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Karyological characterization of *Mugil trichodon* Poey, 1876 (Pisces: Mugilidae)*

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SUMMARY: Cytogenetic studies were conducted on *Mugil trichodon* from Margarita Island, Venezuela. The species showed a karyotype 2n=48 with entirely acrocentric chromosomes (Arm number, NF=48). Chromosomes gradually decreased in size and did not allow a clear distinction of homologues, except for one marker pair, which showed a conspicuous secondary constriction. C-banding showed heterochromatic blocks restricted at the centromeric regions of all the chromosomes. Some were more obvious than others, with the exception of the chromosome pair that had a secondary constriction with entirely heterochromatic short arms. Sequential staining with Giemsa and $AgNO_3$ demonstrated the conspicuous secondary constrictions corresponding to the NORs, and these had significant intraindividual size variations between both homologous chromosomes. The data obtained here support the contention that Mugilidae have a conservative chromosome macrostructure and reinforce the hypothesis that small structural chromosome rearrangements involving active NOR sites are the main cause of the karyotypic diversification seen in this group.

Keywords: karyotype, NOR, C-banding, *Mugil trichodon*.

RESUMEN: CARACTERIZACIÓN CARIOLÓGICA DE *MUGIL TRICHODON* POEY, 1876 (PISCES: MUGILIDAE). – Se presenta el estudio citogenético de *Mugil trichodon* de la Isla de Margarita, Venezuela. La especie posee un cariotipo 2n=48 constituido por cromosomas acrocéntricos (Número de Brazos, NF=48). El tamaño de los cromosomas decrece gradualmente y no permite una clara distinción entre homólogos, excepto un solo par marcador que muestra una constricción secundaria conspicua. El bandeo-C reveló bloques heterocromáticos restringidos a las regiones centroméricas de todos los cromosomas, algunos más evidentes que otros, con la excepción del par cromosómico que posee la constricción secundaria, el cual mostró brazos cortos enteramente heterocromáticos. La tinción secuencial con Giemsa y $AgNO_3$ evidenció como la constricción secundaria corresponde a las regiones organizadoras del nucleolo (NORs), las cuales presentaron una significativa variación intraindividual del tamaño entre los homólogos. Los datos obtenidos respaldan la naturaleza conservativa de la macroestructura cromosómica en los Mugilidae y refuerzan la hipótesis según la cual pequeñas reorganizaciones estructurales que incluyen las NORs han sido la principal causa de la diversificación en este grupo.

Palabras Clave: cariotipo, NOR, bandeo-C, *Mugil trichodon*.

INTRODUCTION

The Mugilidae is a family made up of species which inhabit marine inshore, estuarine, and freshwater environments in tropical, subtropical, and temperate regions of all continents (Harrison and Howes, 1991). Meristic and morphometric data used to establish taxonomic relationships in Mugilidae have resulted in a confusing taxonomy due to the conservative external morphology of the family. This is evidenced by the synonymy that includes 281 nominal species of which

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TABLE 1. – Chromosome number in 16 Mugilidae species. a: acrocentric, st: subtelocentric, sm= submetacentric, m=metacentric; NF= arm number.

Species	2n	Karyotype	NF	Reference
Chelon labrosus	48	2st+46a	48	Cataudella *et al*., 1974
Liza aurata	48	2st+46a	48	Cataudella *et al*., 1974
Liza macrolepis	48	48a	48	Choudry *et al*., 1979
Liza oligolepis	48	48a	48	Choudry *et al*., 1979
Liza ramada	48	2sm+46a	48	Rossi *et al*., 1997
Liza saliens	48	2st+46a	48	Gornung *et al*., 2001
Mugil cephalus	48	48a	48	Rossi et al 1996
M. speigleri	48	48a	48	Rishi and Singh, 1982
M. corsula	48	48a	48	Khuda-Buksh and Manna., 1974
M. curema [1]	28	20m+4st+4a	48	Le Grande and Fitzsimons, 1976
M. curema [2]	24	22m+2sm	48	Nirchio and Cequea, 1998
M. gaimardianus	48	48a	48	Nirchio *et al*., 2003
M. liza	48	48a	48	Nirchio and Cequea, 1998
M. parsia	48	48a	48	Khuda-Buksh and Manna., 1974
M. platanus	48	48	48	Jordao *et al*., 1992
M. trichodon	48	48	48	This paper
Oedalechilus labeo	48	2st+46a	48	Rossi *et al*., 2000

[1] From the Gulf of Mexico; [2] from Venezuela

Thomson (1997), only recognizes 64 valid species.

