

**COMPARATIVE CYTOGENETIC AND ALLOZYME ANALYSIS OF  
*Mugil rubrioculus* AND *M. curema* (TELEOSTEI: MUGILIDAE) FROM  
VENEZUELA**

Mauro Nirchio, Claudio Oliveira, Irani A. Ferreira, Julio E. Pérez, Juan I. Gaviria,  
Ian Harrison, Anna Rita Rossi and Luciana Sola

**SUMMARY**

A comparative cytogenetic and allozyme analysis of sympatric specimens of *Mugil rubrioculus* and *M. curema* from Venezuela is reported. Specimens of *M. rubrioculus* exhibit a  $2n=48$  karyotype with exclusively acrocentric ( $NF=48$ ) chromosomes, one pair of NORs interstitially located on chromosome pair number 8 and constitutive heterochromatin distributed in pericentromeric position of all chromosomes. Specimens of *M. curema* show cytogenetic features significantly different in comparison to *M. rubrioculus* in terms of chromosome number and morphology ( $2n=24$  biarmed chromosomes,  $NF=48$ ) and NORs location (telomeric region of the largest metacentric pair). Starch gel electrophoresis analysis at 20 presumptive loci reveals a reduced genetic differentia-

tion between the two species. In fact, though a total of ten private alleles are identified; all loci share alleles between the two species and the obtained Nei's genetic distance ( $D=0.060$ ) is lower than the values obtained between other congeneric mullet species. Thus, the cytogenetic and allozyme data sets indicate quite different degrees of genetic divergence between *M. rubrioculus* and *M. curema*. This could either reflect an underestimate of molecular divergence owing to cryptic variation or different rates of molecular/chromosomal evolution. Whatever the explanation, this study confirms the power of karyological data in discriminating species of Mugilidae.

**Introduction**

An earlier cytogenetic analysis, based on Giemsa-stained karyotypes, revealed that specimens identified as *Mugil gaimardianus* (*sensu*

Cervigón, 1993), from Panama and Margarita Island, Venezuela, possess a  $2n=48$  uniarmed chromosome karyotype (Nirchio *et al.*, 2003). In contrast, specimens of *M. curema* (*sensu* Cervigón, 1993), also collected

from Margarita Island, have a  $2n=24$ , entirely biarmed chromosome karyotype (Nirchio *et al.*, 2003). Thus, cytogenetic data provided important support to the hypothesis that nominal *M. gaimardianus*,

although very similar in appearance to *M. curema*, was a distinct species, as previously noted by some authors (Menezes, 1983; Cervigón, 1993).

Subsequent morphological comparisons of the specimens

**KEYWORDS / C-banding / Chromosome / Fluorescent in situ Hybridization / Gene-enzyme / Karyotype / Mulletts / NORs /**

Received: 03/26/2007. Accepted: 09/21/2007.

**Mauro Nirchio.** M.Sc. in Marine Sciences, Universidad de Oriente (UDO), Venezuela. Professor, Escuela de Ciencias Aplicadas del Mar (ECAM-UDO), Venezuela. Address: Apartado Postal 147-Portomar, Margarita, Venezuela. e-mail: mnirchio@cantv.net

**Claudio Oliveira.** Ph.D. in Genetics, Universidade de São Paulo, Brazil. Professor, Instituto de Biociências, Universidade Estadual Paulista (UNESP) Botucatu, SP, Brazil.

**Irani A. Ferreira.** M.Sc. in Genetics and Ph.D. student, UNESP, Botucatu, SP, Brazil.

**Julio E. Pérez.** Ph.D. in Biology, University of Southampton, UK. Profesor, Instituto Oceanográfico de Venezuela, UDO, Venezuela.

**Juan I. Gaviria.** M. Aq. Auburn University, USA. Professor, ECAM-UDO, Venezuela.

**Ian Harrison.** Ph.D. in Zoology, University of Bristol, U.K. Bio-

diversity Scientist. American Museum of Natural History, New York, USA.

**Anna Rita Rossi.** PhD in Evolutionary Biology and Researcher, Università de Roma "La Sapienza", (URLS) Italy.

**Luciana Sola.** Doctor in Biology and Professor, URLS, Italy.

## ANÁLISIS COMPARATIVO CITOGENÉTICO Y ALOENZIMÁTICO DE *Mugil rubrioculus* AND *M. curema* (TELEOSTEI: MUGILIDAE) DE VENEZUELA

Mauro Nirchio, Claudio Oliveira, Irani A. Ferreira, Julio E. Pérez, Juan I. Gaviria, Ian Harrison, Anna Rita Rossi y Luciana Sola

