UNIVERSIDADE ESTADUAL PAULISTA

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ESTUDIOS CITOGENÉTICOS EN TELEOSTEOS MARINOS Y DULCEACUÍCOLAS DE VENEZUELA

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Dedicatoria

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CONTENIDO

RESUMEN

La determinación del número diploide (2n), la fórmula cariotípica y el Número Fundamental (NF) para 68 especies marinas y 26 dulceacuícolas pertenecientes a 39 familias contenidas en 18 ordenes reveló que el número diploide varió entre 2n=24 en *Mugil curema* (Mugilidae) y 2n=60 para *Pygocentrus cariba, Serrasalmus rhombeus* (Characidae) y *Hoplosternum littorale* (Callichthyidae) con una moda de 48 cromosomas representada en 47,3% (43/91) de todas las especies estudiadas. El porcentaje de especies que posee exclusivamente 48 cromosomas acrocéntricos (a) fue de 29,67% (27/91) y el NF varió entre 33/34 en *Stephanolepis setifer* (Tetraodontiformes) a 110 en *Pygocentrus cariba* y *Serrasalmus rhombeus* (Characiformes). En el grupo de peces marinos, el número diploide modal fue 48 y estuvo representado en 66,6% de todas las especies estudiadas, mientras que en los peces dulceacuícolas el complemento diploide modal fue 2n=54 representado en 30% de las especies estudiadas. La mediana del número de cromosomas fue estadísticamente mayor en los peces dulceacuícolas que en los marinos. El el rango medio del NF fue superior en el grupo de peces dulceacuícolas indicando que el grado de variación cariotípica, fue mayor en peces de aguas continentales en contraste con un patrón citogenético más conservado en los peces marinos. La proporción de especies con 48 cromosomas exclusivamente acrocéntricos predominó en el 36,7% de los peces marinos mientras que en los dulceacuícolas apenas alcanzó el 7,7%. Al comparar los Perciformes (linaje compuesto principalmente por peces marinos y uno de los más derivados en la filogenia de los Actinoperygii) contra el resto de los órdenes, se verificó que en aquellos, el 61,54% de las especies analizadas exhibieron cariotipos con 48 cromosomas a. Al graficar el número diploide de cromosomas y el NF contra la ordenación filogenética propuesta por Nelson (2006) para los Actinopterygii, se observó, en ambos casos, una relación inversamente proporcional, desde valores cercanos a 60 en los órdenes más ancestrales aquí estudiados, hasta valores de 48 en los grupos más derivados, principalmente en los Perciformes. La tendencia hacia la reducción del número diploide y del NF en la filogenia de los Actynopterygii conduce a reforzar la noción de que un cariotipo con aproximadamente 60 cromosomas con varios elementos de dos brazos sería la condición plesiomórfica en vez de considerar 48 a como la condición ancestral. Se propone la posibilidad de un escenario en el que un antecesor similar a los actuales *Amphioxus* haya evolucionado dando origen, luego de un evento de duplicación total del genoma, a un ancestro con un complemento diploide entre 64-80, a partir del cual habrían surgido los peces actuales.

ABSTRACT

Diploid number (2n), Karyotype formula and the fundamental number (NF) for 94 fish species, 68 marine and 26 freshwater species from 39 families contained in 18 orders revealed that the diploid number ranged between 2n = 24 in *Mugil curema* (Mugilidae) and 2n=60 for *Pygocentrus cariba*, *Serrasalmus rhombe*us (Characidae) and Hoplosternum littorale (Callichthyidae) with a mode of 48 chromosomes represented in 47.3% (43/92) of all studied species. The percentage of species with exclusively 48 acrocentric chromosomes was 29.67% (27/91) and the NF ranged from 33-34 in *Stephanolepis setifer* (Tetraodontiformes) to 110 in *Pygocentrus cariba* and *Serrasalmus rhombeus* (Characiformes). In the marine fish group the modal diploid number was 48 and was represented in 66.6% of all studied species, whereas in the freshwater fishes modal diploid complement was 2n=54 represented in 30% of the studied species. The median of the number of chromosomes was statistically higher in freshwater fishes than saltwater fishes. The NF was higher in the group of freshwater fishes indicating that the degree of karyotipic variation was greater in fish of inland waters in contrast with a more conserved cytogenetic pattern in the marine fishes. The proportion of species with 48 exclusively acrocentric chromosomes predominated in 36.7% of the marine fishes while it barely reached 7.7% in freshwater fishes. Comparing the Perciformes (a lineage composed mainly of marine fish and one of the most derived in the Actinoperygii phylogeny of the) against the rest of the orders, it was verified that in Perciformes 61.54% of analyzed species exhibited karyotypes with 48 chromosomes. When diploid number of chromosomes and the NF were plotted against the phylogenetic arrangement proposed by Nelson (2006) for actinopterigian fishes, both plottings revealed an inversely proportional relationship with values close to 60 in most ancestral orders up to 48 in the most derived groups mainly in the Perciformes. The trend towards both diploid number and NF decrease in the phylogeny of the Actynopterygii leads to reinforce the notion that a karyotype with approximately 60 chromosomes and with several 2-armed elements would be the plesiomorphic condition rather than considering 48 chromosomes a as an ancestral characteristic. The possibility of a scenario in which a predecessor similar to the current *Amphioxus* evolved yielding, after an event of total duplication of the genome, an ancestor with a diploid complement between 64-80, from which current fishes would have arisen, is proposed.

INTRODUCCIÓN

GENERALIDADES

Desde el punto de vista evolutivo los peces forman un grupo polifilético considerable, con 27.977 especies, que representa más de la mitad del número total de vertebrados conocidos el cual se encuentra en 54.711 especies (Nelson, 2006). Es importante destacar que, mientras la descripción de nuevas especies pertenecientes al grupo de los anfibios, reptiles, aves y mamíferos no es muy frecuente, muchas nuevas especies de peces son descritas continuamente lo cual puede elevar su número total a aproximadamente 32.500 (Nelson, 2006).

Además del gran número de especies, otro aspecto relevante relacionado con los peces es la importancia que revisten para las poblaciones humanas. De hecho, el tema de los peces aperece en diversos aspectos de la cultura humana, incluyendo la mitología, religión, literaura y el arte.Constituyen la dieta principal de muchas comunidades, ocupan un papel importante en la economía de muchas naciones y brindan una significativa fuente de trabajo en las sociedades que dependen en gran medida de la actividad pesquera.

Los peces son extraordinariamente diversos en sus características anatómicas y fisiológicas. Han desarrollado especializaciones espectaculares e innumerables para lograr funciones biológicas básicas, tales como alimentación, movimiento y reproducción. Con esta diversidad en estructura y función, los peces se han adaptado para vivir con éxito en una amplia gama de ambientes acuáticos. Pueden ser encontrados soportando temperaturas que van desde 44 °C, como algunas tilapias de África, a -2 °C en la Antártica. Habitan en lagos, ríos, estuarios y océanos de todo el mundo. La mayoría vive enteramente en agua dulce o en mares y océanos mientras que otros efectúan movimientos migratorios (Nelson, 2006).

Los términos y definiciones siguientes de las migraciones de los peces, propuestos por Meyer (1949), han sido adoptados generalmente:

(a) Diadromos: Peces verdaderamente migratorios que se mueven entre el agua de mar y la dulce.

(b) Anádromos: Peces diadromos que se pasan casi toda la vida en el mar y entran en los ríos a reproducirse (salmón, trucha de mar, alosa, lampreas de mar, esturiones).

(c) Catádromos: Peces diadromos que se pasan casi toda la vida en agua dulce y emigran al mar para la reproducción (anguilas, Salángidos, Galáxidos, Retropínidos).

(d) Anfídromos: Peces diadromos que emigran del mar al agua dulce y viceversa, pero no para desovar (ciertos Exócidos, *Perca fluviatilis*, algunos Mugílidos).

(e) Potamódromos: Peces verdadermente migratorios cuyos movimientos ocurren enteramente en agua dulce (trucha, sargo, Coregónidos).

(f) Oceanódromos: Peces verdaderamente migratorios que viven y se desplazan enteramente en el mar (bacalao, arenque, atún, caballa).

En relación con la diversidad biológica, se presentan formas con los más diversos hábitos. Algunas especies viven en grandes grupos mientras otras son altamente territoriales. Existen especies altamente especializadas como aquéllas que se alimentan exclusivamente de zooplancton, especies generalizadas, especies parásitas, etc. Algunas producen venenos, electricidad, luminiscencia. La mayoría son ectotérmicos, aunque existen otros, como los atunes, que son endotérmicos. Pueden presentar sexos separados y en algunos casos acentuado dimorfismo sexual externo, o pueden ser hermafroditas, madurando inicialmente como uno de los sexos y revirtiendo luego al sexo opuesto (protándricas o protoginas). Ciertas especies presentan fecundación interna mientras que la mayoría presenta

fecundación externa. Pueden presentar cuidado parental, hacer nidos o liberar millones de óvulos (Nelson, 2006)

Desde el punto de vista morfológico, los peces presentan tamaños que varían de 8 a 10 milímetros hasta 12 metros. Algunas especies poseen vistosos colores, mientras otras no. Unas presentan formas graciosas mientras otras, como los peces abisales, presentan formas grotescas. Cerca de 50 especies carecen de ojos (Nelson, 2006).

Debido a esta amplia diversidad y a los relativamente escasos estudios realizados hasta el momento, el conocimiento que se tiene de la ictiofauna es aún muy reducido. Este problema se ha agravado en los últimos tiempos por el hecho de que el ser humano está dañando de forma irreparable el ambiente en muchas áreas, conduciendo a la extinción a muchas especies que todavía no son conocidas. Por estas razones, se torna cada vez más importante y urgente la necesidad de estudiar las especies presentes en nuestros ecosistemas. En este punto es necesario señalar que los estudios requeridos no se circunscriben a la simple identificación de especies que puedan registrarse en un futuro, sino que deben abarcar todos los aspectos posibles, de manera que las futuras generaciones dispongan de información que les permita aprovechar ese recurso al máximo.

CITOGENÉTICA DE PECES

Por constituir un grupo particular entre los vertebrados, debido al número de especies, diversidad de formas, de comportamiento, hábitat y por la posición básica en la filogenia de los vertebrados, uno de los aspectos que ha sido objeto de interés por quienes se dedican al estudio de los peces es la Citogenética.

Hasta el inicio de la década del 60, pocos trabajos fueron hechos en citogenética pues los cromosomas apenas eran visualizados mediante el empleo de cortes histológicos seriados, lo que además de laborioso, resultaba en muchos errores de interpretación (Nogusa, 1960). Los estudios citogenéticos en peces

tuvieron inicio hacia el final del siglo pasado (1890), con el trabajo de Retzius que sugirió la presencia de 50 cromosomas en *Myxine glutinosa* y el trabajo de Kastschenko que insinuó la presencia de 30 a 50 cromosomas en *Pristiurus melanostomus* (ver Denton, 1973), pero la caracterización de los cromosomas en los peces se hizo efectiva a partir de 1960, gracias al desarrollo de técnicas refinadas de cultivos de células y tejidos en mamíferos (Clem *et al*., 1961; Booke, 1968; Wolf y Quimby, 1969; Denton, 1973).

En los últimos años la citogenética de peces se ha expandido significativamente, sobre todo a partir de la aplicación de técnicas de bandeo que, entonces usadas de manera rutinaria en investigaciones de citogenética humana y de mamíferos, presentaban dificultades para adaptarlas a los cromosomas de peces. La introducción en el estudio de los cromosomas de los peces de técnicas como el bandeo C, localización de regiones organizadoras del nucleolo por impregnación con Nitrato de plata (Ag-RONs), el uso de fluorocromos base-específicos, la incorporación de análogos de bases del ADN en el ciclo celular y, en menor escala, los bandeos G y R han suministrado, en conjunto, importantes resultados en la comparación de especies estrechamente emparentadas (Sola *et al*., 1984; Almeida -Toledo *et al*., 1988a), en la visualización de estadios iniciales de diferenciación de cromosomas sexuales (Phillips & Ihssen, 1985; Almeida-Toledo *et al*., 2000), en la identificación de patrones de replicación de los cromosomas (Delany & Bloom, 1984) y, más recientemente, en la identificación de secuencias de ADN en los cromosomas (Martínez *et al*., 1996; Martins *et al*., 2002, 2004). La utilización de características citogenéticas, como el número de cromosomas y fórmula cariotípica, Regiones Organizadoras del Nucleolo (RONs), distribución de heretocromatina constitutiva (bandas C) y de otros marcadores más específicos determinados gracias a la aplicación de técnicas moleculares, han revelado ser de gran importancia en el estudio de los peces permitiendo diagnosticar especies, diferenciar especies crípticas y razas cromosómicas (Nirchio *et al*., 2003a, 2005), establecer las relaciones existentes entre especies dentro de un género o familia (Nirchio *et al*., 2001, Oliveira *et al*., 2003) y clarificar el origen de los híbridos naturales y variedades cultivadas (Nirchio *et al*., 2003b).

CONTROVERSIAS SOBRE EL CARIOTIPO ANCESTRAL

La más reciente revisión (Klinkhardt *et al*., 1995), revela que los datos citogenéticos disponibles cubren solo el 11,5% (aproximadamente 2,700 especies), de las especies existentes; el número diploide varia entre 2n=16 en *Sphaerichtys spromenarides* (Belontiidae) y 2n=446 en *Ptychobarbus* (*Diptychus*) *dipogon* (Cyprinidae) y el número cromosómico más común es 2n = 48.

Aunque la predominancia del cariotipo 2n=48 ha conducido a proponer esa constitución como la más ancestral para los teleósteos (Ohno, 1974), esta generalización debe ser tomada con precaución ya que esa constitución, con un número fundamental variable (NF: número fundamental=número de brazos), está representada en 60-70% de los Perciformes, el cual constituye el grupo más estudiado y en el que, casi el 30% exhibe un cariotipo homogéneo con 2n=48 y FN=48. De hecho, se ha argumentado que esta característica no debería ser asumida como ancestral ya que muchos de los vertebrados basales como los Agnatha, Condrichthyes y Sarcopteygii, poseen los mayores NF y números diploides, así como también en los Chondrostei, Gynglimodi, Halecomorpha, Osteoglossomorpha y Elopomorpha (Brum & Galetti, 1997). Efectivamente, los mayores números diploides y la presencia de microcromosomas han sido encontrados principalmente en los peces más primitivos tales como lampreas (Petromyzontidae), peces cartilaginosos (Elasmobranchiomorphi), peces lagarto (Lepisosteidae), y esturiones (Acipenseridae).

Esto ha conducido a proponer que el número básico de cromosomas en los Teleostei debería ser aproximadamente 2n=60, con pocos elementos metacéntricos y que el número diploide 2n=48 podría haber surgido ,a nivel macroestructural, por fusiones y supresiones a partir del cariotipo ancestral, extendiéndose como carácter sinapomórfico en los Clupeomorpha y Euteleostei los cuales habrían conservado este cariotipo principalmente en las especies marinas pertenecientes a los Atherinimorpha y Percomorpha (Brum & Galetti, 1997).

A la luz de los puntos de vista antes señalados, es obvio que la escasez de información citogenética en los peces y el hecho cierto de que apenas muy pocos grupos han sido estudiados, conduce a establecer que hasta tanto no se realice una exploración más exhaustiva, cualquier conclusión a la que se pueda llegar se mantendrá dentro del campo de la especulación.

CITOGENÉTICA DE PECES NEOTROPICALES DE AGUA DULCE Y MARINOS

El número de estudios citogenéticos en peces neotropicales ha presentado un considerable aumento en los últimos años. En la actualidad se registran datos citogenéticos para 47 familias, 278 géneros y 1.047 especies dulceacuícolas y 39 familias, 73 géneros y 109 especies marinas (Estas listas son actualizadas periódicamente y se encuentran disponibles en Internet en los sitios: http://www.ibb.unesp.br/laboratorios/Freshwater%20Neotropical%20fishes.pdf, y http://www.ibb.unesp.br/laboratorios/Marine%20Neotropical%20fishes.pdf.

La primera revisión sobre datos citogenéticos de peces neotropicales dulceacuícolas, realizada por Almeida-Toledo (1978), incluye los números haploides y/o diploides de 252 formas de agua dulce de las divisiones primarias y secundarias de América del Sur y Central. En una revisión realizada 10 años después, se indican los números haploides y/o diploides de 421 especies, distribuidas en 141 géneros y 32 familias (Oliveira *et al*., 1988a). Para el año 2000 los datos disponibles para peces de agua dulce (Oliveira *et al*., 2000a), recopilan los números diploides y/o haploides de 921 especies (113% más que en 1988), 252 géneros (74% más que en 1988) y 44 familias (33% más que en 1988).

Considerando que el más reciente inventario de ictiofauna neotropical de agua dulce (Reis *et al*., 2003), totaliza unas 6025 especies (4475 especies válidas más 1550 especies no descritas) y que en la actualidad existen registros de datos citogenéticos para 47 familias, 278 géneros y 1.047 especies dulceacuícolas del neotrópico (Datos no publicados de Oliveira, 2006; disponibles en el sitio *http://www.ibb.unesp.br/la-boratorios/Freshwater%20Neotropical%20fishes.pdf*) el conocimiento citogenético en este grupo apenas cubre el 17,37% de las especies

existentes. Esos datos permiten establecer que el número diploide varía entre 2n=20 para *Pterolebias longipinnis* a 2n=134 para *Corydoras aeneus*. Sin embargo, hay una gran discrepancia respecto a la naturaleza de los datos disponibles para cada grupo. Así, por ejemplo, para el género *Hyphessobrycon* se conocen los números diploides y/o haploides de 35 especies/subespecies pero apenas seis tienen su cariotipo descrito, mientras que para el género *Leporinus* son conocidos los números haploide y/o diploide de 37 especies de las cuales apenas para tres de ellas no hay datos respecto a su estructura cariotípica. El género más estudiado ha sido *Corydoras* para el cual se conocen los números haploide y/o diploide de 43 especies. Han sido descritos cromosomas sexuales para 51 especies o poblaciones locales (5,75% del total de especies analizadas) que engloban 36 informes de heterogamia femenina (68%) y 17 de heterogamia masculina (32%). Cromosomas supernumerarios han sido encontrados en 41 especies (4,45%) del total de especies analizadas. El contenido de ADN nuclear se encuentra determinado para 174 especies y/o poblaciones y varía entre 1,04±0,09 pg/núcleo diploide en *Corydoras* cf. *simulatus* (2n=62) a 248,0 pg/núcleo diploide en *Lepidosiren paradoxa*. El número y/o la localización de las regiones organizadoras del nucléolo (RONs) han sido descritos para 1.205 especies y/o poblaciones, variando entre 1 y 13 pares de cromosomas portadores de RONs (con una moda de 1 par). Los estudios que involucran la aplicación de técnicas de bandeo cromosómico han sido aplicados a 1.032 especies y/o poblaciones locales, destacándose un creciente aumento de la aplicación de técnicas de hibridación in situ con sondas fluorescentes (FISH).

En cuanto a los en peces Neotropicales marinos, las caracterizaciones citogenéticas se iniciaron a principio de la década de los 80, con la descripción de los cariotipos de *Mentichirrus americanus* y *Micropogonias furnieri* (Gomes *et al*., 1983a, 1983b) y actualmente, el estudio de estos peces se encuentra en franco aumento. El análisis general de los datos para 109 especies marinas (Datos no publicados de Oliveira, 2006; disponibles en el sitio http://www.ibb.unesp.br/laboratorios/Marine%20Neotropical%20fishes.pdf), indica que las familias más extensamente estudiadas son Carangidae y Mugilidae, para las que se conocen

los números haploide y/o diploide de 10 y 16 especies respectivamente. Los números diploides varían de 2n=24 para *Mugil curema* a 2n=100/102 para *Prionotus punctatus*. Del total de especies analizadas, 49 (60%) presentan 2n=48 cromosomas y en 28 (35%) de ellas todos los cromosomas son acrocéntricos. Han sido descritos cromosomas sexuales para tres especies (3,7% del total de especies analizadas) y cromosomas supernumerarios para una especie (1,2% del total de especies analizadas). Se conoce el contenido de ADN nuclear para dos especies (2,5% del total de especies analizadas) siendo que el mismo varía de 1,24±0,01 pg/núcleo en *Micropogonias furnieri* (2n=48) a 1,57±0,03 pg/núcleo en *Menticirrhus americanus* (2n=48). El número y localización de las regiones organizadoras del nucléolo (RONs) han sido descritos para 79 especies y/o poblaciones, y todas han exhibido RONs simples. Otras técnicas de bandeo cromosómico han sido aplicadas en 56 especies y/o poblaciones. Considerando que la ictiofauna de aguas marinas de la región Neotropical es bastante diversificada, se puede afirmar que el estado actual del conocimiento en esta área es aún muy limitado.

AMBIENTES ACUÁTICOS, ICTIOFAUNA Y CITOGENETICA DE PECES EN VENEZUELA

Venezuela se encuentra entre los primeros 10 países con la mayor biodiversidad en el mundo, tanto en el ambiente terrestre como en acuático (Miloslavich *et al*., 2003).

La costa venezolana abarca aproximadamente una longitud de 3.726 Km., que se extiende a lo largo del Mar Caribe y del Océano Atlántico. Alrededor de 1.700 Km. son playas y 311 son islas que incluyen fondos arenosos, litorales rocosos, praderas de fanerógamas marinas, arrecifes coralinos, comunidades de fondos blandos y bosques de manglar. En el territorio venezolano se destacan extensiones de masas de agua que incluyen: ríos, lagunas andinas y costeras, zonas pantanosas, lagos y embalses. La mayor parte de los cuerpos de agua continentales son ríos, que se agrupan en dos vertientes principales: la del

Océano Atlántico (82% del territorio nacional) integrada por el Orinoco y sus tributarios y, la del Mar Caribe (18%) constituida por los ríos que drenan hacia ese mar. Tanto por su extensión territorial como por el volumen de agua escurrida, la cuenca del Orinoco es la unidad hidrográfica dominante en Venezuela, con un caudal promedio anual de 36.000 m^3/s, ocupando el tercer lugar a nivel mundial luego del Amazonas y Zaire.

En todos estos ambientes acuáticos se ecuentra una gran diversidad ictiológica. Una aproximación al conocimiento de la ictiofauna venezolana se encuentra disponible en la base de datos FISHBASE (http://www.fishbase.org/) que registra la existencia de un total de 1.718 especies, de las cuales 916 son dulceacuícolas y 802 marinas. De estas, ocho son especies introducidas, 68 son endémicas, 29 son especies amenazadas, 113 son especies invasoras y 107 tienen importancia comercial (Froese & Pauly, 2009).

Se conoce el número haploide/diploide para apenas el 7% de las especies marinas (nuestros datos no publicados). Es importante destacar que esta información procede de estudios realizados con esas especies en diversas localidades de la región Neotropical y no necesariamente pertenece a la de las especies locales. Por lo tanto, la Citogenética de peces, en Venezuela, es un campo que requiere urgentemente de más exploración si se quiere incrementar el conocimiento general de las características citogenéticas de los peces y disponer de información básica que contribuya a esclarecer las relaciones entre ellas.

OBJETIVOS:

El presente trabajo forma parte de la línea de investigación en Citogenetica de peces Neotropicales que se desarrolla en los laboratorios de Genética y Citogenética de Peces del Instituto de Biociencias de la Universidad Estadual Paulista Julio de Mezquita Filho en Brazil y el Laboratorio de Citogenética de Peces de la Escuela de Ciencias Aplicadas del Mar de la Universidad de Oriente en Venezuela, entre cuyos objetivos principales destaca ampliar el conocimiento sobre la diversidad citogenética de la ictiofauna Neotropical dulceacuícola y marina.

Con base en este propósito se procura:

1. Ampliar el conocimiento sobre la diversidad citogenética de la ictiofauna venezolana determinando el número diploide, cariotipo, número fundamental, y cuando sea posible, el patrón de distribución de Bandas C y el número y distribución de Regiones Organizadoras del Nucléolo (RONs) para un número significativo de peces marinos y dulceacuícolas.
2. Verificar si al extender las pesquisas en grupos aun no estudiados, se mantiene la hipótesis según la cual el cariotipo más común en los peces marinos es 2n=48 acrocéntricos.
3. Correlacionar el número fundamental y el número diploide de los peces estudiados con la posición que ocupa cada grupo a nivel de Órden en la Filogenia de los peces propuesta por NELSON (2006).
4. Empleando el número diploide, cariotipo y número fundamental, proponer una hipótesis para explicar la tendencia evolutiva del cariotipo en los Actinopterygii

MATERIALES Y MÉTODOS

DATOS

Todos los especímenes analizados fueron colectados alrededor de la Isla de Margarita y en Caicara del Orinoco, Venezuela.

Margarita es una de las tres islas que conforman el Estado Nueva Esparta. Se ubica al Norte de Venezuela, a 38 Km de la costa Nororiental entre las coordenadas 10º51’50’’–11º11’06’’ Latitud Norte y 63º46’40’’–64º24’32’’ Longitud Oeste (Fig. 1).

Por su parte, Caicara del Orinoco es una localidad que limita al norte con el río Orinoco, frente al Estado Guárico, al sur con el Estado Amazonas, al este con el municipio Sucre y al oeste con el Estado Apure. Ocupa el márgen derecho del río Orinoco a 6°10'33'' de longitud oeste y a 7°0'36'' de latitud norte (Fig. 1).

Ejemplares de referencia (voucher) se encuentran depositados en la colección ictiológica del laboratorio de Genética y Citogenética de Peces del Instituto de Biociencias de la Universidad Estadual Paulista Julio de Mezquita Filho en Brazil y en el laboratorio de Citogenética de Peces de la Escuela de Ciencias Aplicadas del Mar, perteneciente a la Universidad de Oriente en Venezuela.

OBTENCION DE CROMOSOMAS MITÓTICOS

ESTIMULACIÓN DE LA MITOSIS

Para la obtención de un mayor número de metafases se empleó la técnica descrita inicialmente por Cole & Leavens (1971) para anfíbios y reptiles utilizada por Lee y Elder (1980) para pequenos mamíferos y por Oliveira *et al*. (1988) para peces y que consiste en:

1- Preparar una solución de levadura (Fleischmann) en la siguiente proporción: 0,5g de levadura, 0,5g de azucar y 7ml de agua destilada;

2- Incubar la solución en baño-maria (40°C) por aproximadamente 20 minutos;

3- inyectar la solución dorso-lateralmente en el pez en proporción de 1 ml por cada 100g de peso del animal;

4- dejar el animal en un acuario bien aireado por durante 48-72h.

PREPARACIÓN DE CROMOSOMAS MITÓTICOS

La técnica utilizada para la obtención de figuras mitóticas es la descrita por Foresti *et al*. (1993) que consiste en:

1- Sacrificar el animal, retirando riñones, bránquias y testículos en el caso de ejemplares de sexo masculino;

2- Colocar los tejidos retirados en placa de Petri contenientes 6 ml de solución de KCl 0,075 M, a temperatura ambiente;

3- Disociar el material, procurando obtener una suspenssión de células; para ello primero disociar el material con pinzas de punta fina y, después, homogeneizar con el auxílio de una pipeta Pasteur;

4- Retirar la suspensión de la placa de Petri y colocarla en un tubo de centrífuga.