The available literature verifies that only 16 of the 64 nominal species of mugilids have been karyotyped so far (see Table 1), which shows that the group has not been studied sufficiently from a cytogenetic point of view.

In Venezuela, the family is represented by six species, namely *Mugil curema, M. curvidens, M. gaimardianus M. incilis, M. liza,* and *M. trichodon,* (Cervigón, 1993). Of these species, *M. curema*, *M. gaimardianus* and *M. liza*, have been karyotyped (Nirchio and Cequea, 1998 Nirchio *et al*., 2001, 2003).

This study was carried out to continue the karyological characterization of Venezuelan mugilids with the diploid chromosome number, chromosome formula, Nucleolus Organizer Regions (NOR) locations and constitutive heterochromatin distribution in *M. trichodon*. This species is distributed from Florida in the United States to the northeast of Brazil, and although it is common in Venezuela it is less known than *M. liza, M. incilis,* and *M. curema*.

The taxonomic status of *M. trichodon* remains unclear. Thomson (1997), synonymized it with *M. gyrans* (fantail mullet) and indicated that *Querimana gyrans* were the young of *M. trichodon*, whereas Rivas (1980), recognized the validity of *M. gyrans* based primarily on soft dorsal fin counts. Froese and Pauly (2004), reported *M. trichodon* and *M. gyrans* as two distinct valid species. Thus we considered it necessary to include the meristic and morphometric characteristics of the samples studied in this paper.

METHODS

Twenty specimens of *Mugil trichodon* (Fig. 1) were collected at Chacachacare Lagoon, Margarita Island, Venezuela, in March 2004. The morphometric measurements were: Total length (TL), Standard Length (SL), Head Length (HL), Body Heigth (BH), Ocular Diameter (OD), Pectoral Fin Length (PecFL), Pelvic Fin Length (PelvFL), Length between Pelvic and Anal Fin (LPAF), Length between Dorsal 1 and Dorsal 2 fins (LD1D2), Length between the Mouth and Dorsal 1 Fin (LMD1), Length between the Mouth and Dorsal 2 Fin (LMD2), Length between the Mouth and Anal Fin (LMA). Voucher specimens were deposited with the fish collection of the Escuela de Ciencias Aplicadas del Mar, Universidad de Oriente.

One male and seven females were used for cytogenetic studies. Each fish was injected with 0.025% colchicine solution (1 ml/100 g body weight), and kept in a well-aerated aquarium for 50 min. The mitotic metaphases were obtained according to the technique described by Bertollo *et al*. (1978). NOR were detected by impregnating chromosomes with silver nitrate (Howell and Black, 1980). C-bands were revealed according to the methods described by Sumner (1972).

Mitotic chromosomes were photographed using a digital camera. The images were digitally processed with the program Adobe Photoshop v. 7.0. The karyogram was constructed with chromosomes organized in decreasing order of size. Chromosomes were classified according to Levan *et al*. (1964).