### RESUMEN

Se presentan los resultados del análisis comparativo citogenético y aloenzimático entre las especies simpátricas *Mugil rubrioculus* y *M. curema* de Venezuela. Los especímenes de *M. rubrioculus* presentan un cariotipo con  $2n=48$  cromosomas exclusivamente acrocéntricos ( $NF=48$ ), NORs intersticiales localizados en el par cromosómico número 8 y heterocromatina constitutiva distribuida en posición pericentromérica en todos los cromosomas. Los especímenes de *M. curema* presentan características citogenéticas significativamente diferentes de *M. rubrioculus* en términos de número cromosómico y morfología ( $2n=24$  cromosomas de dos brazos y  $NF=48$ ) y localización de las NORs (región terminal del par metacéntrico más grande). El análisis electroforético en gel de almidón

de 20 loci presuntivos reveló una diferenciación genética reducida entre las dos especies. De hecho, aún cuando un total de diez alelos específicos hayan sido identificados, no hay loci que no compartan alelos entre las dos especies y el valor de distancia genética (Nei) obtenido ( $D=0,060$ ) es más bajo que el obtenido entre otras especies congénicas de mugílidos. Así, los datos citogenéticos y los alozímicos indican diversos grados de divergencia entre el *M. rubrioculus* y *M. curema*. Esto podría reflejar una subestimación de la divergencia molecular por variación críptica o diferentes tasas de evolución molecular y cromosómica. De cualquier manera, este estudio confirma el poder de los datos cariotípicos para discriminar especies de Mugilidae.

## ANÁLISE COMPARATIVA CITOGENÉTICA E ALOENZIMÁTICA DE *Mugil rubrioculus* AND *M. curema* (TELEOSTEI: MUGILIDAE) DE VENEZUELA

Mauro Nirchio, Claudio Oliveira, Irani A. Ferreira, Julio E. Pérez, Juan I. Gaviria, Ian Harrison, Anna Rita Rossi e Luciana Sola

### RESUMO

Apresentam-se os resultados de uma análise citogenética e alozímica comparativa entre as espécies simpátricas *Mugil rubrioculus* e *M. curema* da Venezuela. Espécimes de *M. rubrioculus* apresentam cariótipo com  $2n=48$  e exclusivamente cromossomos acrocêntricos ( $NF=48$ ), NORs intersticiais localizados no par cromossômico número 8 e heterocromatina constitutiva distribuída em posição pericentromérica de todos os cromossomos. Espécimes de *M. curema* apresentam características citogenéticas significativamente diferentes de *M. rubrioculus* em termos de número cromossômico e morfologia ( $2n=24$ , cromossomos de dois braços e  $NF=48$ ) e localização das NORs (região terminal do maior par metacêntrico). A análise de eletroforeses em gel de amido de

20 presumíveis loci revelou uma diferenciação genética reduzida entre as duas espécies. De fato, apesar de que um total de dez alelos específicos tenham sido identificados, não há loci que não compartilhem alelos entre as duas espécies e a distância genética de Nei ( $D=0,060$ ) é menor do que os valores obtidos entre outras espécies de tainhas. Assim, os dados citogenéticos e alozímicos indicam graus de divergência diferentes entre *M. rubrioculus* e *M. curema*. Isto pode ser reflexo de uma divergência molecular subestimada por variação críptica ou a uma taxa de evolução molecular e cromossômica diferente. De qualquer maneira, esse estudo confirma o poder dos dados cariotípicos na discriminação de espécies de Mugilidae.

identified by Nirchio *et al.* (2003) as *M. gaimardianus* with type specimens or representative material from other nominal species of *Mugil* occurring in the Western Central Atlantic, revealed that the specimens identified as *M. gaimardianus* were not conspecific with any other nominal species. However, as noted by Harrison *et al.* (2007), the original description of *M. gaimardianus* by Desmarest in Bory de Saint-Vincent (1831) was uninformative and the name had been suppressed (Álvarez-Lajonchere *et al.*, 1992; ICZN, 1994). Therefore, a new species description and name were required for Nirchio *et al.*'s (2003) specimens; this description has been made by Harrison *et al.* (2007), with provision of the new name *Mugil rubrioculus*.

This study reports a comparative cytogenetic and allozyme analysis of sympatric specimens of *M. rubrioculus* and *M. curema* from La Restinga lagoon, Margarita Island, Venezuela, carried out in order to provide genetic data on the specimens used for the taxonomic description (Harrison *et al.*, 2007) and to expand the knowledge on the genetic divergence between these two, very similar species. The cytogenetic characterization of *M. rubrioculus* includes data on constitutive heterochromatin by C-banding and on nucleolus organizer regions (NORs) by silver staining (Ag-NORs) and fluorescent *in situ* hybridization (FISH). The chromosomal constitution for the same cytogenetic features has also been monitored in specimens of *M. curema*, in consideration of the important chromosome variation

described among *M. curema* from Venezuela and from Brazil (Nirchio *et al.*, 2005a). Additionally, comparative analyses of specimens of *M. rubrioculus* and *M. curema* are made using starch gel electrophoresis of 11 gene-enzyme systems.