7- Deijar el tubo en el interior de una estufa a 37°C por 30 min;

8- Retirar el tubo de la estufa, colocar 5 gotas de fijador helado (metanol y ácido acético en proporción 3:1 V:V). Agitar levemente la mezcla con una pipeta Pasteur y dejar reposar por 5 min a temperatura ambiente;

9- Adicionar 6ml de fijador y, nuevamente, agitar la mezcla. Centrifugar (1000 $\pm$ 100 rpm) por 10 minutos;

10- Retirar el sobrenadante y resuspender el precipitado en 6 ml de fijador. Centrifugar por 7 minutos más a 1000 $\pm$ 100 rpm;

11- Repetir el item 8 por dos o tres veces;

12- Gotear el material en láminas colocadas sobre un soporte, en el interior de um banho-maria a 60°C.

TÉCNICAS DE COLORACION CROMOSOMICA

COLORACIÓN CON GIEMSA

Para colorear con Giemsa, fue utilizado el siguiente procedimento:
1- Hidrolisar el material en HCl 1N a 60°C por cerca de 3 min;
2- Colorear con una solución de solución de Giemsa al 5% em tampón fosfato (pH = 6,7) por 10 min.

LOCALIZACIÓN DE REGIONES ORGANIZADORAS DE NUCLÉOLO

El procedimiento utilizado siguió la técnica descrita originalmente por Howell & Black (1980), fueron utilizadas dos soluciones:
A: solución coloidal reveladora: 1 g de gelatina bien disuelta en 50 ml de agua deionizada. Agregar 0,5 ml de ácido fórmico.
B: solución de nitrato de plata: 1 g de $AgNO_3$ disuelto en 2 ml de agua deionizada.
Después de preparadas estas soluciones deben ser mantenidas en frascos oscuros, a 4°C.
El procedimento para la coloración de las RONs es el siguiente:
1- Hidrolizar el material por 3 min en HCl 1N a 60°C;
2- Secar las lâminas. Colocar una gota de solución A y dos gotas de solución B sobre el material en la lámina; colocar un cubreobjetos;
3- Dejar reposar las láminas en una cámara húmeda, en el interior de un baño-maria a 60°C. En algunos minutos (aproximadamente 3) la mezcla de las soluciones adquiere una tonalidad marron dorada. Lavar la lámina en agua deionizada y dejar secar al aire;
4- Colorear con Giemsa en proporción 1:30 en tampón fosfato (pH = 6,7) por 30 s.

BANDAS C

Para obtención de bandas C fue utilizada la técnica descrita originalmente por Sumner (1972) que consiste en:

1. Sumergir el material previamente goteado sobre un portaobjetos, en una solución de HCl 0,2 N a temperatura ambiente por 10 a 30 min.
2. Lavar con agua destilada.
3. Incubar la lámina por 10 a 60 s en Solución de Ba(HO)2 a 60º C.
4. Lavar rápidamente con HCl 1N a 60º C.
5. Incubar la lámina por 30 min en Solución de 2xSSC a 60º C.
6. Lavar en agua destilada.
7. Colorear por aproximadamente 30 min con Giemsa en proporción 1:20 en tampón fosfato (pH = 6,7)
8. Lavar con agua corriente y dejar secar.

LOCALIZACIÓN CROMOSÓMICA DE SECUENCIAS DE DNA POR HIBRIDACIÓN *IN SITU* FLUORESCENTE (FISH)

La hibridación *in situ* fluorescente (FISH) fue realizada de acuerdo con la metodología descrita por Pinkel *et al*. (1986), con ligeras modificaciones. El proceso consistió en:

Marcar la sonda con biotina-11-dATP, mediante el método de *nick translation*, utilizando el *kit* Bionick™ Labeling System (Gibco.BRL):

1. En un tubo de 1,5 ml, mantenido en hielo, mezclar:
   - 5μl de dNTP mix 10x
   - aproximadamente 1μg de ADN (sonda) – cantidad para 8 láminas
   - 5μl de la mezcla de ADNasa y ADNpolimerasa concentrada 10x
   - Agua milli-Q para completar 45μl
2. Mezclar muy bien y centrifugar brevemente por 5 s.
3. Incubar a 16 ºC por 1 ó 2 h.

4. Adicionar 5 μl de tampón de bloqueo

5. Adicionar 5 μl de acetato de sodio 3M más 100μl de etanol absoluto bien frio (preferiblemente colocado en ultrafreezer a -70 ºC). Mezclar inviertiendo el tubo varias veces.

6. Mantener a -20 ºC durante no menos de 3 h.

7. Centrifugar el material a 13.000 r.p.m. por 10 min.

8. Descartar el sobrenadante cuidadosamente y adicionar 50μl de etanol 70% bien frío.

9. Centrifugar el material una vez más a 13.000 r.p.m. durante 5 min.

10. Descartar el sobrenadante con mucho cuidado y secar en estufa a 37^{o}C.

11. Resuspender la sonda marcada en 80 μl de agua milli-Q.

Preparación de las láminas

1. Tratar cada lámina con las preparaciones cromosómicas con 100μl de solución de RNAsa 40 μg/ml (0,4 μl de RNAsa 10mg/ml y 99,6 μl de 2xSSC - para cada lámina) durante 1 hora y 30 min, en cámara húmeda con 2xSSC, a 37 ºC.

2. Lavar las láminas en 2xSSC durante 10 min.

3. Repetir el lavado en 2xSSC.

4. Deshidratar las láminas en serie alcohólica (etanol frio70%, 85% y 100%) durante 10 min en cada concentración.

5. Colocar las láminas en formamida 70% diluída en 2xSSC durante 5 min a 70^{o}C (utilizar 28 ml de formamida y 12 ml de 2xSSC) para provocar la desnaturalización del ADN en los cromosomas. Esta solución se puede guardar para ser reutilizada (ver más adelante en el texto).

6. Deshidratar las láminas nuevamente en serie alcohólica (etanol frío 70%, 85% y 100%) por 5 min en cada concentración.

7. Dejar secar las láminas al aire.

HIBRIDACIÓN

1. En el tubo que contiene la sonda (80 μl), agregar 200 μl de formamida (concentración final de 50%), 80 μl de sulfato de dextrano 50% (concentración final de 10%) y 40 μl de 20xSSC (concentración final de 2xSSC).

2. Desnaturalizar la solución de hibridación en baño hirviente durante 10 min y colocar inmediatamente en hielo.
3. Colocar 50 μl de solución de hibridación (conteniendo cerca de 125 ng de la sonda) sobre un cubreobjetos e invertir sobre la lámina.
4. Mantener las láminas, con el material volteado hacia abajo, en cámara húmeda con 2xSSC, a 37 ºC durante 12-14 h.

LAVADO

1. Lavar las láminas en 2xSSC a temperatura ambiente apenas para desprender los cubreobjetos. Desde este momento las láminas no pueden secarse.
2. Lavar las láminas en solución de formamida 50% diluída en 2xSSC por 15 min a 37°C. Puede emplearse las solución del dia anterior - agregar 16 ml de 2xSSC).
3. Lavar en 2xSSC por 15 min a 37 ºC.
4. Lavar en 2xSSC por 15 min a temperatura ambiente.
5. Lavar en 4xSSC por 5-10 min a temperatura ambiente.

DETECCIÓN:

1. Sobre cada lámina, colocar 70μl de FITC 0,07% (Fluoresceína Isotil Cianato - Avidina Conjugada) diluida en tampón C (usar 0,1 μl de solución *stock* de FITC 250 μg/100 μl, y 70 μl de tampón C).
2. Cubrir con un cubreobjetos y dejar por 40 min a 1 hora en cámara húmeda con 2xSSC a 37°C.
3. Lavar en tampón de bloqueo recién preparado a 42 ºC durante 5 min, con agitación.
4. Repetir el lavado en tampón de bloqueo dos veces más.
5. Escurrir las láminas y secarlas por debajo.
6. Aplicar sobre cada lámina 80 μl de anticuerpo anti-avidina biotina conjugada (2 μl de anti-avidina stock y 78 μl de tampón de bloqueo).

7. Cubrir la lámina con un cubreobjetos y dejar en cámara húmeda con 2xSSC a 37 ºC durante 30 min.
8. Lavar en tampón de bloqueo a 42 ºC durante 5 min, con agitación.
9. Repetir el lavado en tampón de bloqueo dos veces más.
10. Aplicar nuevamente el FITC sobre la lámina. (La señal de hibridación puede ser aumentada mediante pasos sucesivos utilizando avidina-FITC y anti-avidina biotinilada sobre la lámina).
11. Lavar las láminas en tampón de bloqueo a 42 ºC durante 5 min, con agitación.
12. Repetir el lavado en tampón de bloqueo dos veces más.
13. Lavar en 4xSSC y Triton 2% a temperatura ambiente durante 3 min, con agitación.
14. Repetir el lavado en 4xSSC y Tritón 2%.
15. Lavar en 4xSSC y Tritón 0,2% a temperatura ambiente durante 3 min, con agitación.
16. Repetir el lavado en 4xSSC y Tritón 0,2%.
17. Escurrir las láminas y dejar secar al aire.

Montaje de las láminas

Aplicar Ioduro de Propidio en las láminas para hacer la contracoloración del material – 25 μl de *antifading* (Vectashield antifade-Vector) y 1μl de solución de ioduro de propidio (50 μg/ml) por cada lámina.

SOLUCIONES

20xSSC

NaCl - 175,3g
Citrato de sodio - 88,2g
Agua destilada – hasta completar 800ml
Ajustar a pH 7,0
Agua destilada – hasta completar 1000ml

Tampón de bloqueo

($NaHCO_3$ 1.26%, citrato de sodio 0.018%, Tritón 0.038% y leche descremada 1%) para 1800ml:

4,41g $NaHCO_3$

0,324 g citrato de sodio

694,28 μl de Tritón 20 o 138,85 μl de Tritón 100

Completar con agua destilada hasta 1.800 ml

Mezclar bien

Medir el pH (debe estar entre 8,0-10,8)

Agregar 3,5 g de leche descremada en polvo

Mezclar bien y mantener a 42 ºC en baño de María

Tampón C

Bicarbonato de sodio 0,1M pH 8,5

NaCl 0,15M

Dividir en alícuotas, esterilizar en autoclave y guardar a -20 ºC.

ANÁLISIS

Analizar las preparaciones de hibridación fluorescente *in situ* en microscopio de fluorescencia, con filtro 450-490 nm.

ANÁLISIS Y MONTAJE DE CARIOTIPOS

Las figuras mitóticas fueron fotografiadas con cámara digital marca Nikon modelo Coolpix 995 en un microscopio Leica, modelo DLMB. Un máximo de diez ejemplares por especie fueron analizados para el estudio Citogenético. Para cada individuo se realizaró el recuento de 10 células metafásicas a los fines de totalizar 100 células para la especie.

Para la organización y montaje de los cariotipos, las imágenes digitales fueron editadas con el Software ADOBE PHOTOSHOP CS2. Para cada cromosoma se midió la longitud del brazo largo (L), el brazo corto (S) y la longitud total con la herramienta de medición del programa antes mencionado, la cual brinda la posibilidad de realizar las mediciones con una apreciación de 0.01 mm. A partir de estos datos fue calculado el cociente L/S (Levan et al, 1964). Dependiendo del valor del índice los cromosomas fueron clasificados en metacéntricos (r de 1,00 a 1,70), submetacéntricos (r de 1,71 a 3,00), subtelocéntrico (r de 3,01 a 7,00) y acrocéntricos (r mayor que 7,01).

ANÁLISIS DE LOS DATOS

A menudo han sido reportadas diferencias en la fórmula cariotípica y en el FN para la misma especie debido, en gran parte, a los diversos criterios usados en la clasificación cromosómica y/o en las diferencias en el grado de condensación de los cromosomas. Tal inconveniente fue minimizado en este trabajo, por cuanto todos los cariotipos obtenidos en este trabajo fueron obtenidos bajo las mismas condiciones de laboratorio (concentración de colchicina, tiempo de exposición, etc) y los cromosomas clasificados con el mismo criterio.

Los análisis estadísticos fueron realizados según Sokal & Rolph (1981). Para verificar la existencia de diferencias estadísticas en el número diploide de cromosomas y en el NF entre peces dulceacuícolas y marinos se empleó el Análisis de Varianza no Paramétrico de Kruskal-Wallis. Mediante el uso del test de independencia Chi-cuadrado se probó si la proporción de especies con 48

cromosomas exclusivamente acrocéntricos dependía del ambiente (marino o dulceacuícola) y si había alguna dependencia del número de especies con 2n=48A, entre el grupo de los Perciformes y el resto de los órdenes. Ante la imposibilidad de realizar análisis de regresión por la ausencia de normalidad en la data examinada, fueron creados gráficos del número diploide de cromosomas, Número Fundamental (NF= número de brazos) y valor C contra el valor ordinal correspondiente a cada Orden en la Filogenia de los Actynopterygii propuesto por NELSON (2006), estableciendo el elipsoide que agrupa el 95% de los datos con el Programa PAST (Hammer *et al*., 2001). Mediante el empleo del Programa G-STAT (disponible en: http://www.e-biometria.com/g-stat/index.html) se procedió a establecer si existía correlación entre esos parámetros y la posición filogenética de los Órdenes mediante el Test de Correlación por Rangos de Spearman.

RESULTADOS Y DISCUSION

CAPÍTULO I.- RESULTADOS GENERALES

Los resultados de este estudio incluyen 92 especies pertenecientes a 62 géneros contenidos en 39 familias comprendidas en 18 Órdenes (Tabla 1). De éstas, 67 especies son marinas mientras que 25 son dulceacuícolas. De las 92 especies incluidas en los análisis de este trabajo, 20 especies corresponden a taxones estudiados con anterioridad al desarrollo de este trabajo de tesis doctoral y 72 especies corresponden a las que fueron analizadas durante el periodo de estudios doctorales.

Se incluyen, los artículos científicos publicados y/o en vías de publicación que fueron generados durante este estudio para especies de las familias Characidae, Callichthyidae, Pseudopimelodidae, Loricariidae, Ariidae, Mugilidae (Nirchio *et al*., 2007; Harrison *et al*., 2007; Sola *et al*., 2008) Lutjanidae (Nirchio *et al*., 2008, 2009), Haemulidae (Nirchio *et al*., 2007), Atherinopsidae (Muñoz *et al*., 2006) y Monacanthidae (Nirchio & Oliveira, 2007). Los resultados citogenéticos se encuentran resumidos en la Tabla 2.

El cariotipo con los cromosomas ordenados según tamaño y tipo para cada una de las especies cuyos datos aun no han sido publicados, así como la fotografía de cada una de esas especies se muestran en las figuras 6 a 39

El número diploide para el conjunto de peces aquí analizados varió entre 2n=24 en *Mugil curema* (Mugilidae) y 2n=60 en *Pygocentrus cariba, Serrasalmus rhombeus* (Characidae) y *Hoplosternum littorale* (Callichthyidae) con una moda de 48 cromosomas representada en 48 de las 92 especies estudiadas (52,2%). El porcentaje de especies que posee exclusivamente 48 cromosomas acrocéntricos fue de 28,3% (26/92) y el número de brazos, (NF) varió entre 110 en *Pygocentrus cariba* y *Serrasalmus rhombeus* (Characiformes) a 33/34 en *Stephanolepis setifer* (Tetraodontiformes) (Tabla 2).

En el grupo de peces marinos, el número diploide modal fue 2n=48 y estuvo representado en 44,57% de todas las especies estudiadas, mientras que en los peces dulceacuícolas el complemento diploide modal fue 2n=54, representado en 30% de las especies estudiadas.

En concordancia con estos resultados, el análisis general de los datos citogenéticos de peces marinos y dulceacuícolas obtenidos por Oliveira *et al*., (2007) para la región neotropical y que se encuentran disponibles en la web http://www.ibb.unesp.br/laboratorios/Freshwater%20Neotropical%20fishes.pdf y http://www.ibb.unesp.br/laboratorios/Marine%20Neotropical%20fishes.pdf), muestra que para los peces dulceacuícolas el número diploide varía entre 2n=20 en *Pterolebias longipinnis* a 2n=134 en *Corydoras aeneus* mientras que en el grupo de peces marinos los números diploides varían de 2n=24 para *Mugil curema* a 2n=100/102 para *Prionotus punctatus* y que, del total de especies analizadas, 49 (60%) presentan 2n=48 cromosomas siendo que en 28 especies (35%) todos los cromosomas son acrocéntricos.

El análisis de varianza no paramétrico (Tabla 3) reveló que la mediana del número de cromosomas fue significativamente mayor en los peces dulceacuícolas que en los marinos ($H=26.6341$; $P=0.0005x10^{-4}$). Cuando fueron comparadas las medianas del número de brazos cromosómicos (NF), el rango medio de este valor fue estadísticamente superior ($H=30.0434$; $P\leq0.0002x10^{-06}$) en el grupo de peces dulceacuícolas (Tabla 4) evidenciando que el grado de diversificación y variación cariotípica también fue mayor en peces de aguas continentales, en contraste con un patrón citogenético más conservado en los peces marinos. Además, el test de independencia Chi-cuadrado (Tabla 5) puso en evidencia que la proporción de especies con 48 cromosomas exclusivamente acrocéntricos dependió de si los peces provenían de ambientes marinos o dulceacuícolas, al quedar estadísticamente demostrado ($\chi^2= 9.9661$; $P\leq0.0016$) que esa condición predominó en el 37,31% de los peces marinos mientras que en los dulceacuícolas apenas alcanzó el 4%.

Al comparar el grupo de Perciformes contra el resto de los órdenes (Tabla 6), se verificó que en los primeros, que constituyen un linaje compuesto principalmente por peces marinos y uno de los más derivados en la filogenia de los Actinoperygii, el 83,3% de las especies de perciformes analizadas poseyeron cariotipos con 48 cromosomas acrocéntricos mientras que solo el 21, 43% de los no perciformes mostraron 2n=48 acrocéntricos (χ^2= 33,8460; P≤0.0001). Esta notable estabilidad cariotípica concuerda con el estimado de 60% para los Perciformes estudiados a nivel global (Galetti *et al*., 2000).

Para explicar estas diferencias en el número de cromosomas y en el número de brazos (NF), se ha argumentado que las barreras originadas por los accidentes topográficos en los ambientes dulceacuícolas obstaculizaría el flujo génico entre poblaciones, conllevando a la fijación de alteraciones macroestructurales de los cromosomas, mientras que la ausencia de barreras geográficas bien definidas, la ocurrencia de poblaciones grandes y por lo tanto, un flujo de genes intenso debido a la gran capacidad de dispersión en el ambiente marino contribuirían a homogeneizar las poblaciones y contendría la diversificación cariotípica (Molina, 2007).

De hecho, incluso entre los peces marinos, los grupos que poseen alta movilidad (huevos, larvas, o adultos), como por ejemplo Mugilidae, Sciaenidae, Lutjanidae, Serranidae muestran un cariotipo conservado con 48 cromosomas acrócentricos y poca frecuencia de reorganizaciones cromosómicas macroestructurales, mientras que en grupos con desplazamientos espaciales más limitados, como Blenniidae, Gobiidae y Scorpaenidae, por ejemplo, se observa una diversidad cromosómica más extensa, con una recurrencia de polimorfismos numéricos y estructurales y varios sistemas de cromosomas sexuales (Galetti *et al*., 2006).

Sin embargo, técnicas de bandeo cromosómico en los grupos marinos que presentan cariotipos conservados, como los Serranidae (ver referencias en Galetti *et al*., 2000), Haemulidae (Nirchio *et al*., 2006), Lutjanidae (Nirchio *et al*., 2008) y Mugilidae (Sola *et al*., 2007), entre otros, han revelado la ocurrencia de cambios

microestructurales que ponen en evidencia que el complemento cromosómico de los peces es más dinámico de lo que se creía anteriormente, lo cual indica que la relativa estabilidad observada en los peces marinos debe ser analizada con precaución, ya que es probable que entre las especies que poseen cariotipos conservados se mantengan cambios cromosómicos debidos a reorganizaciones microestructurales no detectables por métodos convencionales.

La grafica del número diploide de cromosomas (2n) y el Número fundamental (NF) contra la ordenación filogenética propuesta por NELSON (2006) para los Actinopterygii (Figura 4) proporciona un panorama del comportamiento global de los datos y revela, en ambos casos, una relación inversamente proporcional que se encuentra reforzada por el análisis de Correlación por Rangos de Spearman (Tabla 7) con una probabilidad altamente significativa (más allá del 99% de confianza), desde valores cercanos a 2n=60 en los ordenes más ancestrales aquí estudiados, hasta valores de 2n=48 en los grupos más recientes, principalmente en el grupo de los Perciformes.

CAPÍTULO II. Characiformes

Caracterización Citogenética de *Brycon amazonicus* (Spix & Agassiz, 1829) (Teleostei: characidae) de Caicara del Orinoco, Venezuela.

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RESUMEN

El estudio citogenético de *Brycon amazonicus* mediante tinción convencional con Giemsa, impregnación argéntica y bandeo C, reveló un cariotipo caracterizado por 50 cromosomas (22 M, 14 SM y 14 ST) y un Número Fundamental (NF) de 100. Las Regiones Organizadoras del Nucléolo (RONs) se encontraron localizadas en los telómeros de los brazos largos del segundo par de cromosomas subtelocéntricos correspondiente (No. 13). La heterocromatina constitutiva (Bandas C) se encontró en posición paracentromérica en el par metacéntrico de mayor tamaño y en posición pericentromérica en los primeros seis pares de cromosomas submetacéntricos. El cariotipo descrito concuerda con la configuración citogenética observada en todas las especies del género *Brycon* hasta ahora estudiadas reforzando la opinión según la cual existe una marcada estabilidad en la macroestructura cromosómica en la subfamilia Bryconinae, mientras que las del patrón de bandas C, demostró ser diferente a todos los demás patrones reportados para las especies analizadas hasta la fecha, lo que sugiere su utilización como posible marcador citotaxonómico.

Palabras clave: Bandas C, Cariotipo, *Brycon amazonicus*, RONs, Citotaxonomía.

INTRODUCCIÓN

La familia Characidae es la más numerosa dentro de los Characiformes e incluye cerca del 55% de los peces dulceacuícolas de este orden (Fink & Fink 1981). Por su amplia capacidad adaptativa a diversos nichos ecológicos, dentro de esta familia se encuentran peces de gran variedad de formas y hábitos de vida, siendo muy probable que sus integrantes no conformen un grupo monofilético de especies (Nelson 1994). El género *Brycon* está representado por 42 especies nominales válidas (Froese & Pauly 2008) y se encuentra ubicado en la subfamilia Bryconinae, conjuntamente con los géneros *Triportheus* y *Salminus* con los que se

encuentra más cercanamente relacionado que otros charácidos suramericanos (Gery 1977).

Las especies pertenecientes al género *Brycon* son de gran interés para el consumo humano debido a la excelente calidad de su carne (Moreira *et al*. 2001) y a sus hábitos alimenticios omnívoros (Figuereido-Garutti *et al*. 2002), lo que aunado al rápido crecimiento e incremento en peso, en condiciones de cultivo, ha permitido obtener buenos resultados en proyectos de acuacultura, como ha sido demostrado en algunos países de América latina, como por ejemplo Brasil (Baras *et al*. 2000; Silva *et al*. 2002; Criscuolo *et al*. 2003) y Colombia (Beltran-Turriago 2001)

Aunque la mayoría de las especies de este género son de importancia económica para el sustento de muchas poblaciones humanas aledañas a los cauces de los ríos y potencialmente utilizables para el desarrollo de proyectos de acuacultura, no existen muchos estudios sobre los aspectos biológicos de las diferentes especies que lo conforman, siendo la información citogenética escasa y limitada a algunos datos obtenidos en *Brycon orthotaenia* (referida como *B. lundii.*), *B. hilarii* (*referida como B. microlepis*) *B. falcatus* (*referida como B. brevicauda*), *Brycon nattereri* (referida como *B. reinhardti*), *B. orbignyanus, B. cephalus* y *B. insignis,* las cuales representan tan solo el 16,66 % de las especies del género (Vascon *et al.* 1984; Margarido & Julio, 1992; Bigoni *et al.* 1993; Klinkhardt *et al*. 1995; Almeida-Toledo *et al.* 1996; Margarido 1996; Margarido & Galetti Jr. 1996; Margarido & Galetti Jr. 1999; Wasko & Galetti Jr. 2000).

Hasta la presente fecha, en Venezuela se han reportado ocho especies pertenecientes a este género: *Brycon bicolor* Pellegrin 1909, *Brycon coquenani* Steindachner 1915, *Brycon polylepis* Mosco Morales 1988, *Brycon unicolor* Mosco Morales 1988, *Brycon amazonicus* (Spix & Agassiz 1829), *Brycon whitei* Myers & Weitzman 1960, *Brycon falcatus* Müller & Troschel 1844 y *Brycon pesu* Müller & Troschel 1845, de las cuales aparentemente las dos primeras son endémicas de la cuenca del río Orinoco y la tercera y cuarta de la cuenca del lago de Maracaibo en Venezuela (Taphorn *et al.* 1997; Lima 2003; Froese & Pauly 2008).

Con la finalidad de ampliar y complementar la información citogenética en el género *Brycon* en este trabajo se describe el cariotipo (número diploide, formula cariotípica y número fundamental), la localización de las regiones organizadoras del nucléolo (RONs) y el patrón de distribución de heterocromatina constitutiva (Bandas C) de *Brycon amazonicus.*

MATERIALES Y METODOS

Fueron analizados 10 ejemplares (5 machos y 5 hembras) de la especie *Brycon amazonicus* (Fig. 1), los cuales fueron capturados mediante el uso de anzuelos en la Laguna de Castilleros en Caicara del Orinoco, Estado Bolívar, Venezuela. Especímenes de referencia se encuentran depositados en la Colección Ictiológica de la Escuela de Ciencias Aplicadas del Mar de la Universidad de Oriente.

Para la obtención de las suspensiones celulares, se empleó la técnica descrita por Foresti *et al*. (1993). La determinación del número diploide, fórmula cariotípica y el número de brazos cromosómicos (NF) se realizó en las preparaciones teñidas durante 10 minutos con colorante de Giemsa al 10% diluido en buffer fosfato, pH 6,88. La detección de las regiones organizadoras del nucléolo (RONs) se realizó empleando la técnica de impregnación con nitrato de plata (Howell & Black 1980) y la distribución de heterocromatina constitutiva (Bandas C) mediante el método de Sumner (1972).

Las figuras mitóticas fueron fotografiadas utilizando una cámara digital Nikon COOLPIX® 995 y las imágenes analizadas digitalmente con el programa ADOBE PHOTOSHOP CS2, el cual permitió realizar las mediciones de la longitud del brazo largo (L), la longitud del brazo corto (S) y la longitud total, con una apreciación de 0.01 mm. A partir de estos datos fue calculado el cociente L/S (Levan *et al.* 1964), que facilitó la ordenación de los cromosomas de acuerdo al tamaño y posición del centrómero.