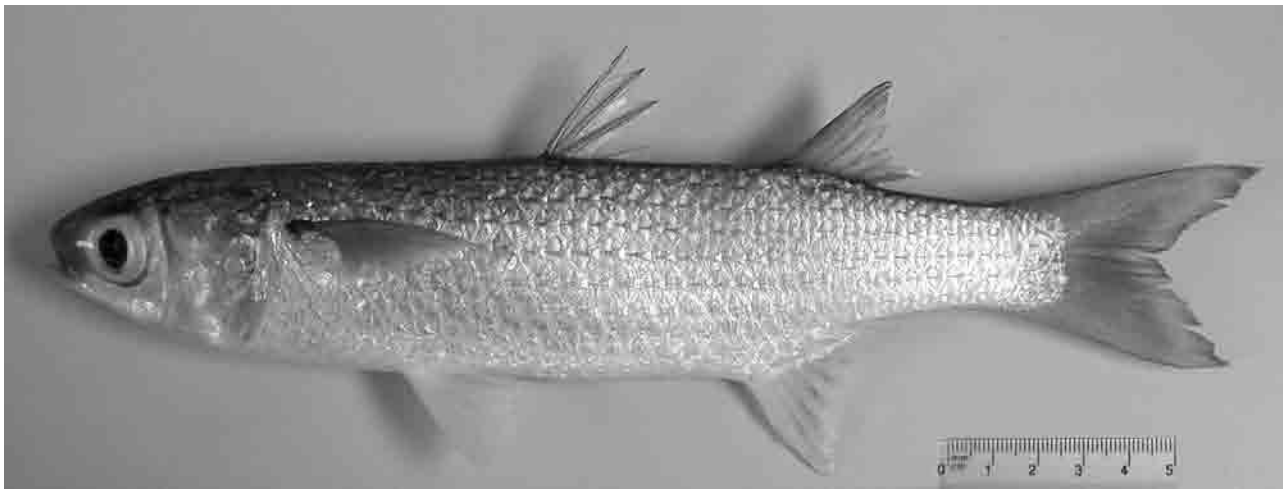

FIG. 1.– Specimen of *Mugil trichodon* from Margarita Island, Venezuela. Scale in cm.

RESULTS AND DISCUSSION

Diagnosis

Description. The fish showed: widely separated spiny-rayed dorsal fins with 4 spines (1st dorsal fin) and 8 soft-rays (2nd dorsal fin); pelvic fins subabdominal with one spine and 5 branched soft rays; anal fin with 3 spines and 8 soft-rays; ctenoid scales in adults; pectoral axillary scale; lateral line with 30 to 33 scales; 2nd dorsal fin and anal fin profusely covered with scales; teeth small but noticeable at first sight, curved but without sharply angled tip; adipose eyelids. Fresh specimens are dorsally grey, white/silver in the ventral portion; pelvic fins can have a yellowish tone, and the base of pectoral fins exhibits a clearly visible dark spot, which is quite large in some individuals (Fig. 1).

Meristic characteristics. Dorsal fin: IV-8; Anal fin: III,8; Pectoral fin: 16; Pelvic fin: I,5; Scale counts in the middle body line: 30-33.

Morphometric measurements (see Methods for abbreviations). TL: 196-268 mm, SL: 163-212 mm. The following measurements are expressed as percentages of the standard length; average values (A) and variation coefficient (CV) are reported in brackets. HL: 23.6–26.0% (A: 25.0; C.V: 3.16); BH: 21.9-28.8% (A: 25.8; C.V: 8.17); OD: 6.7–9.2% (A: 7.7; C.V: 9.12); PecFL: 5.7-6.6% (A: 6.1; C.V: 4.63); PelvFL: 15.1-18.4% (A: 16.8; C.V: 6.83); LPAF: 31.6-36.4% (A: 34.0; C.V: 4.22); LD1D2: 22.9-25.7% (A: 24.1; C.V: 3.98); LMD1: 50.7-53.6% (A: 52.2; C.V: 1.54); LMD2: 74.1-77.3% (A: 75.5; C.V: 1.47); LMA: 67.5-73.2% (A: 71.3; C.V: 2.10).

Cytogenetic

The specimens analyzed in this study displayed a 2n=48 karyotype with entirely acrocentric chromosomes (Arm number, NF=48). The chromosomes gradually decreased in size so that the homologues could not be distinguished clearly, except one marker pair, which showed a conspicuous secondary constriction (Fig. 2A).

Nine out of the 15 species of Mugilidae that have been karyotyped so far have a diploid number of 48 acrocentric chromosomes. The exception to this diploid modal number is *Mugil curema* from Venezuela which has a karyotype 2n=24 with 22 metacentric and 2 submetacentric chromosomes (Nirchio *et al*., 2003). However, specimens also identified as *M. curema* from Brazil (Cirpriano *et al.,* 2002), and from the Gulf of Mexico (Le Grande and Fitzsimons, 1976), were reported to have a karyotype of 2n=28, with 20 metacentric, 4 subtelocentric and 4 acrocentric chromosomes.