### Materials and Methods

Specimens of *M. rubrioculus* and *M. curema* were collected from La Restinga lagoon, Margarita Island, Venezuela ( $10^{\circ}57'46.6''N$ ,  $064^{\circ}10'44.9''W$ ) between April 4, 2004 and March 2, 2005. The voucher specimens deposited in the collections of the Escuela de Ciencias Aplicadas del Mar (ECAM) used for the karyological (K) and allozyme (A) analyses are reported in Table I. Additional specimens were used in electrophoretic studies

for *M. rubrioculus* (21) and *M. curema* (25). The meristics and morphometrics of some voucher specimens are provided in Harrison *et al.* (2007).

### Karyology

A total of 14 specimens of *M. rubrioculus* and 12 specimens of *M. curema* were cytogenetically analyzed. Each fish was injected with 0.0125% colchicine solution (1ml/100g body weight) and maintained in a well-aerated aquarium for 50min, prior to being killed by a sharp blow to the head. The mitotic chromosome preparations followed the technique described by Bertollo *et al.* (1978). C-bands were obtained according to Sumner (1972). Nucleolus Organizer Regions (NORs) were localized by chromosome impregnation

TABLE I  
LIST OF VOUCHER SPECIMENS\*  
USED FOR  
THE KARYOLOGICAL (K)  
AND/OR ALLOZYME (A) ANALYSES

<i>M. rubrioculus</i>	<i>Mugil curema</i>
ECAM-00038 (K)**	ECAM-00034 (K)
ECAM-00039 (K)**	ECAM-00035 (K)
ECAM-00040 (A)	ECAM-00036 (K)
ECAM-00043 (K/A)	ECAM-00037 (K)
ECAM-00045 (K/A)	ECAM-00062 (K)
ECAM-00045 (K/A)	ECAM-00063 (K)
ECAM-00047 (K/A)	ECAM-00064 (K)
ECAM-00052 (K/A)	ECAM-00065 (K)
ECAM-00067 (K)	ECAM-00073 (K)
ECAM-00068 (K)	ECAM-00149 (K)
ECAM-00087 (K)	ECAM-00150 (A)
ECAM-00088 (K)	ECAM-00153 (K/A)
ECAM-00151 (K)	ECAM-00158 (K/A)
ECAM-00152 (K)	ECAM-00159(A)
ECAM-00154 (K)	ECAM-00160 (A)
ECAM-00167 (A)	ECAM-00161 (A)
ECAM-00168 (A)	ECAM-00162 (A)
ECAM-00169 (A)	ECAM-00164 (A)
ECAM-00170 (A)	ECAM-00165 (A)
ECAM-00171 (A)	ECAM-00166 (A)
ECAM-00172 (A)	
ECAM-00176 (A)	
ECAM-00177 (A)	

\* Deposited in the collections of the Escuela de Ciencias Aplicadas del Mar (ECAM), Universidad de Oriente, Venezuela. \*\* Transferred to collections of American Museum of Natural History (see Harrison *et al.*, 2007)

with silver nitrate (Howell and Black, 1980) and by Fluorescent *in situ* Hybridization (FISH). FISH was performed according to the method described by Pinkel *et al.* (1986) with some modifications (Martins and Galetti, 2001). The 18S rDNA probe was obtained from *Oreochromis niloticus* and was labeled by nick translation with biotin-14-dATP according to manufacturer (Bionick labeling system-Invitrogen) instructions. The metaphase chromosome slides were incubated with RNase (40µg/ml) for 1.5h at 37°C. After denaturation of chromosomal DNA in 70% formamide/2xSSC for 4min at 70°C, hybridization mixtures containing 200ng of denatured probe, 10mg/ml Dextran sulfate, 2xSSC and 50% of Formamide were dropped on the slides and the hybridization was performed overnight at 37°C. Hybridization washes included 2xSSC and 50% Formamide at 37°C

TABLE II  
ENZYME SYSTEMS ASSAYED BUFFERS AND TISSUES

Enzyme system	Abbreviation	E.C. No.	N of loci	Buffer	Tissue
Acid phosphatase	ACP	3.1.3.2	1	TC	Liver
Alcohol dehydrogenase	ADH	1.1.1.1	2	TC	Liver
Catalase	CAT	1.11.1.6	1	TC	Liver
Diaphorase	DIA	1.8.14	1	LB	Liver
Esterase	EST	3.1.1.-	4	LB	Liver
Glutamate dehydrogenase	GLUDH	1.4.1.3	1	TEB	Liver
Glucose dehydrogenase	GDH	1.1.1.47	1	TC	Liver
Lactate dehydrogenase	LDH	1.1.1.27	3	TC	Eye
Malate dehydrogenase	MDH	1.1.1.37	2	TC	Muscle
Total protein	Prot		3	TC	Muscle
Superoxide dismutase	SOD	1.15.1.1	1	TEB	Liver

TC: buffer gel 0.97g/l Tris, 0.63g/l citrate, 0.11g/l NaOH, pH 6.7; buffer tray 27g/l Tris, 18.07g/l citrate, 2g/l NaOH, pH 6.3.