RESULTADOS Y DISCUSIÓN

Todos los especímenes analizados presentaron un cariotipo con un número diploide 2n=50 constituido por 22 cromosomas metacéntricos, 14 submetacéntricos y 14 subtelocéntricos (Fig. 2). No fueron detectados cromosomas heteromórficos que pudiesen indicar la existencia de dimorfismo sexual cromosómico.

Los resultados del presente estudio con respecto al número diploide (2n=50) y número de brazos cromosómicos (NF=100), con elementos metacéntricos, submetacéntricos y subtelocéntricos, coinciden con los encontrados por otros autores (Vascon *et al.* 1984; Margarido & Julio, 1992; Bigoni *et al.* 1993; Klinkhardt *et al*. 1995; Almeida-Toledo *et al.* 1996; Margarido 1996; Margarido & Galetti Jr. 1996; Margarido & Galetti Jr. 1999; Wasko &

Galetti Jr. 2000) (Tabla 1), para otros miembros del género *Brycon* e indican una manifiesta estabilidad cromosómica dentro de este grupo. Oliveira *et al.* 1988, señalan que existe cierta tendencia a la prevalencia de un cariotipo 2n=50 dentro del grupo de los Ostariophysi, con cromosomas principalmente del tipo meta-submetacentricos. Por otra parte, con respecto a las diferencias encontradas en la formula cariotípica entre las diferentes especies, Margarido & Galetti Jr. 1996 y Almeida-Toledo *et al.* 1996, señalan que estas diferencias pueden deberse tanto a rearreglos en la macroestructura de los cromosomas, como a dificultades técnicas en la determinación exacta de la posición del centrómero debido a la condensación diferencial de los cromosomas, así como al hecho de que el valor del índice centromérico, generalmente es muy cercano a los límites existentes para los diferentes tipos cromosómicos.

Las metafases sometidas a impregnación argéntica, demostraron la existencia de regiones organizadoras de nucléolo (RONs) en los telómeros de los brazos largos del segundo par de cromosomas subtelocéntricos de mayor tamaño (Fig. 3), lo cual coincide en número y ubicación con lo encontrado en las otras especies de este género analizadas hasta la fecha (Tabla 1). La presencia de un solo par de cromosomas portadores de RONs en la mayoría de los vertebrados ha sido señalada como representativa de la condición primitiva en la mayoría de las especies de vertebrados (Hsu *et al*. 1975; Schmidt 1978), y es también la característica más extendida en la mayoría de los teleósteos (Vitturi *et al*. 1995).

La aplicación de otras técnicas de bandeo, empleando hibridización in situ (FISH), serán necesarias para confirmar estos resultados en esta especie, ya que la tinción argéntica solo revela la ubicación de las RONs que estuvieron activas durante la transcripción en la interfase previa y por lo tanto, cabe la posibilidad de que existan otras RONs que no fueron detectadas. Sin embargo, en un estudio realizado por Wasko y Galetti Jr. (2000), en siete especies del género *Brycon* mediante FISH, se determinó la existencia de un solo par de cromosomas submetacéntricos portadores de estas regiones en los telomeros del brazo largo para todas las especies analizadas, por lo que proponen que esta debe ser una característica primitiva de los integrantes de la subfamilia Bryconinae.

La técnica de bandeo C, ha demostrado mucha utilidad en los estudios citogenéticos de peces, permitiendo la identificación de las regiones formadas por ADN altamente repetitivo, que generalmente no contiene genes mendelianos y no son transcriptas, sino que se replican tardíamente en la fase S, denominadas regiones de heterocromatina constitutiva (Sumner 1990). El patrón de distribución de la heterocromatina demostró la existencia de un par de bloques

heterocromáticos en posición paracentromérica, próximos al centromero, en el par metacéntrico de mayor tamaño y bloques banda-C positivos principalmente en posición paracentromérica en el resto de los cromosomas submetacéntricos (Fig. 1C), resultando en la existencia de al menos 8 pares de cromosomas banda C positivos en *Brycon amazonicus.*

Diversos patrones de distribución de heterocromatina constitutiva han sido señalados para diferentes especies del género *Brycon* analizadas hasta la fecha. De hecho, Margarido y Galetti Jr. (1996) señalan que estos cambios en los patrones de distribución de la heterocromatina constitutiva parecieran haber jugado un importante rol en la evolución cromosómica de este grupo de peces, lográndose distinguir por lo menos dos patrones generales de distribución (Margarido y Galetti Jr. 1999). Un primer grupo caracterizado por la presencia de bandas C positivas paracentroméricas ubicadas principalmente en algunos cromosomas submetacéntricos (e.g. *B. lundi*, *B. brevicauda* y *B. insignis*), en el cual podemos ubicar también a *Brycon amazonicus* en función de los resultados del presente estudio; y el otro, que se caracteriza por la presencia de bandas C positivas en la región telomérica de algunos cromosomas metacéntricos (e.g. *Brycon orbygnyanus*, *B. microlepis* y *B. cephalus*). Estas diferencias, conducen a suponer que los patrones particulares de heterocromatina podrían constituir importantes marcadores cromosómicos para diferenciar poblaciones y/o especies del género.

Por otra parte, en un estudio realizado para evaluar las relaciones filogenéticas existentes entre las especies de los generos *Brycon* y *Salminus* (Margarido y Galetti Jr. 1999), se ha señalado que la presencia de bandas C positivas en los dos pares de cromosomas submetacéntricos, parecen ser un carácter muy conservativo entre las especies del género *Brycon*, por lo que la modificación de este patrón de distribución de la heterocromatina en el género *Salminus* pudiera caracterizar una sinapomorfía del género. Mientras que la presencia de bandas C positivas paracentroméricas en ambos brazos del cromosoma metacéntrico de mayor tamaño en ambos géneros y otras especies de la familia Characidae, pudiera representar una sinapomorfía para la familia.

Lamentablemente, no se logro evidenciar el patrón de distribución de las bandas G, que revelarían las zonas relativamente ricas en A-T (eucromáticas) de los cromosomas. Al parecer, en peces, este procedimiento ha presentado generalmente resultados poco satisfactorios y algunos investigadores señalan que las dificultades encontradas en la aplicación de la técnica de bandeo G en este grupo zoológico están relacionadas a la composición del ADN (Medrano *et*

al. 1988). Por otra parte, también ha sido señalado que cuanto más pequeños y numerosos son los cromosomas, mayor es la dificultad de desnaturalización, por lo que las bandas aparecen difusas en los cromosomas, el procedimiento no es repetible o los cromosomas son completamente resistentes al bandeo G (Ozouf-Costaz y Foresti 1992). Una cuestión a ser discutida es si las bandas G están realmente ausentes en varios grupos, excepto mamíferos, o si su ausencia es simplemente una consecuencia de factores técnicos (Oliveira *et al.* 2002, 2003).

CONCLUSIONES

- El análisis del cariotipo de *Brycon amazonicus* revelo la presencia de 50 cromosomas (2n), con un número fundamental NF = 100.
- La formula cariotípica estuvo caracterizada por 22 cromosomas metacéntricos, 14 cromosomas submetacéntricos y 14 cromosomas subtelocéntricos (22m, 14sm, 14st).
- La técnica de la tinción argéntica reveló la presencia de un solo par de regiones organizadoras del nucleolo ubicadas en los telómeros de los brazos largos del segundo par de cromosomas subtelocéntricos de mayor tamaño.
- El patrón de distribución de las bandas C se caracterizó por la presencia de bloques heterocromatínicos principalmente en la región paracentromérica de los cromosomas submetacentricos y el metacéntrico de mayor tamaño.

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Figura 1. Fotografía de un ejemplar de *Brycon amazonicus* (Spix & Agassiz 1829).

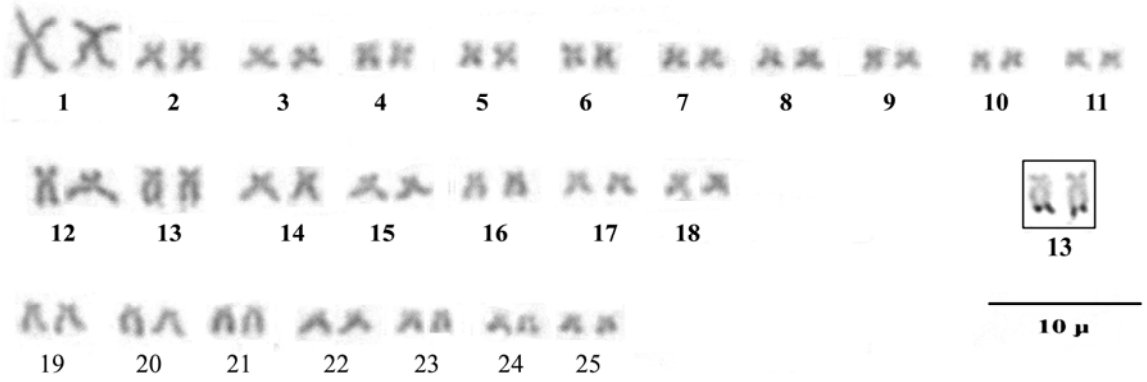

Figura 2. Cariotipo estándar de *Brycon amazonicus* y tinción secuencial de las RONS indicando el par cromosómico en el que se encuentran ubicadas.

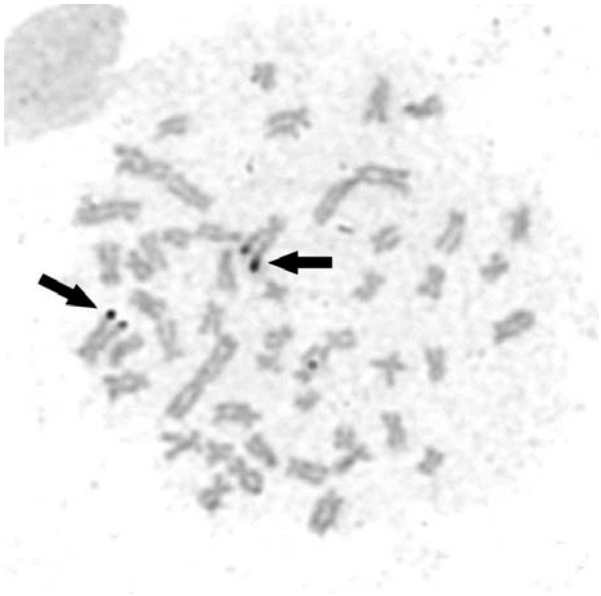

Figura 3. Metafase mitótica mostrando los cromosomas portadores de las regiones organizadoras del nucleolo.

Figura 4. Metafase mitótica mostrando la distribución de las regiones de heterocromatina constitutiva.

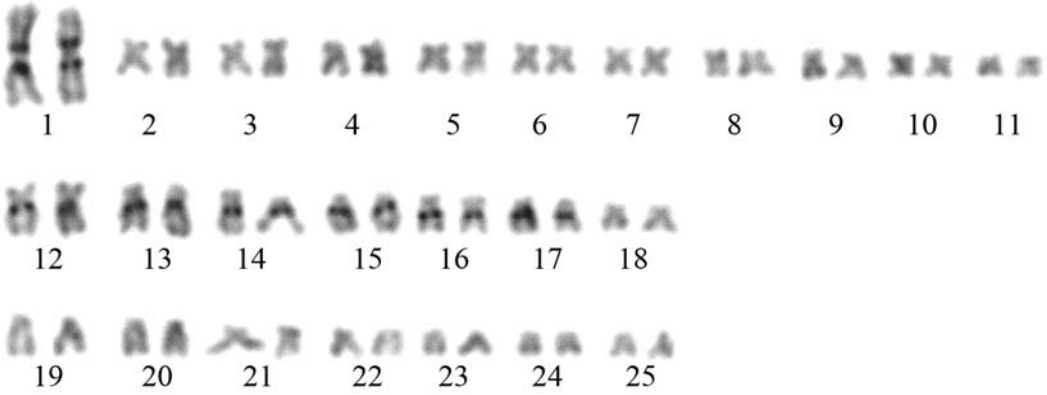

Figura 5. Cariotipo con Banda C de *Brycon amazonicus* indicando los cromosomas en que se encuentran ubicadas.

Tabla 1. Número diploide (2n), Numero de brazos cromosómicos (NF), Fórmula del cariotipo, Número de las regiones organizadoras de nucleolo (RON´s) y Tipos de bandeo realizados en diferentes especies del género Brycon.

Especie	2n	Fórmula	NF	RON´s	Bandeo	Fuente
B. cephalus (Brasil)	50	24m+20sm+6st	94	-------	C, M	Margarido y Galetti Jr.,
B. cephalus (Brasil)	50	-------	-------	1	F	Wasko y Galetti Jr.,
B. falcatus (Brasil)	50	20m+24sm+6st	94	1	C, M	Margarido y Galetti Jr.,
B. hilarii (Brasil)	50	-------	-------	1	C	Margarido et al., 1993
B. hilarii (Brasil)	50	20m+24sm+6st	94	1	C.M	Margarido y Galetti Jr.,
B. hilarii (Brasil)	50	20m+24sm+6st	94	1	C	Daniel-Silva, 2003
B. hilarii (Brasil)	50	20m+24sm+6st	-------	-------	C	Daniel-Silva, 2003
B. hilarii (Brasil)	50	20m+24sm+6st	-------	-------	C	Daniel-Silva, 2003
B. insignis (Brasil)	50	26m+24sm	100	-------	C	Bigoni et al., 1993
B. insignis (Brasil)	50	24m+20sm+6st	94	1	C, M	Margarido y Galetti Jr.,
B. nattereri (Brasil)	50	22m+28sm	100	-------	C	Bigoni et al., 1993
B. orthotaenia	50	22m+24sm+4st	96	-------	C, M	Margarido y Galetti Jr.,
B. orthotaenia	50	-------	-------	-------	F	Wasko y Galetti Jr.,
B. orbignyanus	50	24m+22sm+4st	96	-------	C, M	Margarido y Galetti Jr.,
Brycon amazonicus	50	22m+14sm+14st	100	1	C	ESTE TRABAJO

Nota: Los datos cromosómicos de las diferentes especies del género Brycon fueron tomados de la página web del Instituto de Biociencias de la Universidad Estadual Paulista, Botucatu, Brasil. http://www.ibb.unesp.br/laboratorios/lab_morfo_lbgp.php

Tipos de cromosomas: m= Metacéntrico; sm= Submetacéntrico; st= Subtelocéntrico; **Tipos de bandeo:** C= Bandas C, F = FISH, M= Mitromicina

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Research Article

Extensive polymorphism and chromosomal characteristics of ribosomal DNA in the characid fish *Triportheus venezuelensis* (Characiformes, Characidae)

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Abstract

The karyotype and chromosomal characteristics of the characid fish *Triportheus venezuelensis* were investigated using differential staining techniques (C-banding, Ag-NOR staining) and fluorescent *in situ* hybridization (FISH) with an 18S rDNA probe. The diploid chromosome number (2n = 52), karyotype composition and sex chromosome determination system of the ZZ/ZW type were the same as previously described in other species of the genus *Triportheus*. However, extensive variation regarding nucleolus organizer regions (NOR) different from other species was observed. 18S rDNA sequences were distributed on nine chromosome pairs, but the number of chromosomes with Ag-NORs was usually lower, reaching a maximum of four chromosomes. When sequential staining experiments were performed, it was demonstrated that: 1.) active NORs usually corresponded to segments with 18S rDNA genes identified in FISH experiments; 2.) several 18S rDNA sequences were not silver-stained, suggesting that they do not correspond to active NORs; and 3.) some chromosomes with silver-stained regions did not display any 18S rDNA signals. These findings characterize an extensive polymorphism associated with the NOR-bearing chromosomes of *T. venezuelensis* and emphasize the importance of combining traditional and molecular techniques in chromosome studies.

Key words: 18S rDNA, Ag-NORs, C-band, FISH, fish cytogenetics, sex chromosomes.

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Introduction

Two distinct sets of multiple rRNA genes, usually located on distinct chromosomes, operate in eukaryote genomes: the 18S, 5.8S and 28S major genes and the 5S minor genes (Hadjiolov, 1985). The eukaryotic major rRNA genes are grouped, in order to form RNA polymerase I transcription units, and multiple copies of these units are typically found clustered in long direct tandem arrays cytologically identified as the nucleolus organizer regions (NORs) (Hadjiolov, 1985; Drouin and Moniz-de-Sá, 1995).

The chromosomal sites of the major rRNA genes has been localized using isotopic *in situ* hybridization, fluorochrome dyes, N-banding, silver staining, immunofluorescence, and more recently by means of fluorescent *in situ* hybridization (FISH) using specific probes (Sumner, 1990). However, the technique most commonly used to detected NORs is the silver nitrate (Ag) impregnation method in which silver binds to NOR proteins such as the RNA polymerase I subunit which is part of the active site of ribosomal genes (Roussel and Hernandez-Verdun, 1994; Whitehead *et al*., 1997). Unfortunately, silver nitrate may also bind to other proteins present in the nuclei, thus some chromosome structures visualized by silver nitrate may not correspond to ribosomal genes (Dobigny *et al*., 2002).

The Characidae is a large and diversified family of fish that contains 167 genera and 980 species (Reis *et al*., 2003), including members of the genus *Triportheus* which has 16 species widely distributed in South America (Malabarba, 2004). Members of the genus *Triportheus* possess karyotypes characterized by a constant diploid number of 2n = 52 and a relatively conserved chromosome complement, as well as by the presence of a zz/zw sex chromosome system (Bertollo and Cavallaro, 1992; Sánchez and Jorge, 1999; Artoni *et al*., 2001; Artoni and Bertollo, 2002).

We used silver-staining and FISH with a 18S rDNA probe to investigate the karyotype of *Triportheus*

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venezuelensis and the distribution of the major rDNA gene cluster with the objective of characterizing the number, distribution, and degree of activity of these genes in this species.

Material and Methods

We captured 8 female and 8 male (n = 16) *Triportheus venezuelensis* Spix, 1829 in Castillero Lake (Laguna de Castillero) Caicara del Orinoco, Bolívar State, Venezuela. Voucher specimens were deposited in the fish collection of Escuela de Ciencias Aplicadas del Mar, Universidad de Oriente, Nueva Esparta, Venezuela and the Labortatório de Biologia e Genética de Peixes (LBP 2194 and LBP 2230), Universidade Estadual Paulista, São Paulo, Brazil.

Mitotic chromosome preparations were obtained from kidney and gill tissues using the air-drying technique of Foresti *et al*. (1993). Chromosome morphology was determined on the basis of arm ratio, as proposed by Levan *et al*. (1964), and chromosomes were classified as metacentric (M), submetacentric (SM), subtelocentric (ST), and acrocentric (A). The NORs were identified by silver staining (Ag-NORs), as described by Howell and Black (1980) and C-banding was performed according to Sumner (1972). The FISH experiments were performed according to Pinkel *et al*. (1996). A tilapia (*Oreochromis niloticus*) 18S rDNA sequence (about 1800 base pairs) cloned in pGEM-T was labeled by nick translation with biotin-14-dATP according to the manufacturer's instructions (Bionick Labelling System-Gibco. BRL). The 18S rDNA sequences were located in the chromosomes with Avidin-N-fluorescein

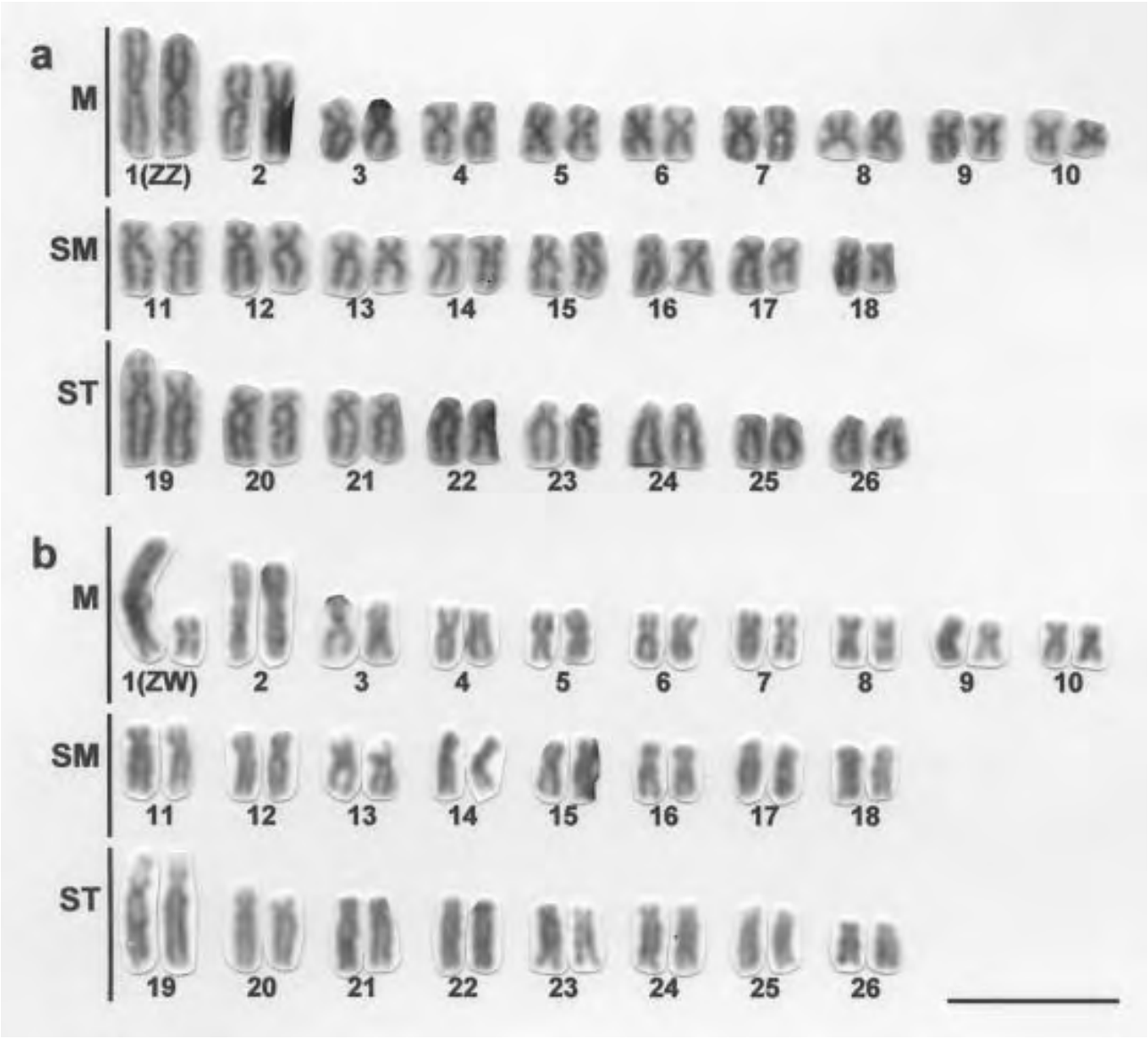

Figure 1 - Giemsa stained karyotypes of a male (a) and a female (b) *Triportheus venezuelensis*. Bar = 10 µm.

Isothiocyanate (FITC) conjugate and the signal enhanced using biotinylated Anti-avidin goat antibodies following a second round of Avidin-FITC detection. Chromosomes were counter-stained with Propidium Iodide (50 µg mL^{-1}) diluted in Antifade. Metaphases were examined in a Zeiss Axiophot photomicroscope and pictures were taken with Kodak Gold Ultra 400 ASA film.

Results

The diploid chromosome number for both sexes was 2n = 52. The male karyotype was 20 M, 16 SM and 16 ST chromosomes, where the largest metacentric pair (pair 1) represented the sex chromosomes (Figure 1). The female karyotype was the same as in the males except that pair 1 was composed of one large metacentric (the Z chromosome) and one small metacentric (the W chromosome) (Figure 1b). Small C-band positive heterochromatic segments were present at the centromeres of almost all chromosomes of both males (not shown) and females (Figure 2). The long arm of the Z chromosomes and the whole W chromosome were entirely C-band positive; the short arms of the chromosome pair 19 were also almost entirely C-band positive (Figure 2).

FISH with 18S rDNA probe showed that the 18S rDNA sites were distributed over nine chromosomes pairs (pairs 1, 6, 7, 11, 12, 18, 19, 20 and 21; Figures 3 and 4): 1.) at the end of the long arm of the W chromosome (pair 1); 2.) on the long arm of a small-sized metacentric chromosome (pair 6); 3.) on both chromosome arms of a small-sized metacentric pair (pair 7); 4.) on the long arm of two large sub-metacentric pairs (pairs 11 and 12); 5.) on the long arm of a small sub-metacentric pair (pair 18); 6) on the short arm of the two largest subtelocentric chromosomes (pairs 19 and 20), 7) and on both arms of a large subtelocentric chromosome pair (pair 21). Chromosome pairs 6 and 7, as well as 20 and 21, were very similar, differing only by the presence of additional 18S sites on the pairs 7 and 21 (Figure 4).

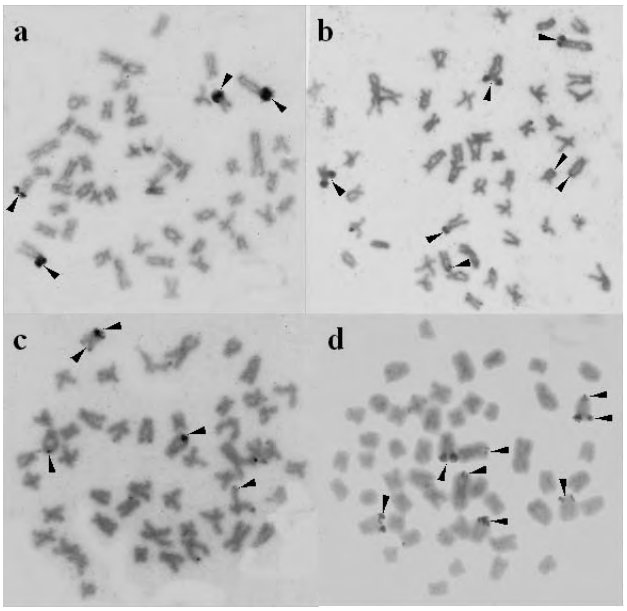

Figure 3 - Chromosome metaphase spreads of *Triportheus venezuelensis*. FISH probed with 18S rDNA. The original colors were artificially transformed. Arrows indicate the detected loci.

Silver-staining showed that the number of chromosomes with Ag-NORs was usually lower than the number of chromosomes with 18S rDNA regions (Table 1). The chromosomes with positive signals after silver-staining were pairs 6, 11, 12, 18 and 19 (Table 1). The most frequently observed chromosome pair with positive Ag-NORs

Figure 2 - C-banded karyotype of a female *Triportheus venezuelensis*. Bar = 10 µm.