Data reported here for *M. trichodon* brings the number of Mugilidae species karyotyped to 16, and 10 species have 48 acrocentric chromosomes in this family. This supports the hypothesis that, with the exception of *M. curema*, a karyotype composed of 48 acrocentric chromosomes is a common feature of Mugilidae (Table 1) and hence, a plesiomorphic condition in the family.

C-banding showed that heterochromatic blocks are mostly restricted to the centromeric regions of all chromosomes, and some were more obvious than others. The chromosome pair characterized by a secondary constriction showed heterochromatic short

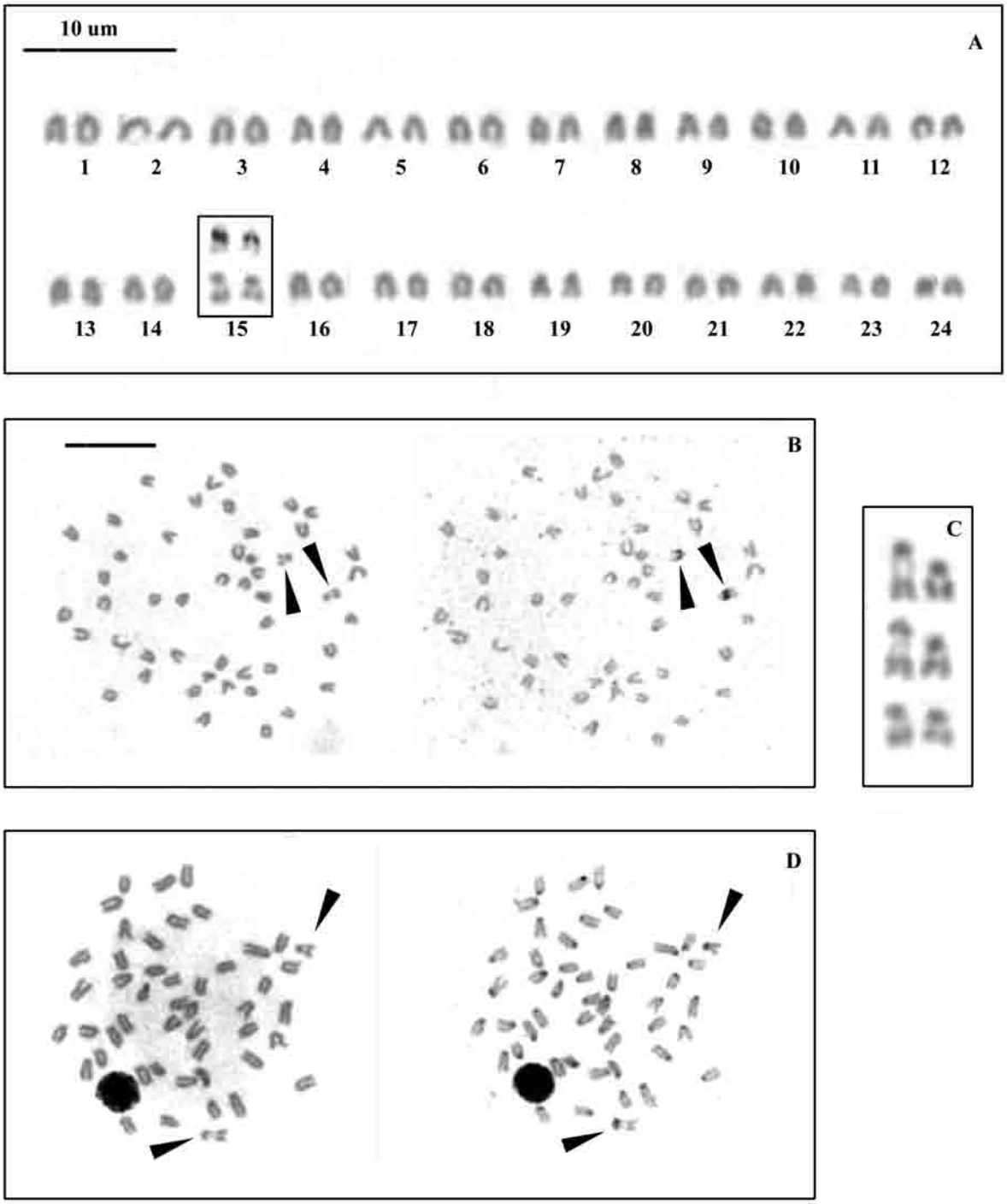

FIG. 2. – Karyotype of *Mugil trichodon*. NOR-bearing chromosomes appear in the squares (A). Sequential Giemsa and $AgNO_3$ staining of the same metaphase (B). Homologous pair No. 15 showing the conspicuous secondary constrictions (C). Sequential Giemsa and C-banding staining of the same metaphase (D). Arrowheads indicate NOR bearing chromosomes.

arms (Fig 2D). This mostly pericentromeric location of heterochromatin was also reported in *Mugil platanus* (Jordao *et al*., 1992), *M. cephalus* (Rossi *et al.,* 1996), *M. liza* (Nirchio, unpublished data), *Liza ramada* (Rossi *et al*., 1996), *L. aurata, Chelon labrosus* (Delgado *et al*., 1990), and *O. labeo* (Rossi *et al.,* 2000).

Cryptic chromosome rearrangements involving the NOR sites have often been described in groups that show an apparently conservative chromosome macrostructure among species [see Galetti *et al*. (2000) for references]. Karyological information about the Mugilidae shows that NORs are located: on the telomeric region of the long arm of acrocen-

tric chromosome pair N° 1 in *M. cephalus* (Rossi *et al*., 1996), *M. platanus* (Jordao *et al*., 1992), and *M. liza* (Nirchio *et al*., 2001); on the telomeric region of the long arm of the metacentric chromosome pair N° 1 in *M. curema* from Venezuela (Nirchio *et al.,* 2001); on the telomeric region of the short arm of the subtelocentric chromosome pair N° 11 in *M. curema* from Brazil (Nirchio *et al*., 2005); and on the short arm of the unique submetacentric chromosome pair (N° 24) of *Liza aurata*, *L. ramada, L. saliens* and *Chelon labrosus* (Rossi *et al*., 1997; Gornung *et al.,* 2001). In *Oedalechilus labeo*, one pair of NORs that are strikingly different in size is located on the short arms of the only subtelocentric pair (N° 9); although there can be an additional, though inactive NOR, in the chromosome complement (Rossi *et al*., 2000).

In *M. trichodon*, sequential Giemsa and silver staining allowed us to identify correspondences between the chromosome pair, the conspicuous secondary constrictions and the one that carries the NORs, with NOR signals corresponding to the secondary constriction and showing significant intraindividual size variations between both homologous chromosomes (Fig. 2B-C).

To stain NORs with silver requires the NOR chromatin to be in a decondensed state (nucleolar constriction) (Clavaguera *et al*., 1983; Medina *et al*., 1983; Sánchez-Pina *et al*., 1984, Jiménez *et al*., 1988), and the ribosomal genes to have transcriptional activity during the preceding interphase (see Hubbel, 1985). Thus, size variations between NORs with homologous chromosomes in *M. trichodon* could be interpreted as differential transcriptional activity of the ribosomal genes.

This correlation between the secondary constrictions in metaphase plates stained with Giemsa and NORs, demonstrated by silver nitrate, occurs in many other fish species (Rodriguez-Daga *et al*., 1993; Oliveira *et al*., 2003), but to the best of our knowledge this is the first report of interstitial localization of NORs in Mugilidae. It is most likely an exclusive apomorphy in the species *M. trichodon,* probably caused by a paracentric inversion involving NOR sites.

It has already been hypothesized that *Liza* and *Chelon* karyotypes are derived from translocation events that affect the acrocentric ancestral chromosome complement found in *Mugil*, which involves NORs (Rossi *et al*., 1996). Our data combined with the preceding information support the hypothesis that structural chromosome rearrangements involving active NOR sites are the main cause of the karyotypic diversification seen in mugilids.

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