LB: buffer gel 3.63g/l Tris, 0.96g/l citrate, 10ml buffer Tray, pH 8.5; buffer tray: 2.52g/l LiOH, 18.55g/l boric acid, pH 8.1.

TEB: buffer gel 1 tray: 3H<sub>2</sub>O, pH 8.7; buffer tray 21.9g/l Tris, 6.18g/l boric acid, 1.17g/l EDTA, pH 8.70.

and 2xSSC and 4xSSC at room temperature.

Detection of hybridized probes was performed with Avidin-FITC conjugate (Sigma) followed by two rounds of signal-amplifica-

tion. After each amplification step, the slides were washed in the blocking buffer (1.26% NaHCO<sub>3</sub>, 0.018% sodium citrate, 0.0386% triton, 1% non-fat dried milk) at 42°C. Chromosomes were counterstained with propidium iodide, and the slides were mounted with Antifade (Vector). Metaphases were examined in a Zeiss Axiophot

photomicroscope and photographed with Kodak Gold Ultra 400 ASA film. The images were digitalized and processed with Adobe Photoshop v. 7.0. This software was also used for constructing the karyogram, with chromosomes organized in decreasing size order. Chromosome morphology followed Levan *et al.* (1964).

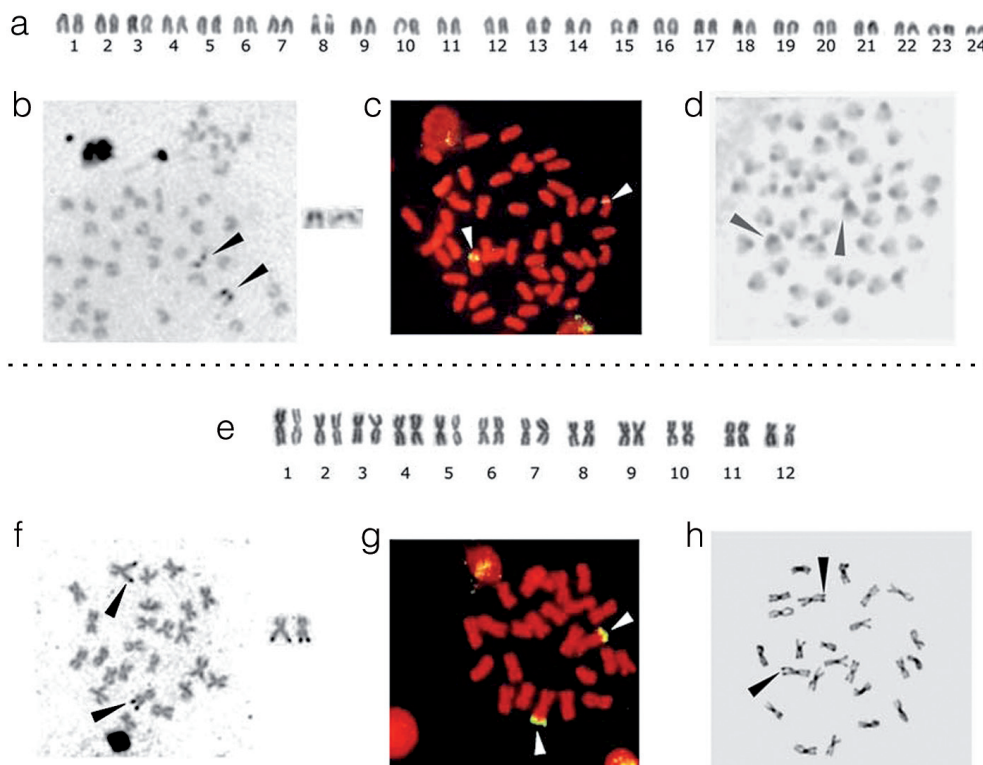


Figure 1. *Mugil rubrioculus* karyotype a: Giemsa-stained and metaphases, b: Ag-stained, c: after FISH with 18S rDNA, and d: C-banded. *Mugil curema* karyotype e: Giemsa-stained, and metaphases, f: Ag-stained, g: after FISH with 18S rDNA, and h: C-banded. Arrows indicate NOR-bearing chromosomes.