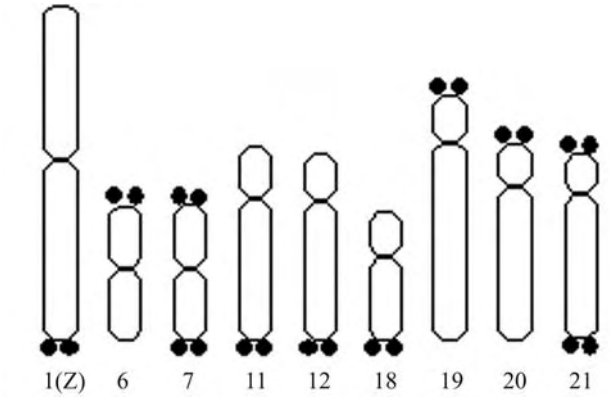

Figure 4 - Ideogram representing the physical mapping of 18S rDNA genes (indicated as black dots) in the chromosomes of *Triportheus venezuelensis*.

was pair 19, identified in 81% of the specimens analyzed (6 females and 7 males), which was the only active pair in 50% of the fish analyzed (2 females and 6 males).

Sequential silver-staining and FISH probing demonstrated that: 1.) positive NOR sites usually correspond to segments with 18S rDNA sites; 2.) several 18S rDNA sites were not silver-stained, suggesting that they do not correspond to active NORs; and 3.) some chromosomes with positive silver-signals do not display the presence of 18S rDNA sites (see Table 1, specimens 3, 6 and 9 and Figure 5).

Discussion

Previous cytogenetic studies on *Triportheus* have shown that all species possess a chromosome complement of 2n = 52 and a ZZ/ZW heteromorphic sex chromosome system (Bertollo and Cavallaro, 1992; Sánchez and Jorge, 1999; Artoni *et al*., 2001; Artoni and Bertollo, 2002). We confirmed this in *T. venezuelensis*, reinforcing the hypothesis that this group represents a monophyletic unit in the Characidae (Malabarba, 2004).

The Z chromosome of *T. venezuelensis* displayed an entirely C-band positive longer arm, a characteristic not yet described in other *Triportheus* species (Artoni *et al*., 2001). The W chromosome was C-band positive throughout all its length, similar to what has been reported for other *Triportheus* species (Artoni *et al*., 2001), with small differences in the extension of the distribution of C-band positive segments. The heterochromatinization of the W chromosome in the *Triportheus* is thought to be associated with a reduction in the size of this chromosome during the evolution of the ZW sex chromosome system (Bertollo and Cavallaro, 1992; Artoni *et al.*, 2001), but a robust phylogeny to test this hypothesis is still lacking for the group. The presence of an entirely C-heterochromatin positive W chromosome has also been reported in *Characidium gomesi* (Maistro *et al*., 2004) and C-heterochromatin positive segments associated with W or Y chromosomes have been described in several fish species, reinforcing the possible important role of heterochromatinization in sex chromosome development in lower vertebrates (Almeida-Toledo *et al*., 2000; Devlin and Nagahama, 2002).

Table 1 - Distribution of Ag-NOR-bearing chromosomes and 18S rDNA-bearing chromosomes in specimens of *Triportheus venezuelensis*. Chromosomes are identified as in Figure 4.

Animal	Sex	Chromosome with Ag - NORs	Total	Chromosome with 188 rDNA	Total
1	♀		2		4
2	♀		2		3
3	♀		4		4
4	♀		3		4
5	♀		3		3
6	♀		3		4
7	♀		3		4
8	♀		2		7
9	♂		4		4
10	♂		2		4
11	♂		2		3
12	♂		3		3
13	♂		2		7
14	♂		2		4
15	♂		2		2
16	♂		2		4

In the karyotypes of representatives of the genus *Triportheus,* the presence of only one pair of NOR-bearing elements (*e.g.* pair 18) has been reported by Artoni and Bertollo (2002), a characteristic that was considered evolutionarily conserved in the genus. However, extensive cytogenetic screening in *Triportheus guentheri* by Bertollo and Cavallaro (1992) has shown that although *T. guentheri* karyotype usually had one Ag-NOR-bearing chromosome pair additional signals were occasionally observed on a second autosomal pair and on the Z chromosome. Our study showed that the *T. venezuelensis* specimens displayed a very conspicuous polymorphism associated with the number of major rDNA sites. The combination of silver-staining and 18S rDNA FISH showed that one chromosome pair (probably pair 19) nearly always had the Ag-positive NORs in this species. However, other chromosome pairs also had Ag-positive NORs, and some had 18S rDNA sites but did not display positive signals after silver-staining.

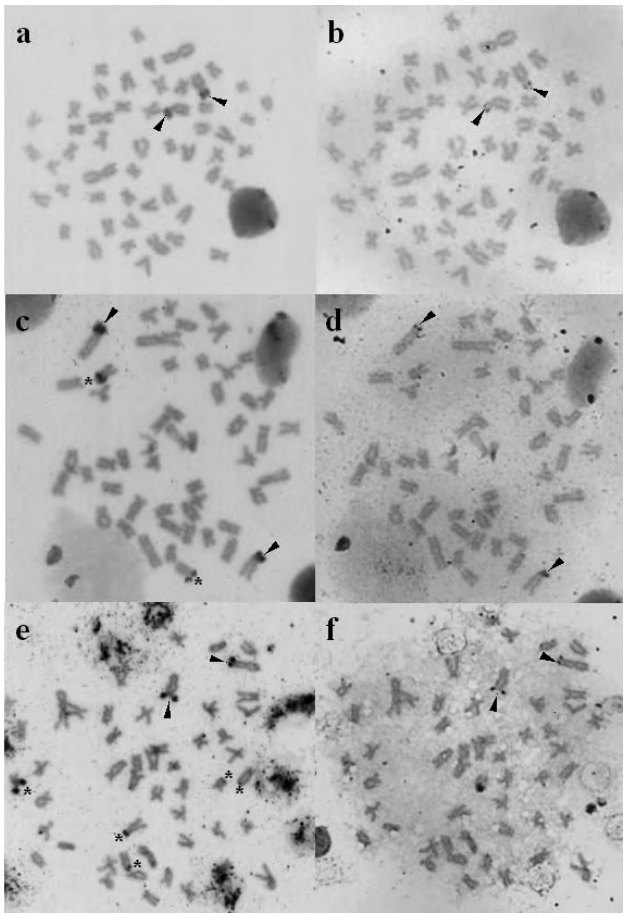

Figure 5 - Metaphases of *Triportheus venezuelensis* sequentially stained with the FISH technique using an 18S rDNA probe (a, c, e - the original colors were artificially transformed) and with the Ag-NOR technique (b, d, f). Arrows point active Ag-NOR. Asterisks show segments identified with the 18S rDNA probe and not stained in the silver staining experiments.

Studies conducted in fishes have shown that in some species, *e.g. Lepisosteus osseus* (Ráb *et al*., 1999), with only one Ag-NOR-bearing chromosome pair, the FISH technique using 18S or 28S sequences usually stains the same chromosome pair. Nevertheless, the presence of a number of major ribosomal cistrons identified by the FISH technique using 18S or 28S sequences higher than that observed with the Ag-NOR technique are more common, having been reported in *Salmo truta* (Pendás *et al*., 1993), *Astyanax scabripinnis* (Ferro *et al*., 2001), *Hyphessobrycon anisitsi* (Centofante *et al*., 2003), *Prochilodus lineatus* (Jesus and Moreira-Filho, 2003), *Colossoma macropomum*, *Piaractus brachypomus* and its interspecific hybrids (Nirchio *et al*., 2003), and *Lebias fasciata* (Tigano *et al*., 2004). These differences have been attributed to the presence of NORs that are usually unexpressed.

Recent studies conducted with human and chimpanzee cells showed that three mechanisms produce inactivation of NORs: 1.) elimination of rDNA; 2.) DNA methylation; and 3.) gene silencing due to positional effects induced by heterochromatin (C-bands) and/or telomeres (Guillén *et al*., 2004). Our results suggest that in *T. venezuelensis* the elimination of rDNA sequences is the most frequent rearrangement involved in NOR inactivation. Gene silencing due to positional effects does not seem to occur, since the more common pair of chromosome with active NORs is pair 19, which has a conspicuous C-band positive segment in the same position as the NORs. The occurrence of DNA methylation was not investigated in our present study.

An unusual finding in our study was the presence of putative active NORs that were not detected by the FISH technique. One hypothesis to explain these data is that silver might be staining proteins not related to NORs, the existence of some nuclear proteins with silver-affinity have been reported by several authors (Sumner, 1990; Dobigny *et al*., 2002). However, the chromosomes with Ag-NORs-stained in the specimens 3, 6 and 9 were usually found to bear 18S genes in the other specimens, leading to an alternative hypothesis suggesting that specimens 3, 6 and 9 might have a very small copy number of major NOR genes not detectable with FISH but observable after intense transcription or as a result of gene amplification. If this second hypothesis is correct, the use of the silver-staining technique to study NORs may be more important than is currently believed.

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CAPÍTULO III. Siluriformes

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CONVENTIONAL KARYOTYPE, CONSTITUTIVE HETEROCROMATIN, AND NUCLEOLAR ORGANIZER REGIONS IN *Hoplosternum littorale* (PISCES: CALLICHTHYIDAE) FROM CAICARA DEL ORINOCO, VENEZUELA

CARIOTIPO CONVENCIONAL DE HETEROCROMATINA Y REGIONES DE ORGANIZACIÓN DEL NUCLEOLO EN *Hoplosternum littorale* (PISCES: CALLICHTHYIDAE) DE CAICARA DEL ORINOCO, VENEZUELA

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ABSTRACT

This paper reports on the cytogenetic study of Hoplosternum littorale by conventional Giemsa staining, C-banding, and silver staining. The species has a diploid chromosome number 2n=60 consisting of 6M, 2SM, 2ST, and 50A elements with an arm number of 70. The ST pair corresponds to the 5th pair and presents a visible secondary constriction that coincides with the silver-stained NORs, which are evidently heteromorphic. C-banding showed heterochromatin blocks in the centromeric and pericentromeric regions of almost all chromosomes. Telomeric and interstitial C-positive regions were also observed in some chromosomes. The comparison of these results with those of a previous report for a population from Camaleao Lake on Marchantia Island in the Amazon river would reflect a subtle chromosomal alteration arising possibly as a consequence of the isolation existing between the Orinoco and the Central Amazon basins, suggesting that both NOR-bearing chromosomes and karyotype formulae would be good chromosomal markers for a population study of this species.

Key words: Karyotype, chromosomes, NOR, C-bands, *Hoplosternum littorale.*

RESUMEN

Se reporta el estudio citogenético de Hoplosternum littorale mediante la utilización de tinción convencional de Giemsa 2n=60, compuesto por 6M, 2ST Y 50A, con un número de brazos de 70. El par ST correspondió al quinto par de cromosomas y presentó una constricción secundaria visible que coincide con las RONs teñidas mediante tinción argéntica, las cuales fueron evidentemente heteromórficas. El bandeo C mostró bloques de heterocromatina en las regiones centroméricas y pericentroamérica de casi todos los cromosomas. Regiones banda C positiva teloméricas e intersticiales fueron también observadas en algunos cromosomas. La comparación de estos resultados con aquellos de un reporte previo de una población del Lago Camaleao en la Isla Marchantia, localizada en el río Amazonas, pudiera reflejar que una sutil alteración cromosómica ha surgido posiblemente como consecuencia del aislamiento entre las cuencas del río Orinoco y del Amazonas central, sugiriendo que tanto la ubicación de las RONs como la fórmula cariotípica pueden ser buenos marcadores cromosómicos para estudios poblacionales en esta especie.

Palabras clave: Cariotipo, cromosomas, RON, bandas C, *Hoplosternum littorale.*

INTRODUCTION

The family Callichthyidae is a group of Neotropical fishes that comprises seven genera: *Aspidoras, Brochis, Callichthys, Corydoras, Dianema, Hoplosternum, Lepthoplosternum,* and *Megalechis* (Reis, 1998).

The genus Hoplosternum contains three species: *H. littorale, H. magdalenae,* and *H. punctatum* (Reis, 1997). These species live in streams, rivers, swamps, and floodplain areas, environs sometimes strewn with stagnant and oxygen-deprived waters, a condition that, according to Porto & Feldberg (1992) seems to have promoted morphological and molecular adaptations.

H. littorale is the most widely distributed species of the genus *Hoplosternum*, being present in all of South America east of the Andes and north of Buenos Aires, including the Orinoco River, Trinidad, the coastal rivers of Guiana, the Amazon River Basin, the Paraguay River, the lower Paraná River and the coastal systems in southern Brazil (Reis, 1997).

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As far as we know, cytogenetic information regarding *H. littorale* is limited to the studies carried out in Brazil in the localities of Camaleao Lake (Porto & Feldberg, 1992). This paper expands the cytogenetic information on *H. littorale* describing the diploid number, chromosome formula, Nucleolus Organizer Region (NOR) locations, and constitutive heterochromatin of specimens from the locality of Caicara del Orinoco, Venezuela**.**

MATERIALS AND METHODS

Eighteen sexually mature specimens of *Hoplosternum littorale* (12 males and six females) were captured with seine nets in the lowland floodplains near Caicara del Orinoco, Bolivar State, Venezuela. Voucher specimens were deposited at the Ichthyology Collection of the Escuela de Ciencias Aplicadas del Mar, Universidad de Oriente.

Chromosome preparations were obtained according to Foresti *et al.* (1993). For the conventional karyotype, the preparations were stained during 20 minutes with 10% Giemsa in phosphate buffer pH 6.88. For detection of the Nucleolus Organizer Regions (NOR), slides were stained with silver nitrate using the method of Howell and Black (1980). C-bands were obtained according to the methods described by Sumner (1972).

The mitotic figures were photographed using a green filter. From digitalized photographs, long arm (L), short arm (S), and whole chromosome lengths were measured for each chromosome to the nearest 0.01 mm, using the measuring tool in ADOBE PHOTOSHOP Software v.7.0. The length (RL%) of each chromosome pair relative to total chromosome length was obtained from these values. Chromosomes were identified according to the arm ratio criteria proposed by Levan *et al.* (1964).

DISCUSSION AND RESULTS

Counts of diploid metaphasic cells revealed a modal chromosome complement 2n=60, consisting of 6M, 2SM, 2ST, and 50A elements with an arm number of 70 (Fig. 1-A). No differences in the karyotype between males and females were observed.

Chromosome size and arm ratio of the M series, as well as the SM and ST, allow the unequivocal classification of pairs as homologous, but minimal differences in size in the acrocentric series do not permit to classify homologous with such certainty.

Two NORs were located on the subtelocentric chromosomes (pair 5). C-banding revealed positive segments in centromeric, telomeric, and/or interstitial position.

The diploid chromosome number of *H. litoralle* reported here coincides with that of the previous descriptions (2n=60) by Porto & Feldberg (1992), but with a slight discrepancy: the NORs of *H. littorale* from the Central Amazon Basin in Brazil were located on a large-sized acrocentric pair (7th), coinciding with a secondary constriction, while for the population from the Orinoco Basin in Venezuela, the silver stained regions were located on the 5th pair, which was classified by us as a large subtelocentric and which also presented a secondary constriction. After Ag-impregnation this pair was much more easily identifiable as being subtelocentric. Secondary constrictions and NORs are usually coincident (Feldberg *et al.* 1999), and this was the case with our fish, since Ag-impregnation revealed that the short arms of pair 5 bears the NORs, as expected from their Giemsa features (Fig. 1-B). These NORs are markedly different in size, a feature that has already been described in fish (Moreira-Filho *et al.* 1984; Rossi *et al.* 2000). Such heteromorphism has been commonly explained as the result of differential transcriptional activity of rDNA genes (Feldberg *et al.* 1999), evidenced by silver staining, since this is a procedure that reveals the residues of the Ag-stainable rRNAprotein complex synthesized only by the active NORs in chromosomes in the preceding interphase.

This subtelocentric pair would increase in two the arm number of the Venezuelan *Hoplosternum* population as compared with the population surveyed by Porto & Feldberg (1992) in Brazil (FN=68, 4M + 4SM + 52 ST-A). This difference, although seemingly a minor one, could be employed as a chromosome marker for differentiating stock/populations of *Hoplosternum littorale*.

C-banding showed heterochromatin blocks in the centromeric and pericentromeric regions of almost all chromosomes. Telomeric and interstitial C-positive regions were also observed in some chromosomes (Fig. 1-C). Although NORs are usually coincident with heterochromatic blocks in fishes (Sola *et al.* 1997, Rossi *et al.* 2000), this was not the case in the population of *H. littorale* studied here, whose NORs were not associated with the C-positive heterochromatin blocks, as expected. This exception has also been reported for the coregonid Coregonus albula (Jankun *et al.* 2001).

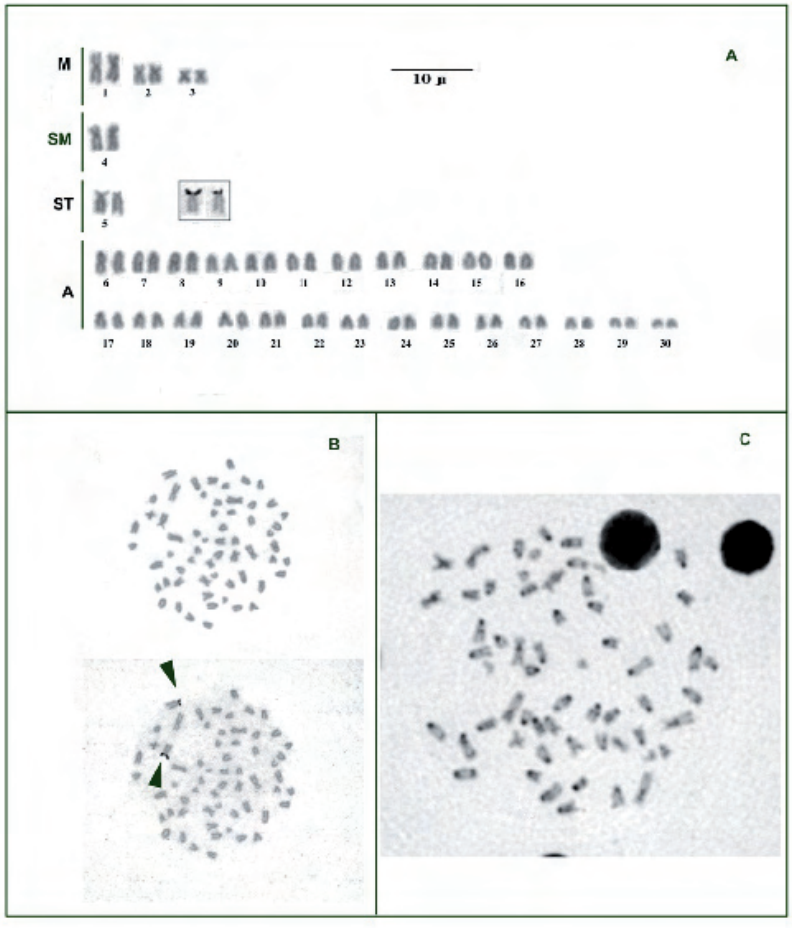

Figure 1. Karyotipe of *Hoplosternum littorale*. NOR-bearing chromosomes appear in the aquare (A). Sequential staining of chromosomes for identifying correspondence among NOR bearing chromosomes when stained with $AgNO_3$ with the same chromosomes previously stained with Giemsa (B). Metaphase plates after sequential C-banding (C).

Interestingly, the distribution of constitutive heterochromatin sites in the karyotype of H. littorale indicates some degree of genome compartmentalization in the species, an uncommon feature in fish, but further analysis of other congeneric species are required to assess whether this heterochromatin distribution is common in the genus.

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Short Communication

Cytogenetic analysis of three catfish species of the family Pseudopimelodidae (Teleostei, Siluriformes)

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Abstract

Cytogenetic analyses performed in *Cephalosilurus apurensis, Microglanis* aff. *cottoides* and *Pseudopimelodus bufonius* revealed that the three species have 2n = 54 chromosomes: *C. apurensis* presented six metacentric (M), 28 submetacentric (SM), 14 subtelolocentric (ST), and six acrocentric (A) chromosomes, while *M.* aff. *cottoides* showed 10M, 32SM, 10ST and 2A, and *P. bufonius* had 12M, 30SM and 12ST. The nucleolus organizer regions (NORs) were present on the short arm of a middle-sized ST pair, identified as pair 19, in *C. apurensis* NORs were found on the short arm of a middle-sized ST (pair 23) and on the long arm of a middle-sized ST (pair 22) in *M.* aff. *cotttoides* and on the short arm of three middle-sized ST pairs, identified as pairs 9, 10 and 11, in *P. bufonius*. C-banding revealed a very small amount of constitutive heterochromatin in the chromosomes of all species, including the NORs. The occurrence of 2n = 54 in all species of the family Pseudopimelodidae and its absence among species of the closely related Pimelodidae and Heptapteridae may be important in identifying Pseudopimelodidae species.

Key words: karyotype, chromosomes, C-banding, Ag-NOR, fish.

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The order Siluriformes (catfishes) has 3,093 species, divided into 36 families and 478 genera, and distributed worldwide, except for the coldest areas in the Northern and Southern hemispheres (Ferraris, 2007). Recent phylogenetic studies showed that the old family Pimelodidae comprised three monophyletic units: Pimelodidae, Heptapteridae and Pseudopimelodidae (Lundberg *et al.*, 1991; de Pinna, 1998). According to Ferraris (2007), Pseudopimelodidae is composed of the genera *Batrochoglanis* (five species), *Cephalosilurus* (four species), *Lophiosilurus* (one species), *Microglanis* (14 species) and *Pseudopimelodus* (five species). A new genus and species, *Cruciglanis pacifisi*, has been recently described by Ortega-Lara and Lehmann (2006). This family is widely distributed in South America and is considered the least known family among the naked Neotropical freshwater catfishes (Shibatta, 2003). Currently, the only species to have their karyotypes reported are *Microglanis garavelloi* (cited as *M. cottoides* - Vissotto *et al.*, 1999a) and *Pseudopimelodus mangurus* (Martinez *et al.*, 2004). The objective of the present study was to analyze the karyotypes of *Cephalosilurus apurensis, Microglanis* aff. *cottoides* and *Pseudopimelodus bufonius*.

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The following specimens were karyotyped: one male specimen of *Cephalosilurus apurensis* from the Orinoco River, Caicara del Orinoco, Bolívar, Venezuela (07°38'11.6" N, 66°19'04.2" W, LBP 3034); two males and four females of *Microglanis* aff. *cotttoides* from Ribeirão Cavalo Stream, Jaraguá do Sul, Santa Catarina, Brazil (26°28,250' S, 49°10,958' W, LBP 731) and two males and two females of *Pseudopimelodus bufonius* from the Amazon (aquarium trade, LBP 2345). The specimens were identified and deposited in the fish collection of the Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, São Paulo, Brazil.

Mitotic chromosome preparations were obtained according to the technique described by Foresti *et al.* (1993). Nucleolar organizer regions (Ag-NORs) were revealed by the silver-staining method (Howell and Black, 1980) and C-banding was performed according to Sumner (1972). The chromosomes were classified according to their arm ratios as metacentrics (M), submetacentrics (SM), subtelocentrics (ST), and acrocentrics (A) (Levan *et al.*, 1964).

The three species analyzed possessed 2n = 54 chromosomes. *Cephalosilurus apurensis* had 6M, 28SM, 14ST and 6A (Figure 1a), *Microglanis* aff. *cottoides* presented 10M, 32SM, 10ST and 2A (Figure 2a) and *Pseudopimelodus bufonius* showed 12M, 30SM and 12ST (Figure 3a). A 2n = 54 is characteristic for the family Pseudopimelodidae and the karyotypes of *C. apurensis* and *M.* aff. *cottoides* are similar to those observed in other species of the family, which typically have chromosomes of all morphological types, except for *M. garavelloi* and *P. bufonius* that do not have any acrocentric chromosome (Table 1).

The 2n = 54 present in Pseudopimelodidae contrasts with the modal 2n = 56 found in most catfish families (Oliveira and Gosztonyi, 2000) and specially with the diploid numbers found among representatives of Heptapteridae and Pimelodidae, which are closely related to Pseudopimelodidae (Sullivan *et al.*, 2006) (Table 1).

Pseudopimelodid species have single or multiple Ag-NORs (Table 1). *Cephalosilurus apurensis* showed a single pair of Ag-NORs on the short arms of a middle-sized ST pair, identified as pair 19 (Figure 1a). The remaining pseudopimelodid species analyzed also had a single Ag-NOR: *Lophiosilurus alexandrii* showed Ag-NORs on the short arm of a SM (Marques, Garcia and Moreira Filho, personal communication); *Microglanis garavelloi* (Vissotto *et al.*, 1999a) had Ag-NORs on the long arm of M; and *Pseudopimelodus mangurus* (Martinez *et al.*, 2004) presented Ag-NORs on the short arm of SM/ST (Table 1). Single Ag-NORs were also identified in all species of Pimelodidae and all but one species of Heptapteridae (Table 1). This is also the most common condition in Siluriformes (Oliveira and Gosztonyi, 2000) and even in Teleostei (Klinkhardt, 1998). The Ag-NORs of *M.* aff. *cotttoides* were found on the short arm of a middle-sized ST pair,

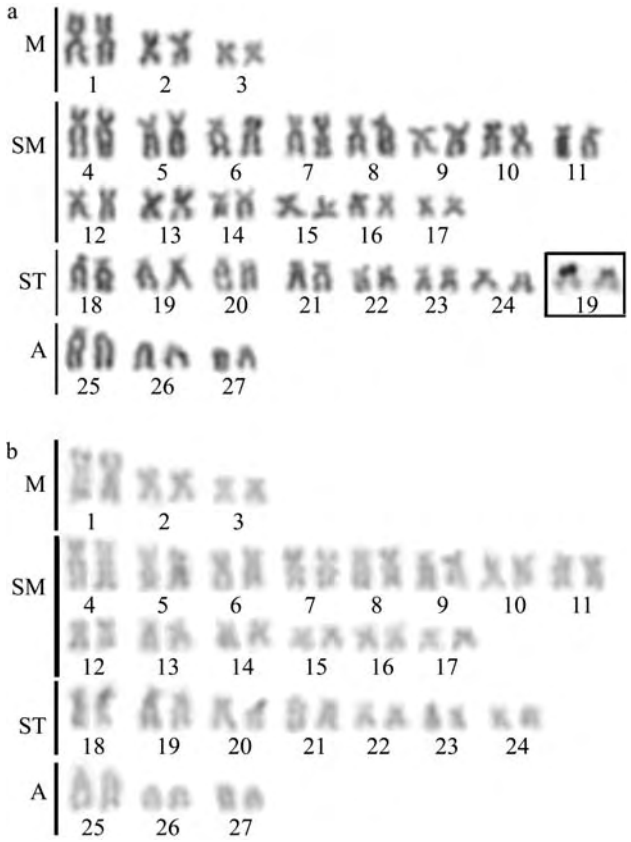

Figure 1 - Karyotype of *Cephalosilurus apurensis* (2n = 54) after: (a) conventional staining and (b) C-banding. In the inset, silver stained chromosomes showing the terminal Ag-NOR on the short arms of pair 19.

