics (m), 14 pairs of submetacentrics (sm) to subtelocentrics (st) and 3 pairs of subtelocentric-acrocentrics (a) (Fig. 1A, Fig. 2A). The largest chromosome pair of the complement was characteristically st- to a. In the male specimen, the nucleolar organizer regions (NORs) detected by Ag- and CMA3-stainings were in the telomeres of the p-arms of two middle-sized sm chromosome (pair No 13 in Fig. 1A). CMA3-positive sites in the karyotype corresponded to the Ag-positive signals (Fig. 1C, 1D). In the female specimen, the NOR phenotype detected by these staining methods showed at least eight positive sites on p-arms of sm chromosomes (pairs Nos 12, 14, 15, 16, 22 in Fig. 2), including characteristic large p-arms of st-a pair (No. 23, Fig. 2). All observed positive signals were located at the telomeres of the p-arms of NOR-bearing elements where pairs Nos. 12 and 14 possessed positively stained sites on both homologues while on one only in the remaining ones. The CMA3-fluorescence revealed also in this female an entirely CMA3-positive longer arm of one homologue of pair No. 13. While all other CMA3-positive sites in the karyotype corresponded to the Ag-positive signals, this entirely CMA3-positive arm had shown no other specific features after Giemsa- (Fig. 2B) and Ag- (Fig. 2D) stainings. We failed to stain satisfactorily our preparations with C-banding to reveal possible heterochromatic composition of this unusual and remarkable chromosomal segment. A nature of such phenomenon (variation) is unknown at present but an involvement of some type of retrotransposon-derived repetitive sequences might be hypothesized (Ziegler *et al*., 2003). The complicated NOR phenotype in this specimen suggests either hybrid situation or possible involvement of an association between mobile element(s) and rDNA loci. No heteromorphic sex chromosomes were detected although the observed presence of entirely CMA3-positive arm in female complement might be hypothesized as sex-associated heteromorphism.

The similar karyotype structure of the examined male of *P. arrigonis* with those of the most other representatives of the European leuciscine fishes may indicate karyotypic conservation within this cyprinid lineage even in narrowly distributed endemic species. However, the NOR phenotype of female, i.e. number and position of major ribosomal sites, NORs, is not apparently derived from ubiquitous leuciscine pattern which is characterized by a single, smaller NOR-bearing sm chromosome pair with NORs situated on the p-arms present in a number of representatives of the genera e.g. *Alburnus, Alburnoides, Abramis, Leucaspius, Leuciscus, Petroleuciscus, Rutilus, Scardinius, Vimba*, etc. (Ráb and Collares-Pereira, 1995; Rábová *et al*., 2003; Bianco *et al*., 2004). The exceptions to this pattern include European representatives of phoxinin lineage of leuciscins *Phoxinus phoxinus* and *Eupallasella perenurus* (Boroń, 2001). Recently, Gante *et al*. (2004) described double NOR phenotype in *Ch. macrolepidotum*, where both sites are situated pericentromerically on one chromosome pair indicating a small inversion of part of the original, simple NOR site around centromere. However, our present finding indicates much larger number and site polymorphism of NOR sites, this unclear phenomenon documenting the need for large scale screening of this chromosomal marker within the former "*Chondrostoma*" group. In fact, the NOR pattern of this