A total of 35 specimens of each *M. rubrioculus* and *M. curema* were used for electrophoretic comparisons of 11 enzyme systems. Samples of liver, muscle and eye lens tissue were obtained from each fish. Each tissue sample was homogenized in a cold buffer solution of Tris-HCl 0.05M pH 7.00 in a 1:3 proportion (weight:volume), centrifuged at 9000rpm for ~10min and the supernatant was electrophoresed in horizontal 12% starch gels employing several buffer systems (Table II). Histochemical staining was performed according to Harris and Hopkinson (1976). Allelic frequencies, effective number of alleles, observed and expected heterozygosity, probability for goodness of fit to Hardy-Weinberg equilibrium, and Nei's (1978) genetic distance index (D) were computed using the software Genes in Populations v. 2 (May and Krueger, 1995).

### Results and Discussion

Specimens of *Mugil rubrioculus* exhibit a 2n=48 karyotype (Figure 1a) with exclusively acrocentric chromosomes (Fundamental Number of arms, NF=48). Chromosomes gradually decrease in size, not allowing a clear distinction of the homologues, with the exception of chromosome pair number 8, which is clearly identifiable by the presence of an interstitial secondary constriction. Silver staining (Figure 1b), as well as FISH with the 18S ribosomal genes (Figure 1c), produce signals located interstitially, close to the centromere, on one pair of chromosomes (apparently chromosome pair number 8), allowing the identification of one single pair of NOR-bearing chromosomes in the chromosome complement. C-band positive segments (Figure 1d), more pronounced on chromosome pair number 8, were observed in pericentromeric position in all chromosomes.

The chromosome complement and the cytogenetic features of the analyzed specimens of *Mugil curema* from Margarita correspond to those previously described (Nirchio *et al.*, 2001, 2005a; Rossi *et al.*, 2005) in conspecific specimens from the same area. These specimens have a 2n=24 (NF=48) karyotype (Figure 1e), with 22 metacentric and two submetacentric chromosomes, and NORs, as detected by Ag-staining (Figure 1f) or FISH with the 18S rDNA probe (Figure 1g), located on the telomeric region of the long arms of the largest metacentric pair. C-banding (Figure 1h) produce strong positive signals in the pericentromeric position of all chromosome pairs and at the terminal region of pair number 1, corresponding to NORs.

Cytogenetic studies in Mugilidae, reviewed in Sola *et al.* (2007), show that a karyotype composed of 2n=48 uniarmed chromosomes, NF=48, is conserved through several species of mugilids. Thus, the karyotypes with 2n=24, or 2n=28, NF=48, observed in *M. curema* appear to be an apomorphic condition. More extensive taxonomic sampling within *Mugil* and basal mugilid lineages such as *Agonostomus* and *Joturus* (Harrison and Howes, 1991) are required in order to make any substantial phylogenetic conclusions about these karyological characters. Nevertheless, the karyological information available for Mugilidae (references in Sola *et al.*, 2007) shows that NORs are generally born by a single chromosome pair, and two groups of species can be identified according to the location of their NORs. In the first group, which includes *M. cephalus*, *M. platanus* (= *M. cephalus* according to Thomson, 1997), *M. liza*, and *M. curema* from Venezuela (Nirchio *et al.*, 2005a), NORs are located in a terminal position on the long arm of the largest chromosome pair. In the second group, which includes *Liza aurata*, *L. ramado*, *L.*

TABLE III  
ALLELE FREQUENCIES AT THE 13 POLYMORPHIC  
LOCI IN *Mugil rubrioculus* AND *M. curema*

Locus	Allele	<i>M. rubrioculus</i>	<i>M. curema</i>
ACP*	(n)	34	34
	98	0	0.162
	100	0.309	0.838
	102	0.529	0
ADH-1*	104	0.162	0
	(n)	30	35
	100	0.900	0.829
	102	0.100	0.171
ADH-2*	(n)	22	28
	100	1.000	0.750
	102	0	0.250
	(n)	35	35
CAT*	100	0.700	0.700
	102	0.300	0.300
	(n)	34	35
	98	0.015	0
DIA*	100	0.044	0.743
	102	0.618	0.171
	104	0.221	0.029
	106	0.103	0.057
	(n)	30	35
EST-1*	98	0.067	0.068
	100	0.917	0.914
	102	0.017	0
	(n)	35	35
EST-2*	100	0.971	0.686
	102	0.029	0.314
	(n)	35	35
EST-3*	98	0	0.014
	100	1.000	0.986
	(n)	33	35
	96	0.167	0.057
EST-4*	98	0.030	0.057
	100	0.803	0.857
	102	0	0.029
	(n)	34	34
	96	0.059	0.147
GDH-2*	98	0.397	0.103
	100	0.412	0.588
	102	0.188	0.147
	104	0.015	0.015
	(n)	35	35
MDH-2*	98	0.029	0
	100	0.971	1.000
	(n)	35	35
PROT-3*	100	0.586	0.886
	102	0.414	0.114
	(n)	35	35
SOD*	98	0	0.014
	100	1.000	0.986
	102		