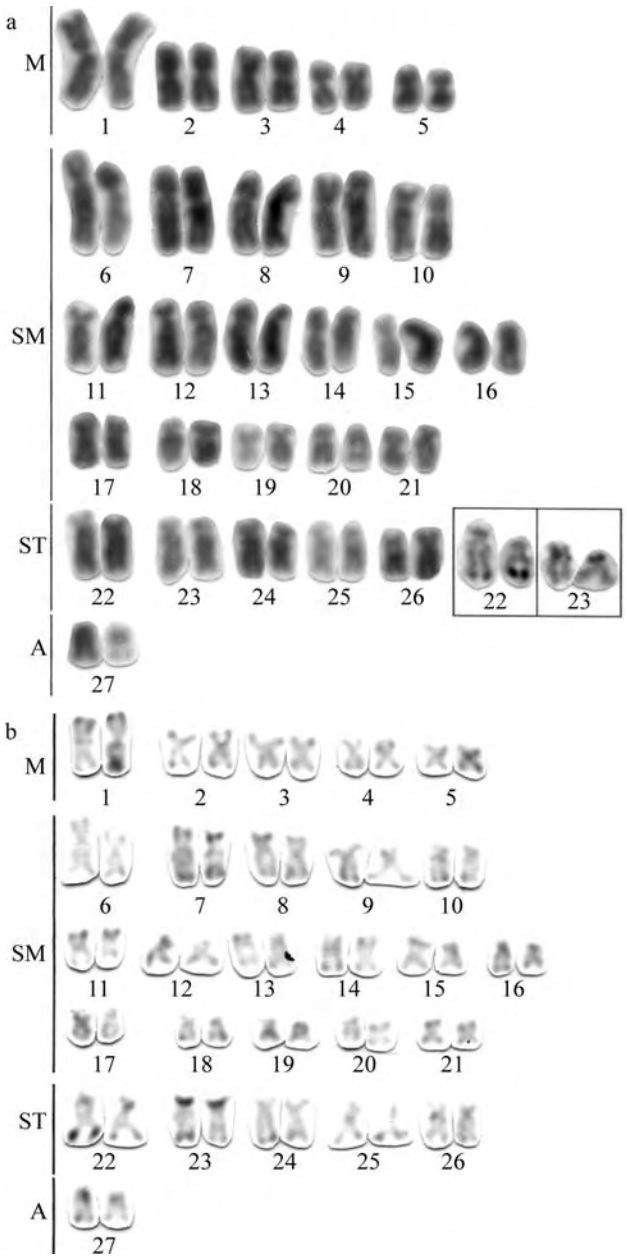

Figure 2 - Karyotype of *Microglanis* aff. *cottoides* (2n = 54) after: (a) conventional staining and (b) C-banding. In the inset, silver stained chromosomes showing the terminal Ag-NORs on the short arms of pair 23 and long arms of pair 22.

Table 1 - Cytogenetic data for Pseudopimelodidae, Heptapteridae and Pimelodidae.

Family/species	Locality	2n	Karyotype	NOR	References
Pseudopimelodidae					
Cephalosilurus apurensis	Orinoco River, Caicara del Orinoco, Bolívar, Venezuela	54	6M+28SM+14ST+6A	1	Present study
Lophiosilurus alexandrii	Três Marias Reservoir, Minas Gerais, Brazil	54	54M, SM, ST, A	1	Marques, Garcia and Moreira Filho (personal communication)
Microglanis aff. *cottoides*	Ribeirão Cavalo Stream, Jaraguá do Sul, Santa Catarina, Brazil	54	10M+32SM+10ST+2A	2	Present study
Microglanis garavelloi	Araquá and Capivara Rivers, Botucatu, São Paulo, Brazil	54	22M+20SM+12ST	1	Vissotto *et al.* (1999a)
Pseudopimelodus bufonius	Amazon Basin	54	12M+30SM+12ST	3	Present study
Pseudopimelodus mangurus	Mogi-Guaçu River, Pirassununga, São Paulo, Brazil	54	6M+26SM+12ST+10A	1	Martinez *et al.* (2004)
Heptapteridae					
Pimelodella avanhandavae	Araquá River, São Paulo, Brazil	46	20M+20SM+6ST	1	Vissotto *et al.* (1999a)
Pimelodella aff. *meeki*	Couro do Boi River, Paraná, Brazil	46	34M+12ST	1	Dias and Giuliano-Caetano (2002)
Heptapterus longicauda	Quinta Stream, Itatinga, São Paulo, Brazil	52	22M+26SM+4ST	2	Vissotto *et al.* (1999a)
Pimelodella aff. *avanhandavae*	Tibagi River, Paraná, Brazil	52	30M+22SM	1	Swarça *et al.* (2003a)
Imparfinis cf. *piperatus*	Juquiá River, Juquiá, São Paulo, Brazil	56	22M+26SM+4ST+4A	1	Vissotto *et al.* (2001)
Rhamdella microcephala	Machado River, São João da Mata, Minas Gerais, Brazil	56	18M+30SM+8ST, A	1	Fonseca *et al.* (2003)
Cetopsorhamdia iheringi	Capivara River, Botucatu, São Paulo, Brazil	58	28M+24SM+6ST	1	Vissotto *et al.* (1999a)
Imparfinis mirini	Quinta Stream, São Paulo, Brazil	58	M24M+34SM/F23M+35SM	1	Vissotto *et al.* (1997)
Imparfinis piperatus	Araras River, Araras, São Paulo, Brazil	58	32M+26SM	1	Vissotto *et al.* (2001)
Pimelodella kronei	Iporanga, São Paulo, Brazil	58	54M, SM+4ST	1	Almeida-Toledo *et al.* (1992)
Pimelodella transitoria	Iporanga, São Paulo, Brazil	58	54M, SM+4ST	1	Almeida-Toledo *et al.* (1992)
Rhamdia quelen	Quadros Lagoon, Rio Grande do Sul, Brazil	58	52M, SM, ST+6A	1	Hochberg and Erdtmann (1988)
Pimelodidae					
Calophysus macropterus	Negro River, Amazonas, Brazil	50	22M+18SM+10A	1	Ramirez-Gil *et al.* (1998)
Pirinampus pinirampu	Tibagi River, Sertaneja, Paraná, Brazil	50	26M+12SM+2ST+10A	1	Swarça *et al.* (1999)
Pseudoplatystoma fasciatum	Solimões River, Amazonas, Brazil	56	18M+14SM+10ST+14A	1	Fenocchio and Bertollo (1992)
Pseudoplatystoma tigrinum	Solimões River, Amazonas, Brazil	56	18M+16SM+8ST+14A	1	Fenocchio and Bertollo (1992)
Sorubim lima	Solimões River, Amazonas, Brazil	56	18M+12S+14ST+12A	1	Fenocchio and Bertollo (1992)
Bergiaria westermanni	São Francisco River, Minas Gerais, Brazil	56	42M, SM+14ST	1	Dias and Foresti (1993)
Pimelodus heraldoi	Tibagi River, Paraná, Brazil	56	22M+22SM+6ST+6A	1	Souza *et al.* (2004)
Pimelodus maculatus	São Francisco River, Minas Gerais, Brazil	56	40M, SM+16ST, A	1	Dias and Foresti (1993)
Pimelodus argenteus	Paraguai River, Corumbá, Mato Grosso do Sul, Brazil	56	34M, SM+22ST, A	1	Souza *et al.* (2003)
Pimelodus mysteriosus	Paraguai River, Corumbá, Mato Grosso do Sul, Brazi	56	26m+20SM+2ST+8A	1	Souza *et al.* (2003)
Pseudoplatystoma corruscans	Porto Rico, Paraná, Brazil	56	18M+16SM+10ST+12A	1	Martins-Santos *et al.* (1996)
Hemisorubim platyrhynchos	Porto Rico, Paraná, Brazil	56	22M+18SM+6ST+10A	1	Martins-Santos *et al.* (1996)
Zungaro zungaro	Foz do Iguaçu, Paraná, Brazil	56	26M+10SM+6ST+14A	1	Martins-Santos *et al.* (1996)
Iheringichthys labrosus	Jurumirim Reservoir, Itatinga, São Paulo, Brazil	56	22M+18SM+10ST+6A	1	Vissotto *et al.* (1999b)
Steindachneridion sp.	Iguaçu River, Usina Salto Segredo, Paraná, Brazil	56	20M+24SM+2ST+10A	1	Swarça *et al.* (2003b)

2n = diploid number; M = metacentrics; SM = submetacentrics; ST = subtelocentrics; A = acrocentrics; NOR = number of chromosome pairs with nucleolus organizer regions.

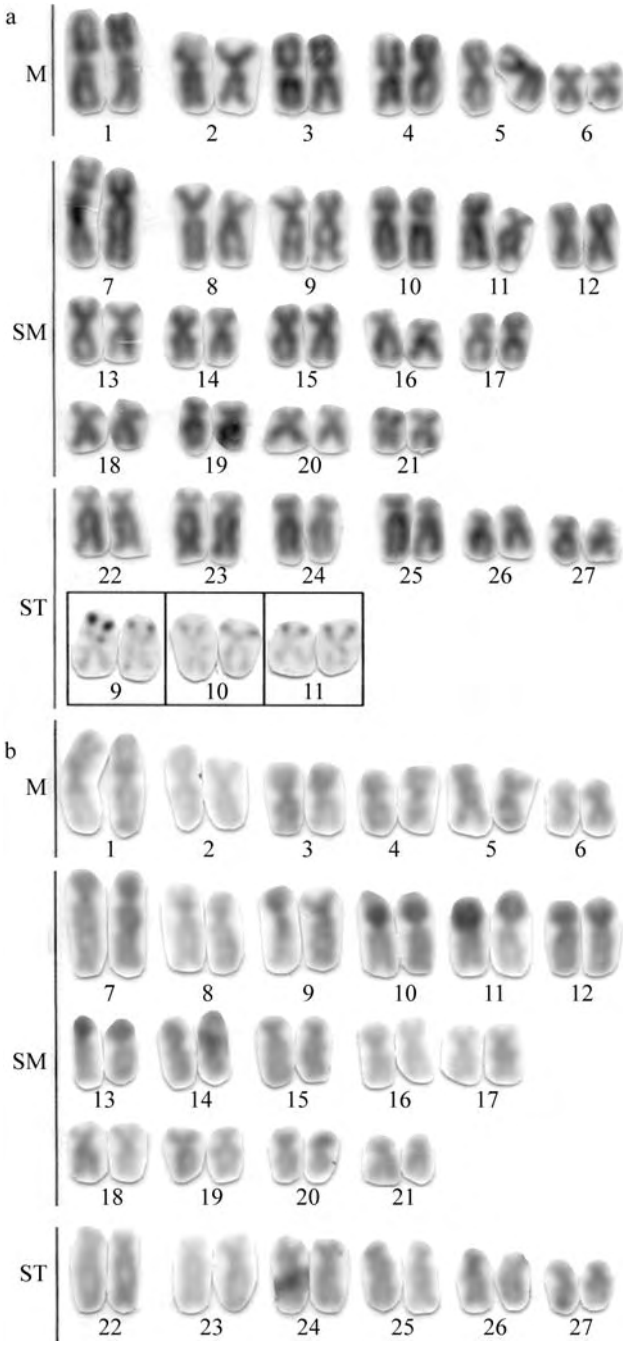

Figure 3 - Karyotype of *Pseudopimelodus bufonius* (2n = 54) after: (a) conventional staining and (b) C-banding. In the inset, silver stained chromosomes showing the terminal Ag-NORs on the long arms of pairs 9, 10 and 11.

identified as pair 23, and on the long arm of another middle-sized ST pair, identified as pair 22 (Figure 2a). The Ag-NORs of *P. bufonius* occurred on the short arm of three middle-sized ST pairs, identified as pairs 9, 10 and 11 (Figure 3a). Multiple Ag-NORs were identified in one species of Heptapteridae (Vissotto *et al.*, 1999a) and were not found among Pimelodidae (Table 1). The number and position of NORs are species-specific and do not seem to follow any pattern during karyotypic evolution.

C-banding showed the occurrence of a small amount of constitutive heterochromatin in the chromosomes of the three species (Figures 1b, 2b, 3b). In *Cephalosilurus apurensis*, positive C-banded segments were observed on the short arms of the largest ST pair (pair 19) and in the Ag-NORs. In *Pseudopimelodus bufonius,* C-banding evidenced segments on the short arms of the six larger SM pairs (pairs 9, 10, 11, 12, 13 and 14) and in *Microglanis* aff. *cottoides*, C-banding revealed positive segments on the short arms of one large ST pair (pair 23) and on the long arms of several ST pairs. The small amount of heterochromatic segments in the chromosomes of *P. bufonius, M.* aff. *cottoides,* and *C. apurensis,* as well as in other representatives of the family Pseudopimelodidae, *P. mangurus* (Martinez *et al.*, 2004), and *M. garavelloi* (Vissotto *et al.*, 1999a), suggests that this may be a characteristic of this catfish family. The occurrence of a very small amount of C-banded positive segments reported herein resembles the data reported for many teleost species, including siluriforms (Gold *et al.*, 1990).

The presence of 2n = 54 chromosomes may be an important characteristic to differentiate Pseudopimelodidae species from species of Heptapteridae and Pimelodidae. Further analysis of additional Pseudopimelodidae species with different staining techniques will provide important information for a better understanding of the chromosome evolution in the group and will help to confirm the conservative nature of the diploid number in this fish family.

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Karyotypic relationships among the tribes of Hypostominae (Siluriformes: Loricariidae) with description of XO sex chromosome system in a Neotropical fish species

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Abstract

The family Loricariidae, with about 683 species, is one the largest fish families in the world. The subfamily Hypostominae was recently reviewed and is now divided in five tribes. With the main objective of contributing to a better understanding of the relationships of the members of the subfamily Hypostominae, cytogenetic analyses were conducted in seven species (three Hypostomini, three Pterygoplichthini and two Ancistrini) from Brazil and Venezuela. In Pterygoplichthini, all species show $2n = 52$ chromosomes. In Hypostomini *Hypostomus ancistroides* has $2n = 68$, *H. regani* $2n = 72$ and *Hypostomus goyazensis* $2n = 72$ chromosomes. In Ancistrini *Ancistrus* n. sp. 1 has $2n = 39/40$ with a sex chromosome system of the type XX/X0, which is a novelty for neotropical fishes, and *Ancistrus* n. sp. 2 has $2n = 52$ chromosomes. Six species have single Ag-NORs and two multiple Ag-NORs. The possible cytogenetic relationships among the species of Hypostominae are discussed.

Introduction

The family Loricariidae, with 683 valid species (Reis, Kullander and Ferraris Jr, 2003), represents one of the largest fish families in the world. This large diversity has resulted in problems of species identification and many new species have been continuously described (Pereira and Oyakawa, 2003; Cardoso and Silva, 2004). Traditionally, Loricariidae has been divided in six subfamilies: Hypostominae, Ancistrinae, Loricariinae, Hypoptopomatinae, Neoplecostominae and Lithogeninae (Reis, Kullander and Ferraris Jr, 2003). However, recent studies conducted by Armbruster (2004) and Reis, Pereira and Armbruster (2006) showed that this division should be reevaluated, and a new proposal resumed in Figure 1 was presented.

To become a natural group, the subfamily Hypostominae was redefined with the exclusion of several genera and with the incorporation of the subfamily Ancistrinae (Armbruster, 2004). Thus, Hypostominae is now divided in five tribes: Corymbophanini, Rhinelepini, Hypostomini, Pterygoplichthini, and Ancistrini (Figure 1). Cytogenetic studies conducted in the subfamily Hypostominae showed that among 32 species analyzed the diploid number ranges from $2n = 38$ in *Ancistrus* sp. to $2n = 80$ in *Hypostomus* sp. (Table 1).

Although most fish species do not display differentiated sex chromosomes, eight systems involving male heterogamety (XY, X0, X1X2Y and XY1Y2), female heterogamety (ZW, Z0 and ZW1W2) and an unusual WXZ in the platyfish were described in fishes (Bertollo et al., 2000;

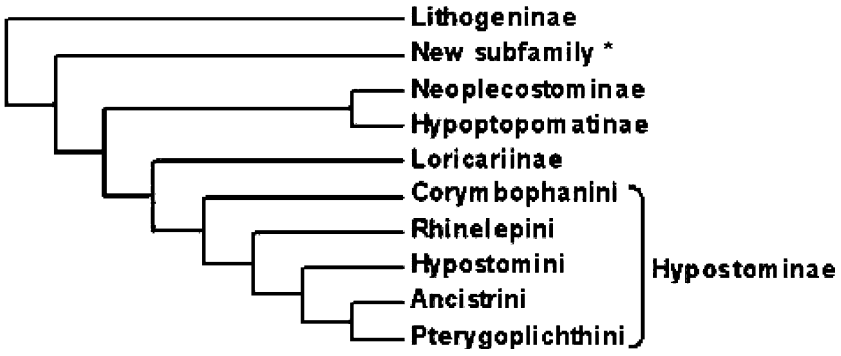

Figure 1. Phylogeny of the family Loricariidae proposed by Armbruster (2004) and modified by Reis et al. (personal communication). * The name of this new subfamily is not available yet.

Devlin and Nagahama, 2002). The ZZ/ZW system is the most common sex chromosome system and the XX/X0 (10 species) and ZZ/Z0 (3 species) systems, are rarer (Devlin and Nagahama, 2002). Sex chromosomes were described in five species of Loricariidae that exhibited XX/XY or ZZ/ZW sex chromosome systems (Table 1).

In the present study, three species of the genus *Hypostomus*, two of the genus *Ancistrus*, two of the genus *Liposarcus* and one of the genus *Glyptoperichthys* were karyotyped. The results obtained were employed to discuss some aspects of the chromosome evolution in the subfamily Hypostominae, and the description of an XX/X0 sex chromosome system which is a novelty for neotropical fish species.

Material and methods

Cytogenetic studies were conducted with the following species: *Hypostomus ancistroides*, four males and five females from the Araquá river, Botucatu, São Paulo, Brazil (LBP 2147); *H. regani*, five males and three females from the Araquá river, Botucatu, São Paulo, Brazil (LBP 2148); *H. goyazensis*, three males and two females from the Vermelho river, Goiás Velho, Goiás, Brazil (LBP 1656, LBP 2150); *Glyptoperichthys gibbiceps*, five males and four females from the Orinoco river, Caicara del Orinoco, Bolivar, Venezuela (LBP 2174); *L. anisitsi*, three males and five females from the Tietê river, Botucatu, São Paulo, Brazil (LBP 2160); *L. anisitsi*, three males and two females from the Miranda river, Corumbá, Mato Grosso do Sul, Brazil (unpreserved fishes); *L. multiradiatus*, three males and three females from the Orinoco river, Caicara del Orinoco, Bolivar, Venezuela (LBP 2173); *Ancistrus* n sp. 1, two males and two females from the Vermelho river, Goiás Velho, Goiás, Brazil (LBP = 2151); *Ancistrus* n sp. 2, four males and three females from the Guaruva river, Guaruva, Santa Catarina, Brazil (LBP 2149). The specimens were studied by taxonomists, which provided the species identification. Two species (here identified as *Ancistrus* n. sp. 1 and *Ancistrus* n. sp. 2) were not named because they are new to science. The fishes were deposited in the fish collection of Laboratório de Biologia e Genética de Peixes (LBP), UNESP, Botucatu, São Paulo, Brazil.

Chromosome preparations were obtained from kidney tissues using the technique described by Foresti, Oliveira and Almeida-Toledo (1993). Silver staining of the nucleolus organizer regions (Ag-NORs) was performed according to the technique proposed by Howell and Black (1980). Chromosome morphology was determined on the basis of arm ratio, as proposed by Levan, Fredga and Sandberg (1964) and the chromosomes were classified as metacentrics (M), submetacentrics (SM), subtelocentrics (ST) and acrocentrics (A).

Results

Among Pterygoplichthini, *Glyptoperichthys gibbiceps* from the Orinoco River has $2n = 52$ chromosomes (20M, 24SM, 8ST) and subterminal Ag-NORs on the short arm of the chromosome pair 21 (SM) (Figure 2a). *Liposarcus multiradiatus* from the Orinoco River has $2n = 52$ chromosomes

Table 1. Cytogenetic data available for the subfamily Hypostominae

Species	Locality	2n	Karyotypic Formulae	Reference
Rhinelepini				
Rhinelepis aspera	Paraná River, Paraná, Brazil	54	20M, 26SM, 8ST	Artoni and Bertollo (2001)
Pogonopoma wertheimeri	Mucuri River, Bahia, Brazil	54	20M, 30SM, 4ST	Artoni and Bertollo (2001)
Hypostomini				
Hypostomus emarginatus	Araguaia River, Mato Grosso, Brazil	52	16M, 30SM, 6ST	Artoni and Bertollo (2001)
H. plecostomus	–	54	24M/SM, 12ST, 18A	Muramoto, Ohno and Atkin (1968)
Hypostomus sp.	Araguaia River, Mato Grosso, Brazil	64	14M, 24SM, 26ST/A	Artoni (1996)
H. ancistroides	Mogi-Guaçu River, São Paulo, Brazil	68	16M, 18SM, 34ST/A	Artoni and Bertollo (1996)
H. ancistroides	Mogi-Guaçu River, São Paulo, Brazil	68	16M, 18SM, 34ST/A	Artoni and Bertollo (1996)
H. ancistroides	Araquá River, São Paulo, Brazil	68	18M, 10SM, 12ST, 28A	Present study
H. macrops	–	68	10M, 14SM, 44ST/A	Michele, Takahashi and Ferrari (1977)
Hypostomus sp. A	Rincão Stream, São Paulo, Brazil	70	18M, 14SM, 38ST/A	Artoni and Bertollo (1996)
H. regani	Mogi-Guaçu River, São Paulo, Brazil	72	10M, 20SM, 42ST/A	Artoni and Bertollo (1996)
H. regani	Araquá River, São Paulo, Brazil	72	12M, 18SM, 26ST, 16A	Present study
Hypostomus goyazensis	Vermelho River, Goiás, Brazil	72	10M, 16SM, 10ST, 36A	Present study
Hypostomus sp. B	Mogi-Guaçu River, São Paulo, Brazil	72	12M, 18SM, 42ST/A	Artoni and Bertollo (1996)
Hypostomus sp. C	Mogi-Guaçu River, São Paulo, Brazil	72	10M, 18SM, 40ST/A	Artoni and Bertollo (1996)
Hypostomus sp. D1	Mogi-Guaçu River, São Paulo, Brazil	72	10M, 26SM, 36ST/A	Artoni and Bertollo (1996)
Hypostomus sp. D2	Mogi-Guaçu River, São Paulo, Brazil	72	14M, 20SM, 38ST/A	Artoni and Bertollo (1996)
H. albopunctalus	Mogi-Guaçu River, São Paulo, Brazil	74	10M, 20SM, 44ST/A	Artoni and Bertollo (1996)
H. paulinus	–	74	10M, 20SM, 44ST/A	Michele, Takahashi and Ferrari (1977)
H. strigaticeps	Mogi-Guaçu River, São Paulo, Brazil	74	8M, 6SM, 60ST/A	Michele, Takahashi and Ferrari (1977)
H. aff. *auroguttatus*	Mogi-Guaçu River, São Paulo, Brazil	76	8M, 30SM, 38ST/A	Artoni and Bertollo (1996)
Hypostomus sp. F	São Francisco River, Minas Gerais, Brazil	76	10M, 16SM, 50ST/A	Artoni and Bertollo (1999)
Hypostomus sp. E	Mogi-Guaçu River, São Paulo, Brazil	80	8M, 16SM, 56ST/A	Artoni and Bertollo (1996)
Pterygoplichthini				
Glyptoperichthys gibbiceps	Orinoco River, Venezuela	52	20M, 24SM, 8ST	Present study
Liposarcus anisitsi	Preto River, São Paulo, Brazil	52	16M, 24SM, 8ST, 4A	Artoni and Bertollo (1996)
L. anisitsi	Tietê River, Botucatu, São Paulo	52	28M, 12SM, 8ST, 4A	Present study
L. anisitsi	Miranda River, Mato Grosso do Sul, Brazil	52	8M, 14SM, 14ST, 16A	Present study
L. multiradiatus	Orinoco River, Venezuela	52	22M, 18SM, 12ST	Present study
Ancistrini				
Ancistrus n sp.	São Francisco River, Acre, Brazil	38	30M/SM, 8ST	Alves, Oliveira and Foresti (2003)
Ancistrus n sp. 1	Vermelho River, Goiás, Brazil	40	33M, 6SM	Present study
		39	34M, 6SM	
Ancistrus n sp. 2	Garuva River, Santa Catarina, Brazil	52	10M, 16SM, 12ST, 14A	Present study
Ancistrus n sp.	Betari River, São Paulo, Brazil	52	32M/SM, 20ST/A	Alves, Oliveira and Foresti (2003)

Table 1. (*Continued*)

Species	Locality	2n	Karyotypic Formulae	Reference
Ancistrus multispinnis	Itapocu River, Santa Catarina, Brazil	52	28M/SM, 24ST/A	Alves, Oliveira and Foresti (2003)
Hemiancistrus sp.	Araguaia River, Mato Grosso, Brazil	52	20M, 20SM, 8ST, 4A	Artoni and Bertollo (2001)
Panaque cf. *nigrilineatus*	Araguaia River, Mato Grosso, Brazil	52	26M, 20SM, 6ST	Artoni and Bertollo (2001)

$2n$ = diploid number; M = metacentric; SM = submetacentric; ST = subtelocentric; A = acrocentric.

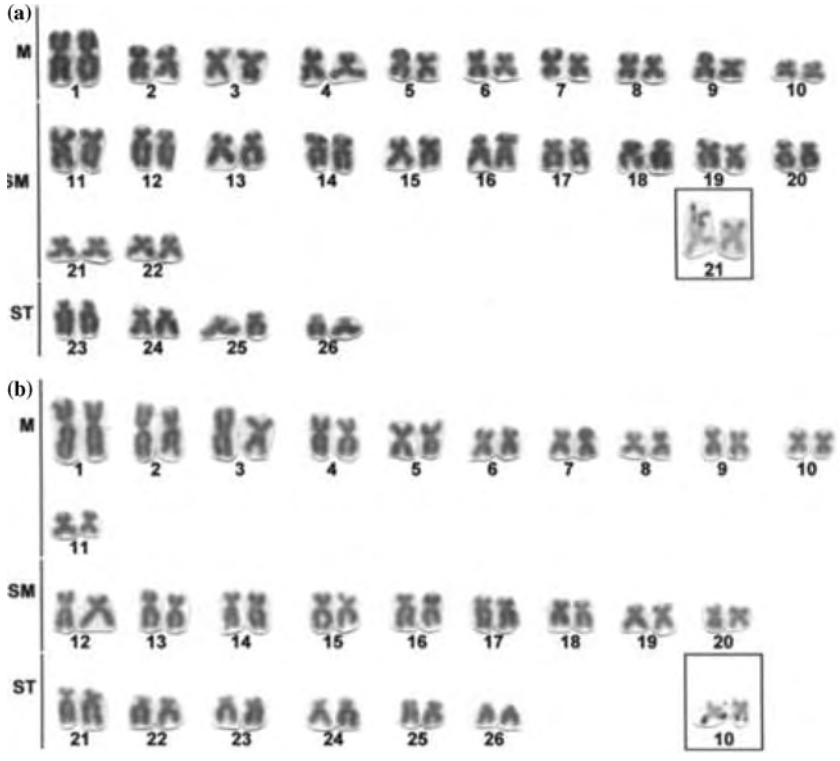

Figure 2. Giemsa stained karyotypes of Pterygoplichthini. (a) *Glyptoperichthys gibbiceps* with $2n = 52$ chromosomes; (b) *Liposarcus multiradiatus* with $2n = 52$ chromosomes. In the insets, the NOR-bearing chromosome pairs.