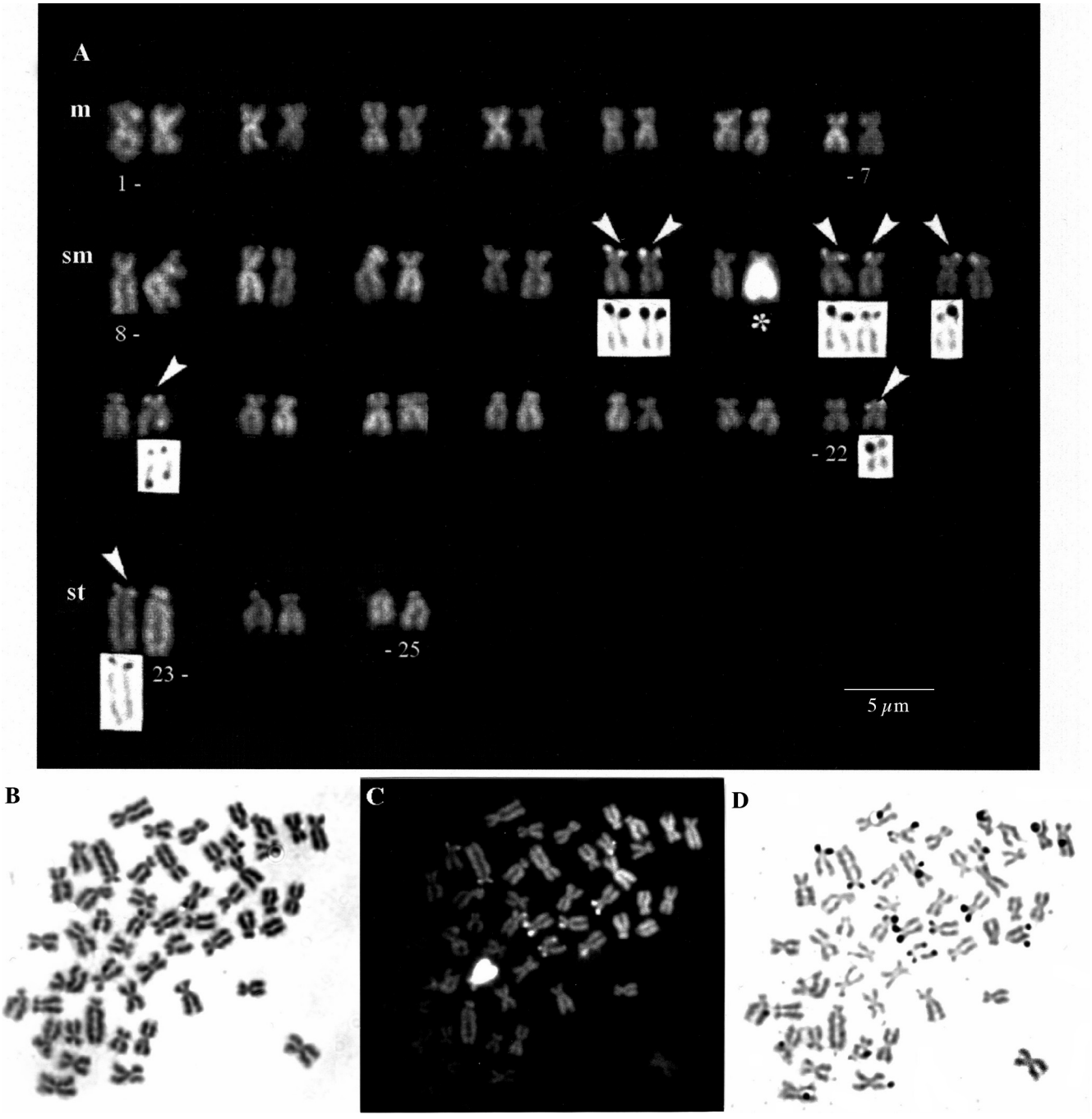

Figure 2. - Karyotype of female morphologically identified as *Parachondrostoma arrigonis* arranged from CMA_3 stained chromosomes, sequentially Ag-stained chromosomes with positive signals are shown in the second row; arrows indicate the CMA_3 positive sites, star indicates entirely CMA_3-positive longer arm. Sequentially Giemsa- (**B**), CMA_3- (**C**), Ag- (**D**) stained metaphase, from which the karyotype (**A**) was arranged. m: metacentric; sm: submetacentric; st: subtelocentric chromosomes. *[Caryotype d'une femelle identifiée morphologiquement comme étant* P. arrigonis *réalisé à partir des chromosomes colorés au CMA_3. Les chromosomes colorés positivement par l'Ag sont présentés au deuxième rang. Les flèches indiquent les sites positifs au CMA_3, l'astérisque indique un long bras entièrement positif au CMA_3. Le caryotype (**A**) a été réalisé à partir de métaphases colorées par le Giemsa (**B**), CMA_3 (**C**) et l'AG (**D**). m : métacentrique ; sm : submétacentrique ; st : subtélocentrique.]*

specimen has never been observed in any of leuciscine cyprinids and without *fluorescent in situ hybridization* (FISH) analyses with relevant rDNA probes it seems to be impossible to reveal the nature of observed pattern. The case is even more complicated because recent findings in leuciscine cyprinids (Gromicho *et al*., 2004) show that Ag- and

CMA3-positive sites do not always correspond to major rDNA positive sites detected by FISH or in mammals where in some rodents Ag-NOR sites do not correspond again to actual rDNA sites (Dobigny *et al*., 2002). Anyhow, *P. arrigonis* is so critically endangered species that to obtain more specimens for deeper cytogenetic analyses of observed variation is unlikely. As a result, we decided to publish this short report being aware that the study is incomplete.

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