*saliens*, *Chelon labrosus* and *Oedalechilus labeo*, NORs are located on the short arm of a unique subtelocentric chromosome pair. Also in *M. curema* from Brazil, NORs have been recently localized (Nirchio *et al.*, 2005a) on the short arms of one of the two subtelocentric chromosome pairs of the chromosome complement. Thus, *M. rubrioculus*, though sharing with the other studied mugilid species the presence of a single NOR-bearing chromosome pair, shows a

completely different location of NORs, compared to most other species. The interstitial position of NORs in *M. rubrioculus* has been seen among other mugilids only in *M. trichodon* (Nirchio *et al.*, 2005b).

As far as the analysis of the allozymes is concerned, seven (*GLUDH\**, *LDH-1\**, *LDH-2\**, *LDH-3\**, *MDH-1\**, *PROT-1\**, *PROT-2\**) out of the 20 presumptive loci investigated were monomorphic in both species. The allele fre-

quencies at the remaining 13 polymorphic loci are reported in Table III. Among these loci, four showed more than one allele only in one of the two species: *MDH-2\** in *M. rubrioculus*; *ADH-2\**, *EST-3\** and *SOD\** in *M. curema*. Mean observed heterozygosity (*H<sub>o</sub>*) among all loci is 0.153 ± 0.055 in *M. rubrioculus* and 0.129 ± 0.047 in *M. curema*. Allele distribution shows the presence of five private alleles at four loci in *M. rubrioculus* (*ACP\**, *DIA\**, *EST-1\**, *MDH-2\**), and five, scattered on five loci in *M. curema* (*ACP\**, *ADH-2\**, *EST-3\**, *EST-4\**, and *SOD\**). The presence of 10 private alleles among the 37 alleles observed does provide indication of divergence between the two taxa; nevertheless, the absence of loci with no shared alleles between the two species represents a result inconsistent with the number of diagnostic loci detected in other congeneric comparisons for mugilids. Between 5 and 12 diagnostic loci have been identified for species comparisons within *Mugil* (Rossi *et al.*, 1998b; Turan *et al.*, 2005), and between 2 and 16 have been identified within *Liza* (Lee *et al.*, 1995; Papatropoulos *et al.*, 2001; Rossi *et al.*, 2004; Turan *et al.*, 2005).

Nei's (1978) genetic distance (D) between *M. rubrioculus* and *M. curema* is 0.060. This value is one order of magnitude lower than the values of genetic distances reported in other congeneric comparisons, including *Mugil*. Within *Liza*, D= 0.237 has been obtained between *L. affinis* and *L. macrolepis* (Lee *et al.*, 1995), and D values ranging from 0.249 to 0.530 (Papatropoulos *et al.*, 2001), from 0.466 to 0.659 (Rossi *et al.*, 2004) and from 0.319 to 0.714 (Turan *et al.*, 2005) have been observed in multispecies comparisons. Within *Mugil*, the lowest genetic distances are as high as D= 0.620 between *M. cephalus* and *M. curema* from East Pacific (Rosenblatt and Waples, 1986), rising to a maximum of D= 0.898 be-

tween *M. gyrans* (probably = *M. trichodon*; Thomson, 1997; Harrison, 2002) and *M. curema* from Florida (Rossi *et al.*, 1998b). Turan *et al.* (2005) reported D= 0.606 between *M. cephalus* and *M. soiuy* (from the Black Sea); however, the latter species is not validly accepted and most reports of *M. soiuy* from the Black Sea are misidentifications of *Liza haematocheilus* (Bogutskaya and Naseka, 2004; Harrison, 2004). Therefore, it is unclear what material Turan *et al.* (2005) had before them. The D value obtained between *M. rubrioculus* and *M. curema* falls within the wide range of distance values obtained by Rossi *et al.* (1998a) for global populations of *M. cephalus*, from 0 to 0.242 (mean= 0.117). The most differentiated of the populations of *M. cephalus* examined by Rossi *et al.* (1998a) have, however, been proposed to be at a stage of incipient speciation.

Thus, the cytogenetic and allozyme data sets suggest quite different degrees of genetic divergence between *M. rubrioculus* and *M. curema*. The cytogenetic features, that are conservative in most species of fishes and particularly in Mugilidae (Sola *et al.*, 2007), reveal striking differences between the two species, both in the chromosome complement and in the position of ribosomal genes. On the other hand, allozyme data, which are generally quite variable at intraspecific and interspecific levels, reveal an unsubstantial genetic differentiation between *M. rubrioculus* and *M. curema* which, in the absence of chromosomal and morphological data, would suggest an ongoing gene flow between them.