(22M, 18SM, 12ST) and subterminal Ag-NORs on the long arm of the chromosome pair 10 (M) (Figure 2b). *Liposarcus anisitsi* from the Tietê River has $2n = 52$ chromosomes (28M, 12SM, 8ST, 4A) and interstitial Ag-NORs on the long arm of the chromosome pair number 16 (SM) (Figure 3a). *Liposarcus anisitsi* from the Miranda River has $2n = 52$ chromosomes (8M, 14SM, 14ST, 16A) and interstitial Ag-NORs on the long arm of the chromosome pair 5 (SM) (Figure 3b).

Among the species of the Ancistrini *Ancistrus* n. sp. 1 from the Vermelho River has $2n = 40$ chromosomes in females (34M, 6SM) and $2n = 39$ chromosomes in males (33M, 6SM), and subterminal Ag-NORs on the long arm in the chromosome pair 20 (SM) (Figure 4a and b). *Ancistrus* n. sp. 2 from the Garuva River has $2n = 52$ chromosomes: (10M, 16SM, 12ST, 14A) and subterminal Ag-NORs on the short arm of the chromosome pair 15 (ST) (Figure 4c).

Among Hypostomini the results showed that *Hypostomus ancistroides* from the Araquá River has $2n = 68$ chromosomes (18M, 10SM, 12ST, 28A), subterminal Ag-NORs on the short arm of the chromosome pair 6 (M), and subterminal Ag-NORs on the short arm of the two chromosome pairs 13 and 14 (SM) (Figure 5a). *Hypostomus regani* from the Araquá River has $2n = 72$ chro-

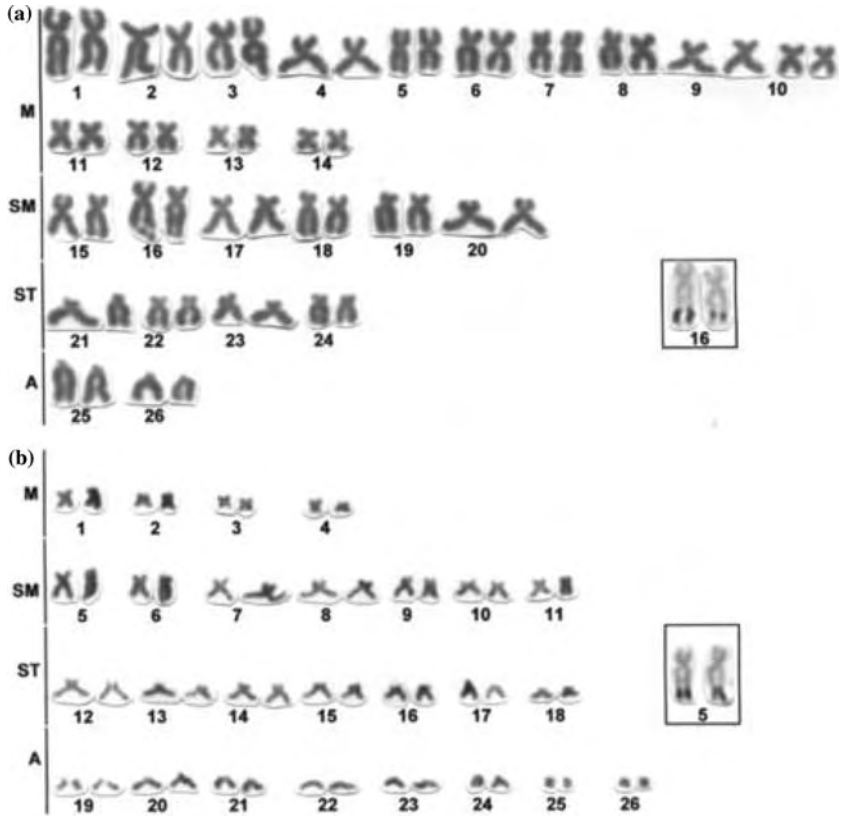

Figure 3. Giemsa stained karyotypes of Pterygoplichthini. (a) *Liposarcus anisitsi* from Tietê River with $2n = 52$ chromosomes. (b) *Liposarcus anisitsi* from Miranda River with $2n = 52$ chromosomes. In the insets, the NOR-bearing chromosome pairs.

mosomes (12M, 18SM, 26ST, 16A) and subterminal Ag-NORs on the long arm of chromosome pairs 35 and 36 (A) (Figure 5b). *Hypostomus goyazensis* from the Vermelho River has $2n = 72$ chromosomes (10M, 16SM, 10ST, 36A) and subterminal Ag-NORs on the short arm of the chromosome pair 18 (ST) (Figure 6).

Discussion

Alves, Oliveira and Foresti (2005), showed that the species of the subfamilies Neoplecostominae (sensu Armbruster, 2004) and Hypoptopomatinae are conserved from the cytogenetic point of view, with almost all species exhibiting $2n = 54$ chromosomes, mainly biarmed and usually with single interstitial Ag-NORs. Among Loricariinae species a high diploid number variation is observed, with values ranging from $2n = 36$ to $2n = 74$ (Alves, Oliveira and Foresti, 2003). Diploid number variation in Hypostominae is also very high ranging from $2n = 38$ to $2n = 80$ (Table 1). However, when the different tribes of Hypostominae are analyzed separately it is possible to note that this variation is not widespread among the them.

No cytogenetic data is available for representatives of the Corymbophanini, the most primitive tribe of Hypostominae (Figure 1). Alves, Oliveira and Foresti (2005), suggested that a species identified as *Corymbophanes* n. sp. had $2n = 54$ chromosomes. However, a re-examination of the animals showed that they belong to a new species, probably of the genus *Pareiorhina* (Reis and Pereira, personal communication).

In this paper we have not studied representatives of the Rhinelepini, but Artoni and Bertollo (2001) found $2n = 54$ for *Rhinelepis aspera* and *Pogonopoma wertheimeri*. The presence of $2n = 54$ chromosomes in Rhinelepini is an interesting feature considering the common occurrence of this diploid number among Neoplecostominae and Hypop-

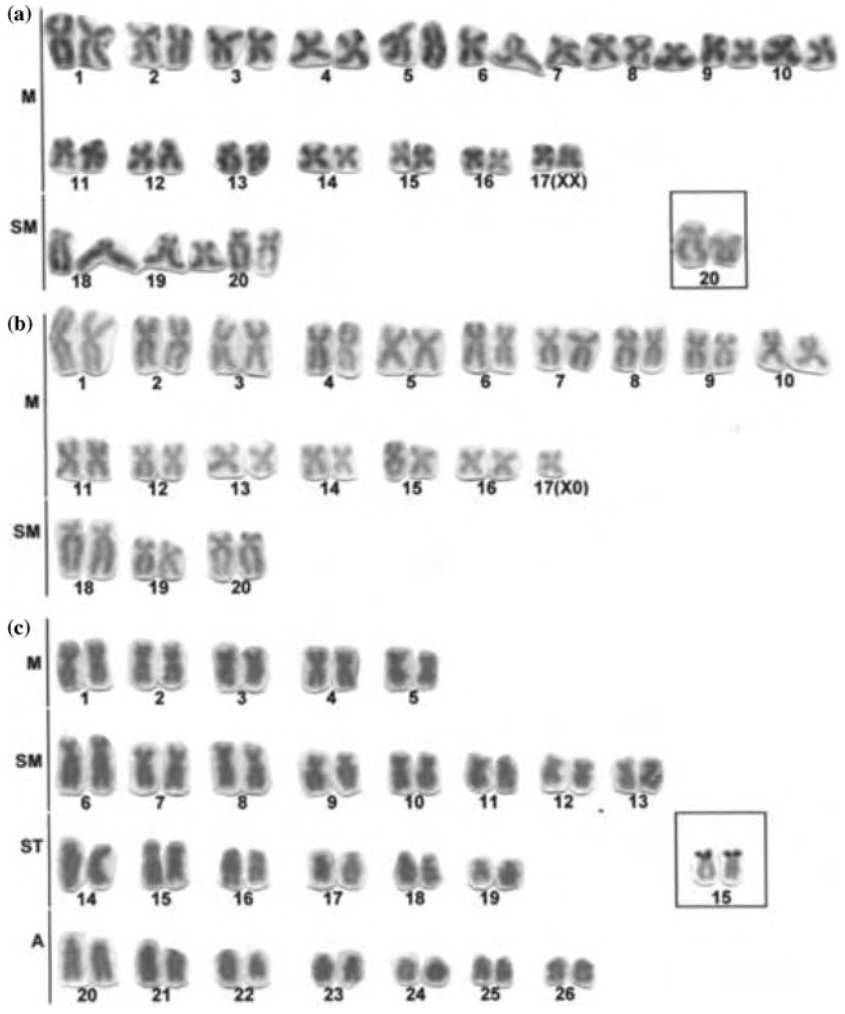

Figure 4. Giemsa stained karyotypes of *Ancistrus*. (a) Female of *Ancistrus* n. sp. 1 with $2n = 40$ chromosomes; (b) Male of *Ancistrus* n. sp. 1 with $2n = 39$ chromosomes; (c) *Ancistrus* n sp. 2 with $2n = 52$ chromosomes. In the insets, the NOR-bearing chromosome pairs.

topomatinae (as discussed above) and the phylogenetic position of Rhinelepini as the second most basal in the subfamily Hypostominae (Figure 1).

According to Armbruster (2004), the genus *Hypostomus* may be the only valid genus of the tribe Hypostomini. The species of this genus display a large variation in diploid numbers, with values ranging from $2n = 52$ to $2n = 80$ chromosomes (Table 1). A general analysis shows that two species, *H. emarginatus* with $2n = 52$ and *H. plecostomus* with $2n = 54$, have reduced diploid numbers and the other species have larger diploid numbers ranging from $2n = 64$ to $2n = 80$ (Table 1). Among the *Hypostomus* species the high diploid number is coincident with a high number of uniarmed chromosomes (Table 1), suggesting the occurrence of a large number of centric fissions in the karyotypic evolution of the group (Artoni and Bertollo, 1996).

Muller and Weber (1992), suggest the division of the genus *Hypostomus* in two groups, according to their morphology and color pattern, one group named *plecostomus* and other named *regani*. Molecular data obtained by Montoya-Burgos (2003), corroborate the existence of these two groups but the author showed that the species *H. emarginatus* and *H. squalinus* were more closely related to other Hypostominae than to other species of *Hypostomus*. The cytogenetic data fit very well in this hypothesis, since *H. emarginatus* has a diploid number that is more common among

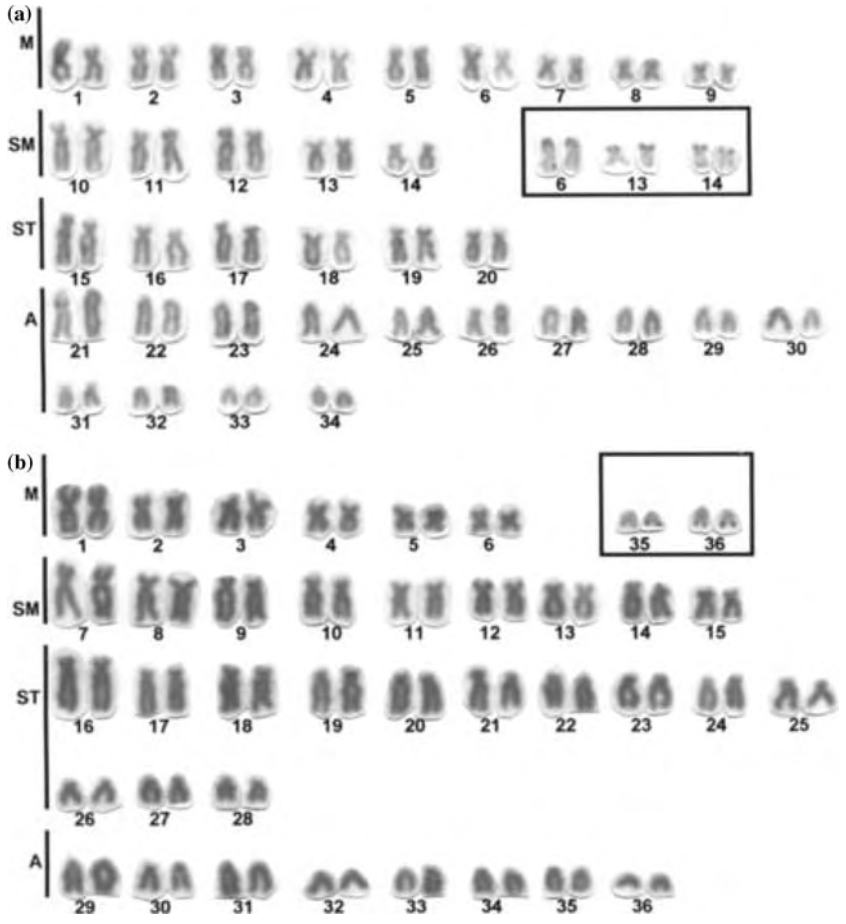

Figure 5. Giemsa stained karyotypes of *Hypostomus*. (a) *H. ancistroides* with $2n = 68$ chromosomes; (b) *H. regani* with $2n = 72$ chromosomes. In the insets, the NOR-bearing chromosome pairs.

other tribes of Hypostominae, suggesting that it may not belong to the tribe Hypostomini. In the group *plecostomus* the karyotyped species have diploid numbers ranging from $2n = 66$ to $2n = 68$ while in the group *regani* the karyotyped species have diploid numbers ranging from $2n = 72$ to $2n = 74$.

The analyses of the Ag-NORs distribution among Hypostomini show that some species have single Ag-NORs as *Hypostomus goyazensis* ($2n = 72$), while others have multiple Ag-NORs, as *H. ancistroides* ($2n = 68$). This last condition, i.e., the presence of multiple Ag-NORs, is more common among the *Hypostomus* species as the presence of terminal Ag-NORs is as well (Artoni and Bertollo, 1996; present study). Considering that the identification of many *Hypostomus* species is usually difficult and that many species are still undescribed, the simultaneous analysis of cytogenetic and molecular data will be very useful for a better understanding of the evolution in the group.

The tribes Pterygoplichthini and Ancistrini are sister groups and compose the most derived clade of the Hypostominae (Armbruster, 2004). The cytogenetic data obtained for species of Pterygoplichthini show that all have $2n = 52$ chromosomes (Table 1). The presence of $2n = 52$ chromosomes in Pterygoplichthini is probably an apomorphic characteristic, suggesting the reduction in the diploid number in the ancestor of this tribe. The species *Liposarcus anisitsi* (Artoni and Bertollo, 1996; present study) displays single Ag-NORs in interstitial position as observed in Neoplecostominae and Loricariinae (Alves, Oliveira and Foresti, 2003; Alves, Oliveira and Foresti, in press).

The tribe Ancistrini has about 24 genera, but the taxonomy of the group is still not well resolved

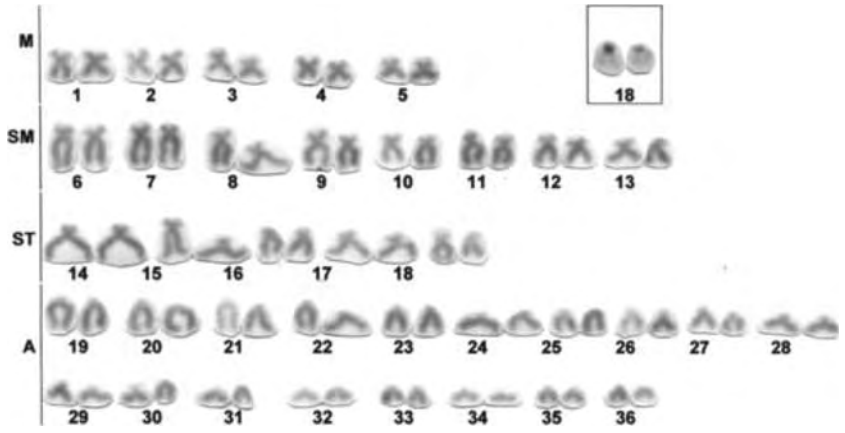

Figure 6. Giemsa stained karyotypes of *Hypostomus goyazensis* with $2n = 72$ chromosomes. In the insets, the NOR-bearing chromosome pairs.

(Armbruster, 2004), with many new species to be described. Cytogenetic data is available only for seven species belonging to three genera. An interesting characteristic is the presence of $2n = 52$ chromosomes in five species and a lower diploid number in the other two species (Table 1). This reduction in the diploid number found in two *Ancistrus* species, may be related to centric fusions events, since the number of uniarmed chromosomes in these species is smaller than that observed in the species with $2n = 52$ (Table 1).

The analysis of the Ag-NORs distribution in the species of Ancistrini showed the occurrence of single Ag-NORs (Figure 3), mainly found in subterminal position, although the species *Ancistrus* n. sp. with $2n = 38$ chromosomes, shows single interstitial Ag-NORs (Alves, Oliveira and Foresti, 2003). The presence of single interstitial Ag-NORs in Ancistrini, suggests that the primitive NOR state remained in this species or, on the contrary, that a convergent evolution would have occurred, considering that *Ancistrus* n. sp. presented a derived diploid number ($2n = 38$) for the Loricariidae. The cytogenetic data available for the species of Pterygoplichthini and Ancistrini corroborate the sister-group relationship hypothesized by Armbruster (2004).

In the family Loricariidae, sex chromosome systems were previously described in six species (Table 2). Among the species with XX/XY and ZZ/ZW systems, some species have sex chromosomes morphologically different, as in *Hyp-*

Table 2. Sex chromosome systems described for species of the family Loricariidae

Species	2*n*	Karyotypic Formulae	Sex chromosome system	References
Hypostominae				
Ancistrus n sp. 1	40	33M, 6SM	XX/XO	Present study
	39	34M, 6SM		
Hypostomus macrops	68	10M, 14SM, 44ST/A	XX/XY	Michele, Takahashi and Ferrari (1977)
Hypostomus sp.	64	14M, 24SM, 26ST/A	ZZ/ZW	Artoni, Vênere and Bertollo (1998)
Loricariinae				
Loricariichthys platymetopon	54	6M, 20SM, 4ST, 24A	ZZ/ZW	Scavone and Júlio Jr. (1995)
Hypoptopomatinae				
Hisonotus leucofrenatus	54	22M, 26, 4ST, 2A	ZZ/ZW	Andreata et al. (1993)
Hisonotus leucofrenatus	54	24M, 26, 2ST, 2A	ZZ/ZW	Andreata et al. (1993)
Pseudotocinclus tietensis	54	28M, 20SM, 6ST	XX/XY	Andreata et al. (1992)

2*n* = diploid number; M = metacentric; SM = submetacentric; ST = subtelocentric; A = acrocentric.

ostomus sp. (Artoni, Vênere and Bertollo, 1998) while in other species the sex chromosomes are identified by an additional C-band in one chromosome of the sex pair as in *Pseudotocinclus tietensis* (Andreata et al., 1992). The sex chromosome system of the XX/X0 type in *Ancistrus* n. sp. 1 is very uncommon (Devlin and Nagahama, 2002) and not yet described in Neotropical fishes. This suggests an independent origin of this sex chromosome system in this species.

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Cytogenetic analysis of three sea catfish species (Teleostei: Siluriformes: Ariidae)

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Abstract

Despite their ecological and economical importance, fishes of the family Ariidae are still less studied from the genetic and cytogenetic point of view. Among 133 known species of ariids, only eight have been karyotyped. Cytogenetic analyses performed on *Genidens barbus* and *Sciades herzbergii* revealed that both species have 2n=56 chromosomes and *Cathorops* aff. *mapale* has 2n=52 chromosomes: *Genidens barbus* has 10 Metacentrics (M), 14 Submetacentrics (SM), 26 Subtelocentrics (ST), and 6 Acrocentrics (A)*, Sciades herzbergii* has 14M, 20SM, 18ST and 4A, while *Cathorops* aff. *mapale* has 14M, 20SM, and 18ST. The nucleolus organizer regions (NORs) were found in *Genidens barbus* in a single chromosome pair *on* the short arm of a large-sized ST pair and in *Cathorops* aff. *mapale* on the short arm of a middle-size SM pair. Multiple NORs were found in *Sciades herzbergii* on the short arm of two large-sized ST pairs. The occurrence of diploid numbers ranging from 2n=52 to 2n=56 chromosomes and the presence of different karyotypic formulae, number, and position of NORs suggest that several numeric and structural chromosome rearrangements were fixed in the evolutionary history of this fish family.

Key words*:* Cytogenetic, Karyotype, Ag-NOR, Chromosome evolution, Chromosome Rearrangements, fish

Introduction

The order Siluriformes (catfishes) has 3,088 species, divided into 36 families and 477 genera worldwide distributed, except for the coldest areas in the Southern and Northern hemispheres (Ferraris 2007). Only two families are predominantly marine: Plotosidae and Ariidae. The family Ariidae, sea catfishes, with 133 species distributed in 26 genera, presents a worldwide distribution and their species live in marine and brackish water (Ferraris 2007). Recent phylogenetic studies showed that the family Ariidae is monophyletic, but its relationships with other siluriforms remain unclear. Some authors suggest a close relationship with Pangasiidae, Ictaluridae, and mainly Anchariidae (de Pinna, 1998; Kailola, 2004; Hardman, 2005; Sullivan *et al.,* 2006; Betancur-R *et al.,* 2007).

Karyotypes of only eight ariid species showing diploid numbers ranging from 2n=54 to 2n=56 and karyotypes mainly constituted of biarmed chromosomes (Table 1) have been described so far. Considering the worldwide distribution of Ariidae and its importance as a commercial fish, the main objective of the present study was to describe the karyotypes of *Cathorops* aff. *mapale*, *Genidens barbus,* and *Sciades herzbergii* adding data for a better understanding of the karyotypic evolution and relationships among genera and species in the family.

Material and methods

The species studied were: *Genidens barbus* (3 males and 3 females) from Ubatuba, São Paulo, Brazil (23°26'00.8''S 45°01'01.7''W, LBP 2338),*Cathorops* aff. *mapale* (4 males) from Isla Margarita, Nueva Esparta, Venezuela (10°57'39.6''S 64°10'26.4''W, LBP 6061), and *Sciades herzbergii* (8 specimens unsexed) from Isla Margarita, Nova Esparta, Venezuela (10°57'39.6''S 64°10'26.4''W, LBP 6060). Fishes were identified and deposited in the fish collection of the Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, São Paulo, Brazil and Escuela de Ciencias Aplicadas del Mar, Universidad de Oriente, Isla Margarita, Venezuela.

Mitotic chromosome preparations were performed according to the technique described by Foresti *et al*. (1993). Nucleolar organizer regions (Ag-NORs) were revealed by the silver-staining method (Howell and Black 1980). The chromosome morphology was determined based on arm ratios, as proposed by Levan *et al*. (1964), and the chromosomes were classified according to their morphology as Metacentrics (M), Submetacentrics (SM), Subtelocentrics (ST), and Acrocentrics (A).

Results and Discussion

Cytogenetic analyses showed that *Genidens barbus* has 2n=56 (10M+14SM+ 26ST+ 6A - Figure 1), *Cathorops* aff. *mapale* has 2n=52 (14M+20SM+8ST - Figure 2a), and *Sciades herzbergii* has 2n=56 (14M+20SM+18ST+4A - Figure 2b). Early karyotypic studies of *G. barbus* by Gomes *et al*. (1994, cited as *Netuma barba*) and *S. herzbergii* by Molina et al. (2004, cited as *Hexanematichthys herzbergii*) showed the same diploid number in their samples as those found in the present study. However, the karyotypic formulae previously described for these species are different from those found here (Table 1). These differences may be due to technical artifacts, such as different chromosome condensation, or attributed to real differences that should be checked in further studies covering the distribution area of these species.

The data available show that diploid numbers range from 2n=52 to 2n=56 among ariids and that the karyotypes are mainly constituted by biarmed chromosomes (Table 1). The chromosome number 2n=56 is the commonest found among ariids occurring in *Aspistor parkeri*, *Bagre bagre*, *Genidens barbus*, *G. genidens*, and *Sciades herzbergii* (Table 1). Diploid number 2n=54 was reported for three species: *Ariopsis felis*, *Bagre marinus*, and *Cathorops sp*. (Table 1). The occurrence of 2n=52 in *Cathorops* aff. *mapale* (present data) represents the lowest diploid number that has been described for ariids up to the present.

Two species of *Cathorops* were cytogenetically investigated : *C. aff. mapale* (2n=52 - present study) and one unidentified species, *Cathorops sp*., that has

2n=54 chromosomes (Gomes et al., 1992). This difference in diploid number between species of a single genus was also found *in Bagre* (Table 1), showing that this phenomenon is not rare among ariids. On the other hand, the two analyzed species of *Genidens* presented the same diploid number (Table 1). reached

Karyotypes of ariids are composed of all morphological types of chromosomes (Table 1). However, in some species such as *Bagre* and *Sciades herzbergii*, a large number of metacentric and submetacentric chromosomes is observed, while in other species such as *Bagre marinus* for example, a large number of subtelocentric and acrocentric chromosomes is observed (Table 1). This variation allows hypothesizing that many structural chromosome rearrangements were fixed during the karyotypic evolution among species of this family.

Studying the karyological evolution of the order Siluriformes, particularly of the family Diplomystidae, Oliveira and Gosztonyi (2000) reached the conclusion that the ancestral diploid number for this order is 2n=56. Among families closely related to Ariidae, species of Pangasiidae have about 2n=60 chromosomes (Magtoon & Donsakul, 1987; Manosroi et al. 2003), and among Ictaluridae, diploid number ranges from 2n=40 to 72 (LeGrande and Cavender, 1980; LeGrande, 1981; Clark and Mathis, 1982; LeGrande et al., 1984; Amemiya, 1986). This diploid number diversification, as well as that observed among ariids, may suggest that numeric chromosome rearrangements (fusions and fissions) also could have had an important role in the karyotypic evolution of this group.

The present results about Ag-NORs locations are the first described for ariids. The ariid species studied have single or multiple Ag-NORs (Table 1). The NORs were found in a single chromosome pair in *Genidens barbus* on the short arm of a biggest-size ST pair, and in *Cathorops* aff. *mapale,* on the short arm of a middle-size SM pair. Among Ictaluridae species, only single NOR-bearing chromosomes have been observed (Amemiya *et al.,* 1986). This is also the most common condition in Siluriformes (Oliveira and Gosztonyi 2000; Oliveira *et al.,* 2006) and even in Teleostei (Klinkhardt 1998). On the other hand, multiple NORs were found in *Sciades herzbergii* on the short arm of two large-sized ST pairs,

which reinforce the hypothesis that structural chromosome rearrangements also were fixed in the karyotypic evolution of ariids.

The available cytogenetical data support the hypotheses that many numeric and structural chromosomal rearrangements were fixed during the evolution of fishes from the family Ariidae. However, additional data should be added to clarify the importance of these chromosomal rearrangements in the evolution of the species and genera of ariids.