Considering the two data sets, two possible scenarios can be hypothesized. On the one hand, an underestimate of molecular divergence between *M. curema* and *M. rubrioculus* could have been obtained, due to the presence of cryptic variation and alleles with identical electrophoretic mobility, but different nucleo-

tide sequences (Aquadro and Avise, 1982). On the other hand, a significant structural intrachromosomal reorganization might have taken place at the time of speciation and the low divergence of molecular data would mark the recentness of the separation between the two taxa. The application of other molecular markers to *M. curema*, *M. rubrioculus* and other Western Atlantic mugilids is required to test these hypotheses further. Such studies will provide additional characters to ascertain the phylogenetic relationships of these species and to test the assumption that *M. rubrioculus* and *M. curema* are closely related (based on their similar appearance).

Nevertheless, there is little doubt that chromosomal rearrangements play an important role in establishing reproductive barriers. Thus, karyological data constitute a useful diagnostic tool for examining species divergence within Mugilidae, and this has applications to studying their biology and ecology. The data presented confirm the power of karyological data in discriminating sympatric specimens of *M. rubrioculus* and *M. curema*. Moreover, by comparing the data reported with that reported for global populations of *Mugil cephalus* (Rossi *et al.*, 1998a), for which genetically differentiated populations share the chromosome complement and NOR location (Rossi *et al.*, 1996), it is suggested that different rates of molecular/chromosomal evolution might act within the genus *Mugil*.

#### ACKNOWLEDGEMENTS

The authors thank José Marcano for his help with specimen collection and acknowledge financial support provided by the Consejo de Investigación, Universidad de Oriente, Venezuela, by the Italian Ministry of Instruction, University and Research (MIUR), DM n. 219, 22-12-05, National Relevant Interest Research Projects (PRIN), 2005,

and by the Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo à Pesquisa de São Paulo. Ian Harrison is grateful to staff and students at the Universidad de Oriente, Venezuela.

#### REFERENCES

- Álvarez-Lajonchere L, Trewavas E, Howes GJ (1992) *Mugil curema* and *M. liza* Valenciennes in Cuvier and Valenciennes, 1836 (Osteichthyes, Perciformes): proposed conservation of the specific names. *Bull. Zool. Nomenclat.* 49: 271-275.
- Aquadro CF, Avise JC (1982) An assessment of "hidden" heterogeneity with electrophoresis at three enzyme loci in deer mice. *Genetics* 102: 269-284.
- Bertollo LAC, Takahashi CS, Moreira-Filho O (1978) Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erytrinae). *Braz. J. Genet.* 1: 103-120.
- Bogutskaya NG, Naseka AM (2004) *Catalogue of agnathans and fishes of fresh and brackish waters of Russia with comments on nomenclature and taxonomy*. Fishes Russia. Russian Academy of Sciences. Moscow, Russia. 389 pp.
- Bory de Saint-Vincent, JBGM (1831) *Dictionnaire Classique d'Histoire Naturelle*, Vol. 17. Rey & Gravier. Paris, France.
- Cervigón F (1993) *Los peces marinos de Venezuela*. Vol. II. 2<sup>a</sup> ed. Fundación Científica Los Roques. Caracas, Venezuela. 497 pp.
- Harris H, Hopkinson D (1978) *Handbook of enzyme electrophoresis in human genetics*. North-Holland Publishing. Amsterdam, Holland. 205 pp.
- Harrison IJ (2002) Mugilidae. In Carpenter K (Ed.) *The Living Marine Resources of the Western Central Atlantic*. FAO Species Identification Guide for Fisheries Purposes. Vol. 2. Bony Fishes, part 1. FAO. Rome, Italy. pp. 1071-1085.
- Harrison IJ (2004) Mugilidae. Addendum to Volume 8. Part 1. In Miller PJ (Ed.) *The Freshwater Fishes of Europe*. Vol. 8. AULA. Wiebelsheim, Germany. pp. 469-471.
- Harrison IJ, Howes GJ (1991) The pharyngobranchial organ of mugilid fishes; its structure, variability, ontogeny, possible function and taxonomic utility. *Bull. Brit. Mus. Nat. Hist. (Zool.)* 57: 111-132.