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Table 1. Summary of the cytogenetic data available for the family Ariidae. Names in parenthesis are original citations corrected in the present study. 2n= diploid number; M = metacentrics; SM = submetacentrics; ST = subtelocentrics; A= acrocentrics; T=telocentrics; STT=subtelo-telocentrics; NORs= number of chromosome pairs with nucleolus organizer regions.

Species	Locality	2n	Karyotype	NORs	References
Ariopsis felis (=Arius felis)	Caminada Bay, Louisiana, USA	54	26M/SM+28ST/A	-	LeGrande (1980)
Aspistor parkeri (=Arius parkeri)	Cananéia Coast, São Paulo, Brazil	56	16M+16SM+22ST+2T	-	Gomes *et al.* (1994)
Bagre bagre	Cananéia Coast, São Paulo, Brazil	56	24M+26SM+6ST	-	Gomes *et al.* (1990)
Bagre marinus	Northern Gulf of Mexico, Louisiana, USA	54	12M+8SM+34ST	-	Fitzsimons *et al.* (1988)
Cathorops aff. mapale	Isla Margarita, Venezuela	52	14M+20SM+18ST	1	Present study
Cathorops sp.	Cananéia Coast, São Paulo, Brazil	54	13M+13SM+28ST	-	Gomes *et al.* (1992)
Genidens barbus (=Netuma barba)	Cananéia Coast, São Paulo, Brazil	56	18M+18SM+18ST+2T	-	Gomes *et al.* (1994)
Genidens barbus	Ubatuba Coast, São Paulo, Brasil	56	10M+14SM+26ST+6A	1	Present study
Genidens genidens	Cananéia Coast, São Paulo, Brazil	56	12M+20SM+20ST+4T	-	Gomes *et al.* (1994)
Sciades herzbergii (=Hexanematichthys herzbergii)	Maracaibo Lake, Venezuela	56	24M+24SM+6ST+2T	-	Molina *et al.* (2004)
Sciades herzbergii	Isla Margarita, Venezuela	56	14M+20SM+18ST+4A	2	Present study

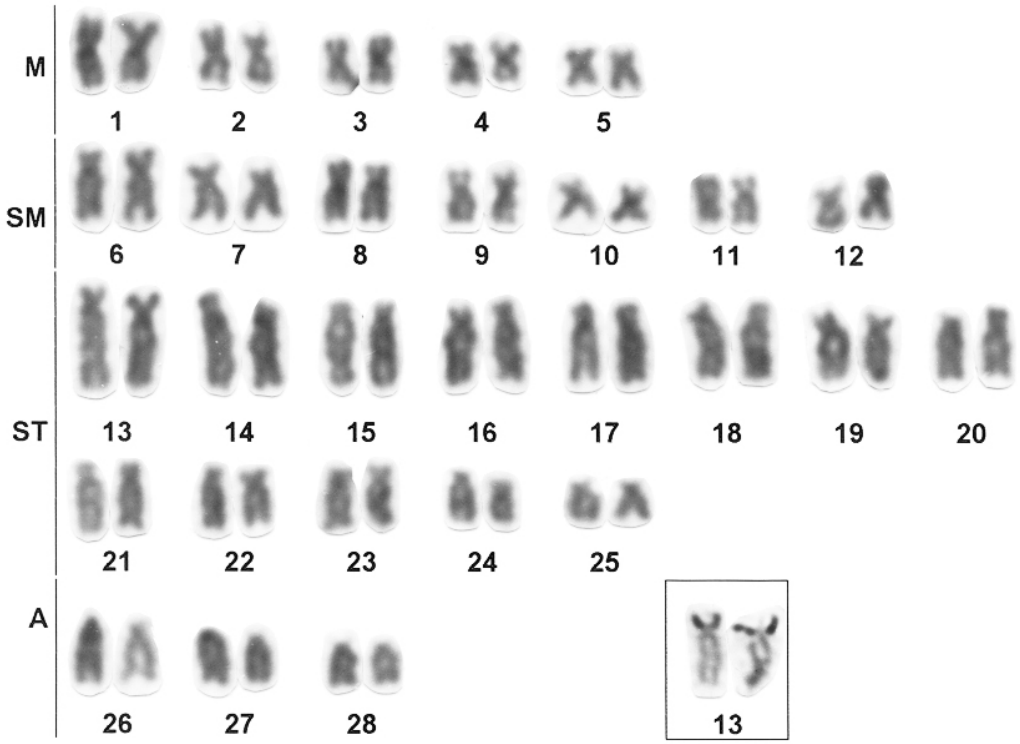

Figure 1. Giemsa stained karyotype of *Genidens barbus* with 2n=56 chromosomes. In the inset, silver stained chromosomes showing the terminal Ag-NORs (black dots).

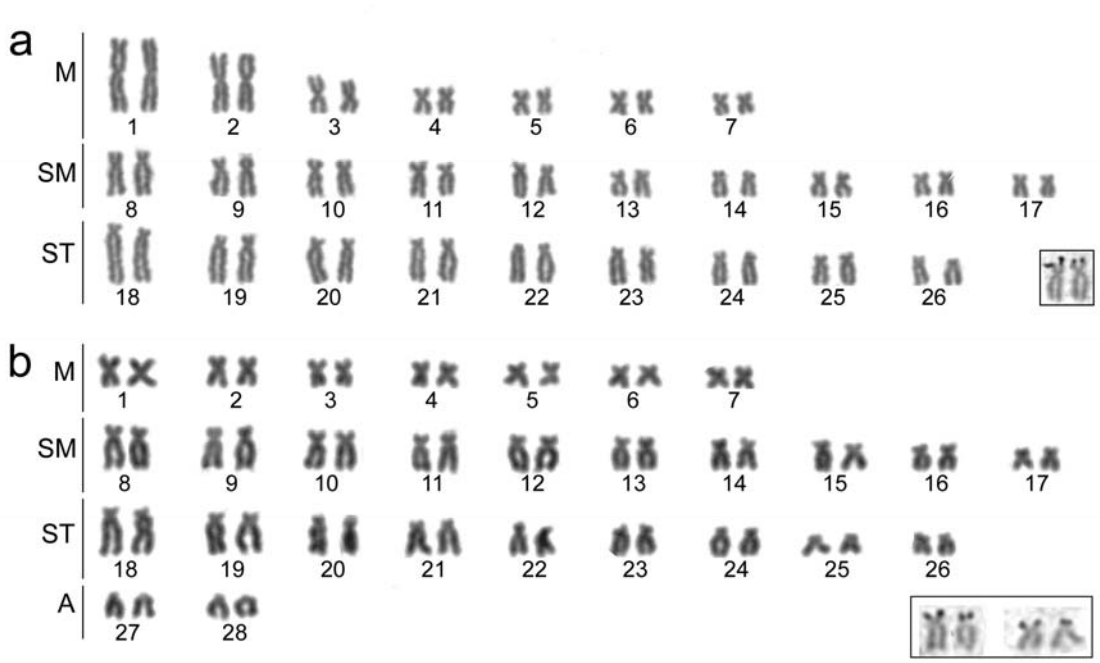

Figure 2. Giemsa stained karyotypes of (a) *Cathorops* aff. *mapale* with 2n=52 chromosomes and (b) *Sciades herzbergii* with 2n=56 chromosomes (b). In the inset, silver stained chromosomes showing terminal Ag-NORs (black dots).

CAPÍTULO IV. Mugiliformes

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Classical and molecular cytogenetic characterization of *Agonostomus monticola*, a primitive species of Mugilidae (Mugiliformes)

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Abstract This study reports the first description of the karyotype of *Agonostomus monticola*, a species belonging to a genus which is considered to be the most primitive among living mugilid fish. Specimens from Panama and Venezuela were cytogenetically analysed by conventional chromosome banding (Ag and base-specific-fluorochrome staining, C-banding) and by fluorescent in situ hybridization (FISH). *Agonostomus monticola* showed a chromosome complement of 2n = 48, composed of 23 acrocentric and one subtelocentric chromosome pairs and a pericentromeric distribution of the C-positive heterochromatin in all chromosomes. Major ribosomal genes were found to be located on the short arms of the subtelocentric chromosome pair number 24 and minor ribosomal genes in a paracentromeric position of a single medium-sized chromosome pair. All these observed cytogenetic features are similar to those previously described in four representatives of two genera, *Liza* and *Chelon*, which are considered to be among the most advanced in the family. Thus, this karyotypic form might represent the plesiomorphic condition for the mullets. This hypothesis regarding the plesiomorphic condition, if confirmed, would shed new light on the previously inferred cytotaxonomic relationships for the studied species of Mugilidae, because the karyotype with 48 acrocentric chromosomes, which has been so far regarded as primitive for the family, would have to be considered as derived.

Keywords Constitutive heterochromatin · FISH · Karyotype · 18S and 5S rDNA · NORs

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Introduction

The family Mugilidae contains approximately 70 fish species (Nelson 2006), distributed in all the tropical and temperate coastal marine and brackish waters of the world. Both the assignment of the family (to a distinct order or to a suborder of Perciformes) and the number of valid genera and species within it have been widely revised over the years (see Sola et al. 2007, for a review). Mugilidae have also been the subject of many cytogenetic investigations. Earlier studies indicated that the family is mainly characterized by the conservative 48 all-uniarmed karyotype originally proposed by Ohno (1974) as the primitive teleostean karyotype, which is typically shared by many marine euteleostean families. More recent studies (reviewed in Sola et al. 2007) have shown that the application of suitable chromosome markers revealed finer chromosome rearrangements, suggesting that the chromosomal evolution in Mugilidae is more complex than was originally assumed.

To summarize, the available information, regarding approximately 25% of the mugilid species, indicates three main cytotypes: cytotype A, composed of 48 exclusively acrocentric chromosomes (NF = 48), which is the most common (ten species belonging to four genera); cytotype B, composed of 46 acrocentric plus two subtelocentric chromosomes (NF = 48), displayed by five species

belonging to three genera; and cytotype(s) C, mainly, or even exclusively, composed of biarmed chromosomes. The latter cytotypes have been found in specimens of *Mugil curema* from Louisiana and Brasil (2n = 28, NF = 48; LeGrande and Fitzsimons 1976; Nirchio et al. 2005) and from Venezuela (2n = 24, NF = 48; Nirchio and Cequea 1998; Rossi et al. 2005; Nirchio et al. 2007, among others). They were originally interpreted (LeGrande and Fitzsimons 1976) to be due to extensive Robertsonian fusions which occurred in an ancestral group with an all-acrocentric chromosome complement (cytotype A) similar to that of *M. cephalus*. The cytotype A has been regarded to date as plesiomorphic in any cytotaxonomic consideration in the family (Sola et al. 2007). In fact, it is shared by all the *Mugil* species investigated (with the exception of *M. curema*) and this genus is phylogenetically more basal (Thomson 1997) compared to the other genera (*Rhinomugil*, *Valamugil*, *Liza*, *Chelon*, *Oedalechilus*) so far karyologically studied.

According to Thomson (1997), all the morphological features suggest that *Agonostomus* is the most primitive among living Mugilidae. Moreover, the mountain mullet, *Agonostomus monticola* (Bancroft 1834), is the only one that ascends far inland and spends all its adult life in freshwater, although it possibly spawns catadromously (Phillip 1993). Distributed from North Carolina, in North America, to Colombia and Venezuela, including the West Indies, in South America, *A. monticola* is the only representative of the genus in the area. The other two species, *A. catalai* and *A. telfairii*, in fact, are found in the South West Indian Ocean. In spite of its peculiar systematic position and bio-ecological traits, no phylogenetic studies using more modern approaches, already applied in other Mugilidae (Caldara et al. 1996; Papasotiropoulos et al. 2002; Rossi et al. 2004; Turan et al. 2005; Sola et al. 2007), have been carried out in this genus.

In this study, specimens of *A. monticola* from Panama and Venezuela were cytogenetically characterized by classical (Ag and base-specific-fluorochrome staining, C-banding) and molecular techniques (fluorescent in situ hybridisation—FISH), in order to verify whether this primitive species shows the chromosome constitution which has, to date, been considered plesiomorphic.

Materials and methods

A total of 27 specimens of *A. monticola* were caught with seine nets in the Changuinola River, Bocas del Toro, Panama (six specimens) and in La Trilla River, Aragua State, Venezuela (21 specimens). Voucher specimens were deposited at the Ichthyology Collection of the Escuela de Ciencias Aplicadas del Mar, Universidad de Oriente.

Chromosome preparations were obtained from cephalic kidney cells using conventional air-drying techniques. Silver-stained nucleolus organizer regions (Ag-NORs) were obtained as described by Howell and Black (1980). Fluorescence staining with the GC-specific stain chromomycin A_3 (CMA_3) and the AT-specific stain 4′,6-diamidino-2-phenylindole (DAPI) was carried out according to Sola et al. (1992). C-banding was performed following the method of Sumner (1972).

The 5S and 18S ribosomal RNA gene (rDNA) loci were identified by FISH according to the method of Pinkel et al. (1986). A 1.8 kb sequence of the 18S rDNA of *Oreochromis niloticus* (Nile tilapia), cloned in pGEM-T plasmid, was used as a probe to localize the major rDNA sites. Repeats used as probes for mapping the minor 5S rDNA were generated by Polymerase Chain Reaction (PCR) with the primers 5SA (5′TAC GCC CGA TCT CGT CCG ATC3′) and 5SB (5′CAG GCT GGT ATG GCC GTA AGC3′) according to Martins and Galetti (1999), employing DNA extracted from muscle of one mountain mullet (Sambrook and Russell 2001).

The probes were labelled by nick translation with biotin-14-dATP, following the manufacturer's instructions (Bionick™ Labelling System-Gibco.BRL). Signals were detected after amplification by a three-round application of Avidin-FITC/biotinilated Anti-avidin (Roche). Chromosomes were counter-stained with propidium iodide (50 μg/ml) diluted in an antifade.

Mitotic chromosomes were photographed using a Motic B400 microscope equipped with a Moticam 5000C digital camera and the images were digitally processed with Adobe Photoshop CS3 (Windows). FISH metaphases were examined with a Zeiss Axiophot photomicroscope, using Kodak Gold Ultra 400 ASA film.

Results

In all of 27 mountain mullet individuals examined, the karyotype (Fig. 1) was found to be made of 48 chromosomes, 46 acrocentrics and 2 subtelocentrics, uniformly decreasing in size. Only the subtelocentric chromosome pair 24, the smallest in the complement, can be identified unequivocally, due to its size and the presence of short arms with prominent terminal achromatic regions, often heteromorphic in size (Fig. 1). No differences in the karyotype were observed among specimens of different sex or from the two geographically distant sampling localities.

As expected from their Giemsa-staining features, Ag-NORs were found to be terminally located on the short arms of chromosomes 24 after silver staining (Fig. 2a). C-banding revealed heterochromatic regions at the centromere of all chromosomes (Fig. 2b).

Fig. 1 Giemsa-stained karyotype of *Agonostomus monticola*. Scale bar = 10 μm

After CMA_3-staining (Fig. 2c), fluorescent signals corresponding both in number and morphology to Ag-NORs were detected on the short arms of chromosome pair number 24. Apart from NORs, CMA_3 produced a uniform staining pattern along chromosomal arms. The AT-specific DAPI also produced a uniform staining pattern of all chromosomes, with a fainter staining of the NOR-bearing short arms of chromosome pair 24 (data not shown).

FISH with 18S rDNA probe produced bright signals, corresponding to the ones obtained after Ag- and CMA_3-staining, on the short arms of chromosome pair number 24 (Fig. 3a), which indicates that the species possesses no additional NOR-sites. FISH with 5S rDNA probe produced paracentromeric signals in a medium-sized acrocentric chromosome pair (Fig. 3b).

Discussion

By adding the chromosome complement of *A. monticola* reported in this study to the Mugilidae database, the genera of the family so far cytogenetically analysed rises to seven, covering a total of at least 17 species. The number could be higher, given that karyological data have probably disclosed the existence of a *M. curema* species complex (Nirchio et al. 2005). The obtained data confirms that the 48 all-uniarmed karyotype is the most common in the family, as it is shared by all the investigated species, with the exception of *M. curema*.

As far as the *A. monticola* cytogenetic features are concerned, the C-positive heterochromatin distribution did not reveal any chromosome-specific heterochromatic block nor differentially AT or GC-enriched DNA, with the exception of the CMA_3-positive short arms of chromosome pair number 24, where NORs are located. The GC-richness of NORs is quite common in fish, although evidence of GC-poor NORs (Souza et al. 2001, Rossi and Gornung 2005), sometimes in combination with GC-rich regions other than NORs (Rab et al. 2002; Gromicho et al. 2005; Kavalco et al. 2005) have also been reported. The major and minor rDNA clusters were found located on distinct chromosome pairs, as in the other Mugilidae so far analysed (Sola et al. 2007). A similar situation is also found in fish, in general, with very few exceptions (Moran et al. 1996; Fujiwara et al. 1998, 2007; Almeida-Toledo et al. 2002). Although the major rDNA location is unambiguous in *A. monticola* and in most of the Mugilidae analysed (Sola et al. 2007), the paracentromeric and interstitial location of minor rDNA clusters on a medium-sized chromosome pair does not permit its unequivocal classification. However, pursuing a parsimonious criterion, the 5S rDNA bearing chromosomes pair observed in *A. monticola* might be homeologous to the chromosome pair of similar size, classified as number 8, which shows a similar minor rDNA location observed in the other mugilid species with this type of chromosome complement (see Sola et al. 2007, for references).

Considering the available cytogenetic data, *A. monticola* shows an apparent similarity in the karyotype constitution, C-positive heterochromatin distribution, major and minor rDNA loci numbers and locations, to *Chelon labrosus* and three *Liza* species, *Liza ramada*, *L. aurata*, *L. saliens*, (see Sola et al. 2007, for references), that is, the karyotype of *A. monticola* can be assigned to cytotype B.

The latter cytotype is therefore shared by a genus, *Agonostomus*, which is the most basal in the family, and by two other genera which are, on the other hand, considered to be the most advanced in the family (Thomson 1997).

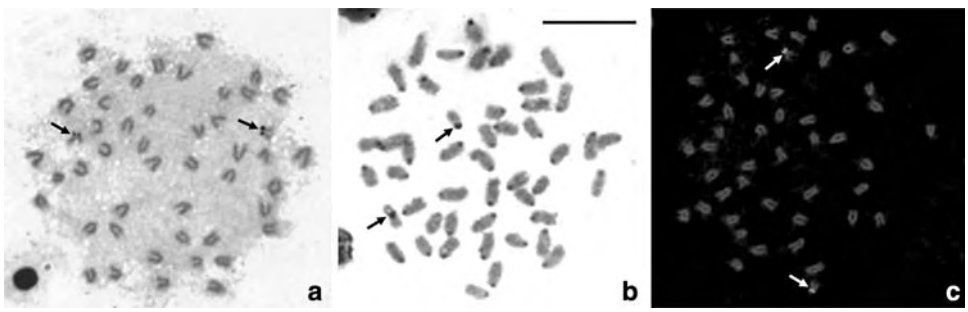

Fig. 2 Metaphase plates of *Agonostomus monticola* after (**a**) Ag-staining, (**b**) C-banding and (**c**) CMA_3-staining. Arrows indicate NOR-bearing chromosomes. Scale bar = 10 μm

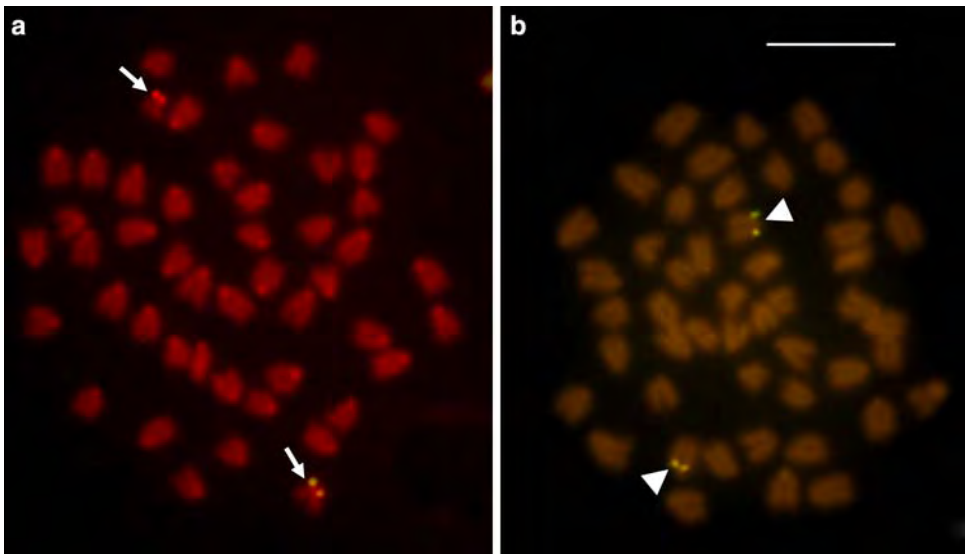

Fig. 3 Metaphase plates of *Agonostomus monticola* after FISH with (**a**) 18S rDNA, (**b**) 5S rDNA. Arrows indicate NOR-bearing chromosomes, arrowheads 5S rDNA clusters. Scale bar = 10 μm

This indicates that the cytotype B might be regarded as the plesiomorphic condition for the karyotype in the family. Consequently, the cytotype A, shared by most of the species of a genus, *Mugil*, in an intermediate systematic position, and which has been so far considered to be the closest to the ancestor' karyotype, should now to be re-considered as derived. This factor should be taken into consideration in any cytotaxonomic reconstruction in the family.

The results obtained in *A. monticola* are therefore of considerable interest and further karyological studies should be carried out on the remaining undescribed species of Mugilidae, in order to provide a more general picture of karyoevolutive trends in the family. This could be useful to gain a more general understanding of chromosomal evolutionary processes in different taxa of fish with the 48-uniarmed-chromosome karyotype.

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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 differentiation 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 / Mullets / NORs /
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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 congenéricas 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.*

ANALISE COMPARATIVA CITOGENETICA 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°57'46.6"N, 064°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 alloyzyme (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

M. rubrioculus	*Mugil curema*
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)

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: $3H_2O$, pH 8.7; buffer tray 21,9g/l Tris, 6,18g/l boric acid, 1,17g/l EDTA, pH 8,70.

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 and 2xSSC and 4xSSC at room temperature.

Detection of hybridized probes was performed with Avidin-FITC conjugate (Sigma) followed by two rounds of signal-amplification. After each amplification step, the slides were washed in the blocking buffer (1.26% $NaHCO_3$, 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).

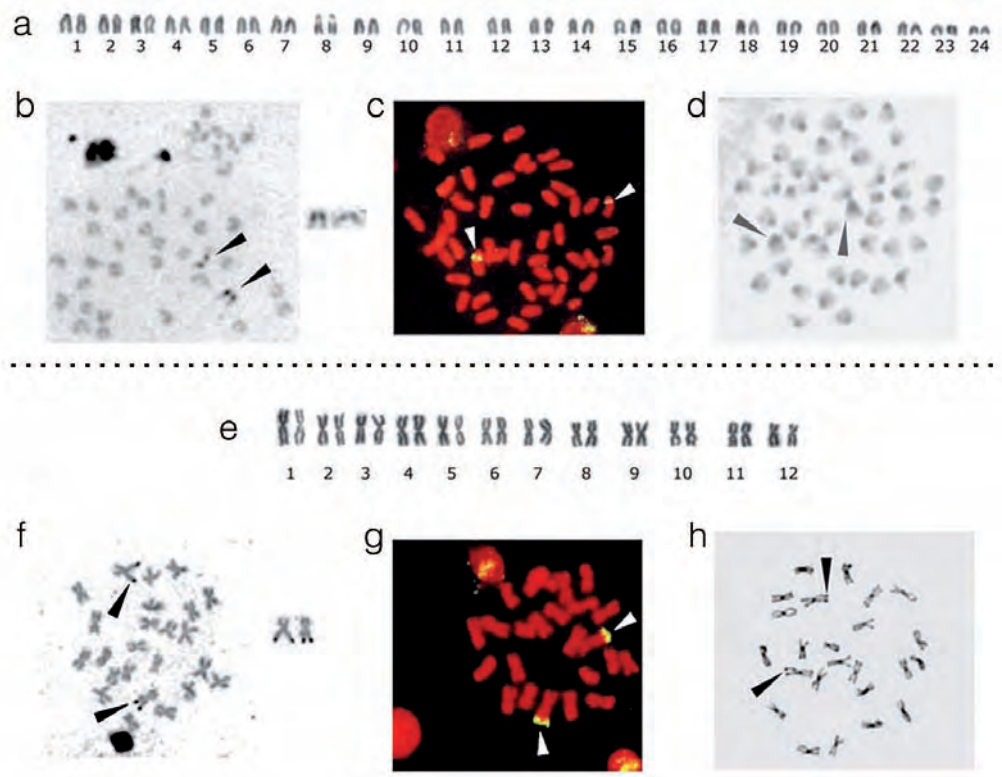

Figure 1. *Mugil rubrioculus* karyotype a: Giemsa-stained and metaphases, b: Ag-stained, c: after FISH with 18S rDNA, and d: C-banded. *Mugil curema* karyotype e: Giemsa-stained, and metaphases, f: Ag-stained, g: after FISH with 18S rDNA, and h: C-banded. Arrows indicate NOR-bearing chromosomes.

Allozyme electrophoresis

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. 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-

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

Locus	Allele	*M. rubrioculus*	*M. curema*
	(n)	34	34
	98	0	0.162
ACP*	100	0.309	0.838
	102	0.529	0
	104	0.162	0
	(n)	30	35
*ADH-1**	100	0.900	0.829
	102	0.100	0.171
	(n)	22	28
*ADH-2**	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
	100	0.044	0.743
*DIA**	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
	98	0.397	0.103
*GDH-2**	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

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 (*Ho*) 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 privates 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; Papasotiropoulos *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 (Papasotiropoulos *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 between *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 nucleotide 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*.

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A new species of mullet (Teleostei: Mugilidae) from Venezuela, with a discussion on the taxonomy of *Mugil gaimardianus*

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The original description of *Mugil gaimardianus* has created various taxonomic problems in the past since the description is ambiguous and the type specimen is apparently lost. The name *M. gaimardianus* could not be reliably applied to any known species and was suppressed by the International Commission on Zoological Nomenclature (ICZN) (Bulletin of Zoological Nomenclature, 51: 286–287, 1994). Nevertheless, karyological evidence has shown that there is a species of mullet in Venezuelan coastal waters that does not conform to the description of any other mullet from the Western Central Atlantic and has the feature of a red eye that was often used by earlier authors to define nominal *M. gaimardianus*. The purpose of this study was to make a morphological description of these unusual specimens, provide a morphological diagnosis from other species of *Mugil* present in the Caribbean and Western Central Atlantic and establish a valid name for the species.

Key words: karyotype; morphology; mugilid; systematic; taxonomy.