- Harrison IJ, Nirchio M, Oliveira C, Pérez J, Gaviria J, Ron E (2007) A new species of mullet (Teleostei: Mugilidae) from Venezuela, with a discussion on the taxonomy of *Mugil gaimardianus*. *J. Fish Biol.* 71 (Suppl A): 76-97.
- Howell WM, Black DA (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* 36: 1014-1015.
- ICZN (1994) Opinion 1787. *Mugil curema* and *M. liza* Valenciennes in Cuvier and Valenciennes, 1836 (Osteichthyes, Perciformes): species names conserved. *Bull. Zool. Nomenclat.* 51: 286-287.
- Lee SC, Chang JT, Tsu YY (1995) Genetic relationships of four Taiwan mullets (Pisces: Perciformes: Mugilidae). *J. Fish Biol.* 46: 159-162.
- Levan A, Fredga A, Sandberg A (1964) Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.
- Martins C, Galetti PM Jr (2001) Organization of 5S rDNA in species of the fish *Leporinus*: two different genomic locations are characterized by distinct nontranscribed spacers. *Genome* 44: 903-910.
- May B, Krueger CC (1995) *Genes in Populations* ver. 2. A computer program for analysis of genetic data. <http://animalscience.ucdavis.edu/extension/Software/Gene.htm>.
- Menezes NA (1983) Guia prático para conhecimento e identificação das tainhas e paratis (Pisces, Mugilidae) do Litoral Brasileiro. *Rev. Bras. Zool.* 2: 1-12.
- Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.
- Nirchio M, González D, Pérez JE (2001) Estudio citogenético de *Mugil curema* y *M. liza* (Pisces: Mugilidae): regiones organizadoras del nucleolo. *Bol. Inst. Oceanog. Venez.* 40: 3-7.
- Nirchio M, Cervigón F, Porto J, Pérez J, Gómez JA, Villalaz J (2003) Karyotype supporting *Mugil curema* Valenciennes, 1836 and *Mugil gaimardianus* Desmarest, 1831 (Mugilidae: Teleostei) as two valid nominal species. *Scientia Marina* 67: 113-115.
- Nirchio M, Cipriano R, Cestari M, Fenocchio A (2005a) Cytogenetical and morphological features reveal significant differences among Venezuelan and Brazilian samples of *Mugil curema* (Teleostei: Mugilidae). *Neotrop. Ichthyol.* 3: 107-110.
- Nirchio M, Ron E, Rossi AR (2005b) Karyological characterization of *Mugil trichodon* Poey, 1876 (Pisces: Mugilidae). *Scientia Marina* 69: 525-530.
- Papasotiropoulos V, Klossa-Kilia E, Kiliass G, Alahiotis S (2001) Genetic divergence and phylogenetic relationships in grey mullets (Teleostei: Mugilidae) using allozyme data. *Biochem. Genet.* 39: 155-168.
- Pinkel D, Straume T, Gray JW (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proc. Nat. Acad. Sci.* 83: 2934-2938.
- Rosenblatt RH, Waples RS (1986) A genetic comparison of allopatric populations of shore fish species from the eastern and central Pacific Ocean: dispersal or variance? *Copeia*: 275-284.
- Rossi AR, Crosetti D, Gornung E, Sola L (1996) Cytogenetic analysis of global populations of *Mugil cephalus* (striped mullet) by different staining techniques and fluorescent in situ hybridization. *Heredity* 76: 77-82.
- Rossi AR, Capula M, Crosetti D, Sola L, Campton DE (1998a) Allozyme variation in global populations of striped mullet, *Mugil cephalus* (Pisces: Mugilidae). *Marine Biol.* 131: 203-212.
- Rossi AR, Capula M, Crosetti D, Campton DE, Sola L (1998b) Genetic divergence and phylogenetic inferences in five species of Mugilidae (Pisces: Perciformes). *Marine Biol.* 131: 213-218.
- Rossi AR, Ungaro A, De Innocentiis S, Crosetti D, Sola L (2004) Phylogenetic analysis of Mediterranean mugilids by allozymes and 16S mt rRNA genes investigation: is the genus *Liza* monophyletic? *Biochem. Genet.* 42: 301-315.
- Rossi AR, Gornung E, Sola L, Nirchio M (2005) Chromosomal evolution in Mugilidae (Pisces, Mugiliformes): comparative molecular cytogenetic analysis of two congeneric species, *Mugil curema* and *M. liza*, characterized by significant karyotype diversity. *Genetica* 125: 27-32.
- Sola L, Gornung E, Mannarelli ME, Rossi AR (2007) Chromosomal evolution in Mugilidae, Mugilomorpha: an overview. In Pisano E, Ozouf-Costaz C, Foresti F, Kapoor BG (Eds.) *Fish Cytogenetics*. Science Publishers, Enfield, NH, USA. pp. 165-194.
- Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.* 75: 304-306.
- Thomson JM (1997) The Mugilidae of the World. *Mem. Queensland Mus.* 41: 457-562.
- Turan C, Caliskan M, Kucuktas H (2005) Phylogenetic relationships of nine mullet species (Mugilidae) in the Mediterranean Sea. *Hydrobiologia* 532: 45-51.