INTRODUCTION

Mugilidae, or mullets, are found in marine inshore, estuarine and freshwater environments in tropical, subtropical and temperate regions of all continents (Thomson, 1997; Harrison, 2003; Nelson, 2006). Of the 280 nominal species of mugilids (Thomson, 1997), *c.* 17 genera and 72 species are recognized as valid (Nelson, 2006). However, ongoing research by I.J.H. and other authors (Senou, 1988; Ghasemzadeh, 1998; Ghasemzadeh & Ivantsoff, 2004) indicates that the taxonomy and nomenclature of mugilids are not stable. Therefore, the total number of valid taxa is likely to change. Differences in meristics and external morphology have frequently been the principal means of establishing taxonomic

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differentiation among Mugilidae. However, these characters appear to be somewhat labile within species, and interspecific differences are often not clearly different from the intraspecific ones, resulting in numerous taxonomic and associated nomenclatural problems.

Within *Mugil*, with 12 species recognized by Thomson (1997), there are at least two species, *Mugil cephalus* Linnaeus and *Mugil curema* Valenciennes, that have widespread distributions and show an unusual amount of variation between geographically distant populations. Crosetti *et al.*'s (1993, 1994) analyses of mitochondrial genes indicate that different populations of *M. cephalus* from around the world are well discriminated genetically, even when they show morphological uniformity. Rossi *et al.* (1998*a*, *b*) have also shown a high level of isozyme variation for specimens of *M. cephalus* collected from around the world, including fixed allelic differences between different populations.

Recent karyological studies have also shown some variation in the karyotype of *M. curema*, with $2n = 24$ (22 metacentric plus two submetacentric chromosomes) in Venezuelan specimens and $2n = 28$ (20 metacentric plus four subtelocentric and four acrocentric chromosomes) in specimens from Brazil and Louisiana, U.S.A. (LeGrande & Fitzsimons, 1976; Nirchio & Cequea, 1998; Nirchio *et al.*, 2001, 2003, 2005). Nirchio *et al.* (2003) noticed that some specimens of *Mugil* collected from Panama had a $2n = 48$ acrocentric karyotype, which is totally unlike any karyotypes recorded for *M. curema*. Cervigón (1993) and some earlier authors (Rivas, 1980; Menezes, 1983) had used the name *Mugil gaimardianus* Desmarest for a species from Western Central Atlantic mullet that is similar to *M. curema* but can be distinguished from it by having a reddish colouration to the iris, longer pectoral fins extending beyond the origin of the first dorsal fin and a wider skull at the level of the sphenotics. Nirchio *et al.* (2003) stated that their $2n = 48$ Panamanian specimens matched the general description of *M. gaimardianus* given in the keys of Cervigón (1993), therefore, they applied that name to their specimens. A $2n = 48$ form of *Mugil* has subsequently been identified also from coastal waters of Margarita Island, Venezuela (M. Nirchio, C. Oliveira, L. Sola, A. R. Rossi, I. A. Ferreira, J. E. Pérez, J. I. Gaviria & I. Harrison, in prep.). The large karyotype differences between the specimens with the $2n = 48$ karyotype and those identified as *M. curema* with the $2n = 24$–28 karyotype confirm that these are different species. However, the name *M. gaimardianus* is not available, for the reasons discussed below.

The nominal species *M. gaimardianus* was first collected from Cuba and the original description by Desmarest appearing in Bory de Saint-Vincent (1831: 129) is:

> ''Cette espèce de Cuba, certainement nouvelle, a été figurée à la demande et sous la direction de M. Desmarest, qui n'en a pas donné la description, la publication de ses Décades ichthyologiques ayant été interrompue''.

Neither this description nor the accompanying figure (Plate CIX, reproduced in this study in Fig. 1) allows easy diagnosis of *M. gaimardianus* from other species of *Mugil* known from the Western Central Atlantic (*i.e. M. cephalus*, *M. curema*, *Mugil curvidens* Valenciennes, *Mugil hospes* Jordan & Cuvier, *Mugil incilis* Hancock, *Mugil liza* Valenciennes and *Mugil trichodon* Poey). Also, no

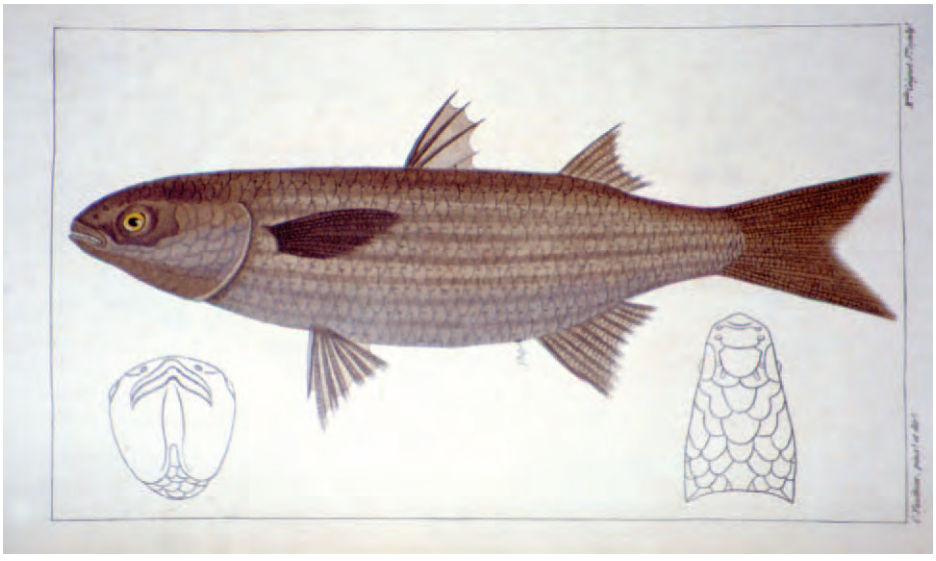

FIG. 1. Original illustration of *Mugil gaimardianus* from Bory de Saint-Vincent (1831: Plate CIX).

type specimens of *M. gaimardianus* are known to exist (Alvarez-Lajonchere *et al.*, 1992). For these reasons, many specimens of Western Central Atlantic mullets that subsequently have been identified as *M. gaimardianus* and preserved in museum collections appear to be misidentifications of one of the other mullet species found in the region, frequently *M. curema* (Harrison, 2002). Moreover, several descriptions of *M. gaimardianus*, published after Desmarest's original description, appear to be based on these misidentified specimens and are therefore misleading. Alvarez-Lajonchere *et al.* (1992) concluded that because of the lack of a reliable diagnosis or type material the name *M. gaimardianus* should be suppressed for purposes of priority and placed on the ICZN Official Index of Rejected Names. This opinion was accepted by the ICZN (1994).

The purpose of this study was to make a morphological description of the $2n = 48$ karyotype specimens described by Nirchio *et al.* (2003), provide a morphological diagnosis from other species of *Mugil* present in the Caribbean and Western Central Atlantic and establish a valid name for the species.

MATERIALS AND METHODS

MATERIALS

Specimens of the species previously referred to as *M. gaimardianus*, and renamed in this study, are listed under the new species description. Other comparative material is listed in the Appendix. Abbreviations for museum names follow Eschmeyer (2006) except for: ECAM (Genetics and Evolution Laboratory, Department of Aquaculture, Escuela de Ciencias Aplicadas del Mar Collection, Universidad de Oriente, Isla de Margarita, Venezuela); MHNN (Museum d'Histoire Naturelle, Neuchâtel, Switzerland).

METHODS

Morphometric measurements were made using dial or digital callipers. Measurements are: Ab, length of base of anal fin; Ad, depth of body at origin of anal fin; Aw, width of body at origin of anal fin; CP, length of caudal peduncle from posterior end of base of anal fin to point of caudal flexure; CPd, minimum depth of caudal peduncle; D2b, length of base of second dorsal fin; D1d, depth of body at origin of first dorsal fin; D1w, width of body at origin of first dorsal fin; D1/CP, horizontal distance from origin of first dorsal fin to caudal peduncle at point of caudal flexure; H, length of head from snout to posterior of opercle; LJL, length of lower jaw measured from dentary symphysis to corner of mouth; Mw, width between corners of mouth; Pb, dorsoventral depth of origin of pectoral fin; Pl, length of pectoral fin from axilla to tip of longest ray; Pw, width of body at origin of pectoral fin; L_S, standard body length; SN, length of snout from tip to anterior margin of eye; SN/A, horizontal distance from tip of snout to origin of anal fin; SN/D1, horizontal distance from tip of snout to origin of first dorsal fin; SN/D2, horizontal distance from tip of snout to origin of second dorsal fin; SN/V, horizontal distance from tip of snout to origin of pelvic fin; ULth, dorsoventral thickness of upper lip at junction of premaxillae; Vl, length of pelvic fin from base of spine to tip of longest ray. ALJ is the angle of the lower jaw, formed at the dentary symphysis by the opposing dentaries, it was calculated as $2 \times [\arcsin((Mw/2)/LJL)]$. All measurements of body parts are given as proportions of standard length (L_S) or head length (H).

Meristic counts are as follows: A, number of unbranched, spinous rays (in roman numerals) and soft branched rays (in arabic numerals) in second dorsal fin; D1, number of spinous rays (in roman numerals) in first dorsal fin; D2, number of segmented rays in second dorsal fin (the first ray is a small segmented spine and is indicated by lower case roman numerals; the remaining, branched rays are indicated by arabic numerals); P, number of rays in pectoral fin [the first (*i.e.* dorsal) ray is reduced to a very short spur that is closely apposed to the second ray and, although not a true spine, appears spinous and is therefore listed in italicized roman numerals, all remaining rays are segmented and listed in arabic numerals]; CPs, number of scales in circumpeduncular series entirely around caudal peduncle, just anterior to point of caudal flexure (the circumpeduncular series start at the scale row on the ventral surface of the caudal peduncle and is taken vertically up the scale rows on one flank, over the dorsum, and vertically down the opposite flank, zigzagging between adjacent, overlapping scale rows so that all rows are included in the count); D2s, number of scales in longitudinal series anterior to origin of second dorsal fin; LL, number of scales in longitudinal series on midline, counted from just behind opercle, above pectoral fin, to point of caudal flexure (*i.e.* not including scales on caudal fin); Ps, number of scales in longitudinal series anterior to tip of pectoral fin; TR, number of scales in transverse series, counted from origin of pelvic fin to origin of first dorsal fin; GR, number of gill rakers in the anterolateral row on the lower part (ceratobranchial) of the first gill arch; VC, vertebral count, given as abdominal vertebrae + caudal vertebrae, including last caudal vertebra which is a modified half centrum, fused to the hypurals of the caudal skeleton (abdominal vertebrae do not have the parapophyses united to form a complete haemal arch and spine). The arrangement of the supraneurals and dorsal proximal pterygiophores (S/DPP) is expressed in numerical sequence, with each number indicating the number of supraneurals or pterygiophores lying between successive vertebral neural spines. The first number represents the supraneurals between the cranial occipital condyles and the neural spine of the first vertebra. The unbracketed numbers of the sequence refer to the supraneurals anterior to the first proximal pterygiophore of the first dorsal fin; subsequently, bracketed numbers refer to the proximal pterygiophores associated with the dorsal fin or posterior to the fin. The last number represents the number of pterygiophores lying in the interneural space which accommodates the first proximal pterygiophore of the second dorsal fin.

Buccal dentition and pharyngobranchial morphology are described according to the terminology of Harrison & Howes (1991).

RESULTS

MUGIL RUBRIOCULUS, SP. NOV. [FIG. 2(a)]

Mugil gaimardianus (non Desmarest): Cervigón, 1993: 374, 377, fig. 172; Nirchio *et al.*, 2003: 114. *Mugil* sp.: Nirchio *et al.*, 2005: 100.

Other accounts of *M. gaimardianus* (e.g., Menezes, 1983) cannot be reliably attributed to *M. rubrioculus* n. sp. Several earlier accounts apparently refer to other species such as *M. curema* (Alvarez-Lajonchere, 1975:6; Alvarez-Lajonchere *et al.*, 1992: 272; Thomson, 1997:489).

In the following list, specimens are noted as 'wide jaw' or 'narrow jaw' morphotypes. This refers to the angle of the lower jaw. See Discussion for further details.

Holotype

AMNH 238167: one specimen; 250 mm L_S (formerly ECAM 000241), wide jaw; Venezuela, Margarita Island, Macanao peninsula at Boca del Rio, 10°57′ 48″ N 64°10′42″ W; local fisherman; 2 March 2005.

Paratypes

AMNH 238168: two specimens; 113·7 mm L_S (formerly ECAM-000039), wide jaw, karyotype $2n = 48$; 142·5 mm L_S (adult male, formerly ECAM-000038), wide jaw, karyotype $2n = 48$; Venezuela, Margarita Island, Macanao peninsula, La Restinga, 10°58′59″ N 64°6′00″ W; M. Nirchio; 4 April 2004. AMNH 238169: three specimens; 226 mm L_S (formerly ECAM-0000173), wide jaw; 220 mm L_S (formerly ECAM-0000174), narrow jaw; 214 mm L_S (formerly ECAM-0000175), narrow jaw; Venezuela, Margarita Island, Macanao peninsula, La Restinga, 10°58′59″ N 64°6′00″; M. Nirchio; 11 September 2004.

Other material (M. rubrioculus sp. nov*)*

ANSP 152244: one specimen; 221 mm L_S, wide jaw; U.S.A., Florida, Dubois Park Cove, Jupiter Inlet (depth 2 feet); D. Nickerson & T. Fucigna; 15 September 1983. ECAM 000466: three specimens; 252 mm L_S, wide jaw; 258 mm L_S, wide jaw; 276 mm L_S, narrow jaw; Antigua, Gallon Beach, seine; O. Bailey; 3 April 1997. AMNH 238173: one specimen; 267 mm L_S, narrow jaw; Belize, coastal waters around Belize City; K. E. Carpenter, M. DeGravelle & T. Orell; 6–9 July 1999.

Mugil aff. *rubrioculus*

AMNH 238174: one specimen; 159 mm L_S, narrow jaw; Panama, Golfo de San Miguel; J. Van Tassell & R. Robertson; December 2002. AMNH 238170: one specimen; 145·1 mm L_S (formerly ECAM 000243), narrow jaw, karyotype $2n = 48$; Panama, La Chorrera district, Puerto Caimito, 8°49′53″ N 79°35′32″ W; November 1999. ECAM 000001: two specimens; 123·6 mm L_S, wide jaw, karyotype $2n = 48$; 144·7 mm L_S, narrow jaw, karyotype $2n = 48$; Panama, La Chorrera district, Puerto Caimito, 8°49′53″ N 79°35′32″ W; 11 November 1999.

Diagnosis

Mugil rubrioculus n. sp. [Fig. 2(a)] has the following features that are typical of *Mugil*. Maxilla straight with posterior tip not curved down below corner of mouth; serrate anterior edge of lachrymal straight (rather than with concave kink as in *Liza*) and posteroventral corner of lachrymal relatively narrow (rather than broad and squarish as in *Liza*); translucent adipose tissue ('adipose eyelid') covering most of eye except for small area over the pupil; long, modified, pectoral axilla scale just dorsal to the origin of the pectoral fin; pharyngobranchial organ with a single valve appearing as a prominent flap on the wall of the pharyngobranchial sulcus.

When compared with other species of *Mugil* from the Western Central Atlantic, *M. rubrioculus* n. sp. can be distinguished from *M. cephalus*, *M. liza*, *M. curvidens* and *M. trichodon* by the presence of three spines and nine soft rays in the anal fin of adults (or two spines and 10 soft rays in juveniles). It is distinguished from *M. incilis* by the presence of fewer than 43 scales in the longitudinal series. *Mugil rubrioculus* n. sp. is most similar to *M. hospes* and *M. curema*. It can be distinguished from the former by a deeper body (Ad = 23·8–25·5% L_S *v.* 21–23% in *M. hospes*), the pectoral fin with more soft fin rays (15–16 in *M. rubrioculus* n. sp. *v.* 13–14 in *M. hospes*) and the pectoral fin is usually slightly shorter (Pl = 17·3–18·9% L_S, 72·3–82·9% Hl in *M. rubrioculus* n. sp. and Ps = 9–11 *v.* Pl = 21–24% L_S, 80–96% Hl and Ps = 11–13 in *M. hospes*). Live *M. rubrioculus* n. sp. can be easily distinguished from *M. curema* because it has a distinctly reddish-orange iris (absent or indistinct in *M. curema*) and only a small goldish spot on the opercle (larger opercular spot in *M. curema*) [Figs 2

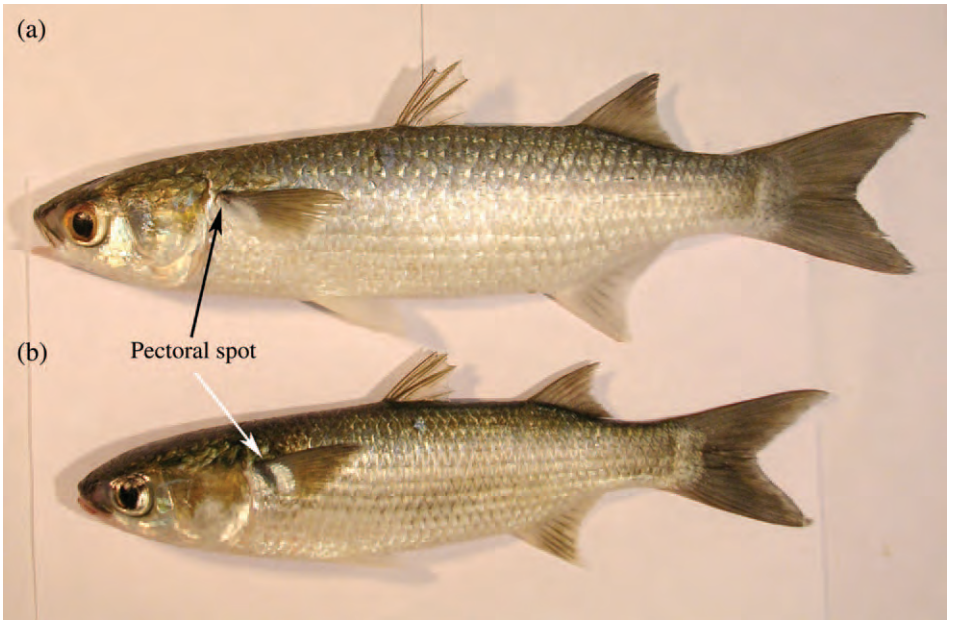

FIG. 2. Fresh specimens of (a) *Mugil rubrioculus* n. sp. holotype (AMNH 238167) from Boca del Rio, Macanao peninsula, Margarita Island, Venezuela, L_S = 250 mm and (b) *Mugil curema* (ECAM uncatalogued) from same locality for comparison.

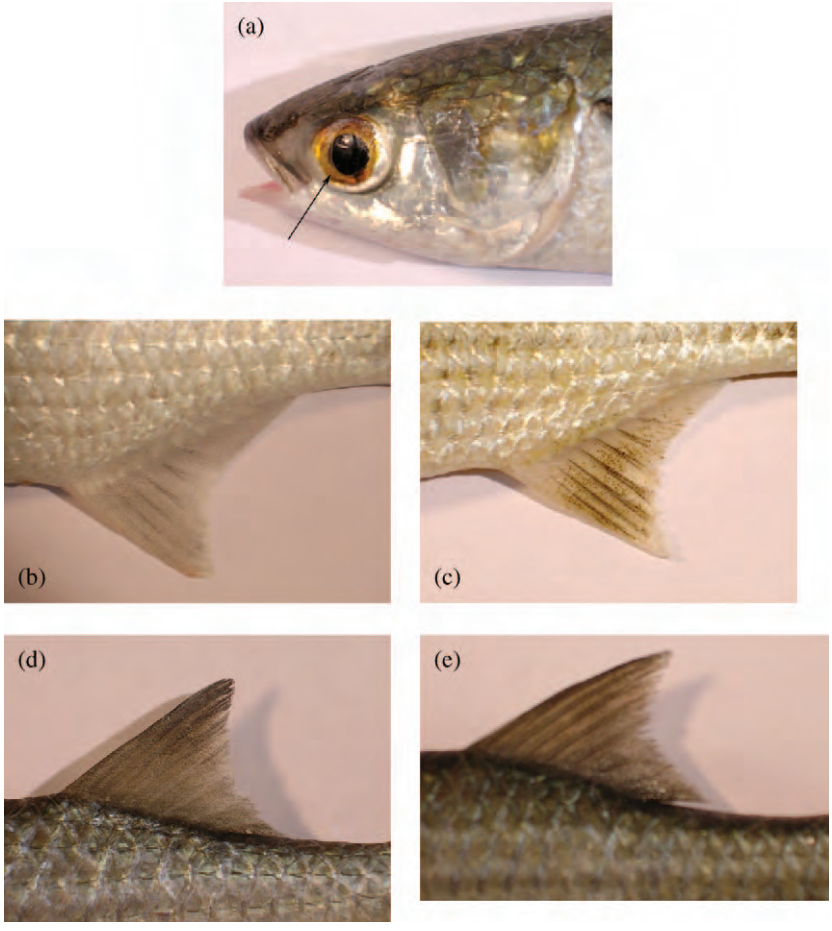

FIG. 3. *Mugil rubrioculus* n. sp. holotype (AMNH 238167; 250 mm L_S), showing (a) red colouration of iris (arrowed) and small goldish opercular spot; (b) anal fin pigmentation; (d) second dorsal fin pigmentation. *Mugil curema* (ECAM uncatalogued) for comparison, showing (c) anal fin pigmentation; (e) second dorsal fin pigmentation.

and 3(a)]. *Mugil rubrioculus* n. sp. also has paler pigmentation on the flanks, without longitudinal bands (flanks darker with *c.* six bands in *M. curema*) (Fig. 2). The pectoral fin has only a small spot at the dorsal part of the base (this spot is deeper in *M. curema*, extending vertically down most of the base of the fin, and the fin tends to be pigmented darker on the fin rays and fin membrane) (Fig. 2); these pectoral differences may also be seen in preserved specimens. The anal fin is pale (dark in *M. curema*) [Fig. 3(b), (c)]; this difference is also evident in preserved specimens. The anterodorsal tip of second dorsal fin is slightly darker than the other parts of the fin [Fig. 3(d)] (the second dorsal fin is usually more uniformly dark in *M. curema*; a slightly darker anterodistal tip to the fin may also be seen in some *M. curema*, but this feature is usually more distinct in *M. rubrioculus* n. sp.). The caudal fin is usually less heavily pigmented than in *M. curema* (Fig. 2). The scales on the flanks usually

lack any minute, overlying, secondary scales, giving a smoother texture to the flanks than in *M. curema* (which usually have the secondary scales). The teeth on the upper and lower lips are usually very fine and short, although this feature may also be seen in some specimens of *M. curema*. The karyotypes of *M. rubrioculus* n. sp. is $2n = 48$ acrocentric chromosomes [Fundamental Number of arms (NF) = 48] compared with $2n = 22$ metacentric + two submetacentric (NF = 48) or $2n = 20$ metacentric + four subtelocentric + four acrocentric (NF = 52) in *M. curema*. Some additional population differences are noted in the Discussion given below.

Etymology

From the Latin, *ruber* (red) and *oculus* (eye) referring to the distinct eye colour. A noun in apposition was used.

Description

General body morphology. Maximum observed size is 258 mm L_S. Morphometric measurements are shown in Table I (Fig. 2). Body moderately deep. Body depth at the origin of the first dorsal fin is 24·7–26·7% L_S and at the origin of the anal fin is 23·8–25·5% L_S. Maxilla straight with posterior tip not curved down below corner of mouth. Serrate anterior edge of lachrymal straight and posteroventral corner of lachrymal relatively narrow. Translucent adipose tissue covering most of eye except for small area over the pupil. Head depth greater than the head width at level of the posterior of operculum. Origin of the first dorsal fin is usually midway between the tip of snout and base of the caudal fin or slightly closer to snout. Origin of second dorsal fin is usually level with anterior third to half of anal fin (AMNH 238168, L_S = 142·5 mm, has origin of second dorsal fin slightly anterior to anal fin). The length of pectoral fin is less than that of head minus snout and not reaching origin of first dorsal fin; pectoral fin 17·3–18·9% L_S, 72·3–82·9% H.

Fins. D1: IV; D2: one small spine (segmented at tip) and seven to nine soft rays (usually eight soft rays); A: III, 9 in adults; P: *I*, 15–16 (usually 16).

Squamation. LL: 35–38 (usually 36 or 37); TR: 11·5–13·5 (usually 12 or 12·5); Ps: nine to 11; D2s: 22–24 (usually 23 or 24); CPs: 18 (rarely 19). Large predorsal scales extending to level of posterior nostril and *c.* four rows of smaller scales extending to level of anterior nostril are seen. Scales on flanks are weakly ctenoid, usually with single longitudinal groove. A few, overlying, minute, secondary scales covering dorsal scales anterior to first dorsal fin, on dorsal part of caudal peduncle, and sometimes (AMNH 238169, L_S = 220 mm) on ventral part of caudal peduncle; flanks and ventral part of abdomen are usually without minute secondary scales and thus have smooth texture to the touch. Long, modified, pectoral axilla scale present just dorsal to the origin of the pectoral fin; long pelvic axilla scale lateral to each pelvic fin; moderate obbasal scale on each side of base of first dorsal fin. Second dorsal fin and anal fin profusely covered with scales.

Lips and jaws. Lips relatively thin (27·9–43·1% SN); mouth in ventral view variable, 84–112° (104° in holotype) at dentary symphysis. Upper lip of juvenile

TABLE I. Comparative morphometrics and meristics for *Mugil rubrioculus* n. sp., *Mugil* aff. *rubrioculus* and *Mugil curema*. *n*, number of specimens examined. See text for further discussion and explanation of other abbreviations

	Mugil rubrioculus n. sp.				*Mugil* aff. *rubrioculus*			*Mugil curema*			
	Wide-jaw form							Wide-jaw form			
	Holotype	Other wide-jaw specimens	Narrow-jaw form	Total specimens	Wide-jaw form	Narrow-jaw form	Total specimens	Lectotype	Other wide-jaw specimens	Narrow-jaw form	Total specimens*
n	1	6	4	11	1	3	4	1	3	25	39
Standard length (L_S)/mm	250	113·7–258	214–276	113·7–276	123·6	144·7–159·0	123·6–159·0	240	194·9–345	88·0–205	30·5–345
Morphometrics As % L_S											
H	24·2	23·6–25·3	22·7–24·4	22·7–25·3	26·2	25·1–26·3	25·1–26·3	22·8	20·5–26·1	22·3–27·9	20·5–27·9
SN/A	70·5	66·7–70·5	68·2–70·9	66·7–70·9		67·3–69·9	67·3–69·9	69·4	69·4–70·8	63·1–72·5	63·1–72·5
SN/D1	49·4	47·8–50·6	47·9–50·9	47·8–50·9		49·1–50·2	49·1–50·2	48·5	49·3	48·6–53·4	48·5–53·4
D1/CP	52·2	48·8–51·5	48·3–51·5	48·3–52·2		50·2–51·7	50·2–51·7	51·8	49·8–52·2	47·1–53·0	47·1–53·0
SN/D2	75·4	72·8–74·5	73·3–75·3	72·8–75·4		73·8–74·5	73·8–74·5	74	73·0–75·9	71·4–78·4	71·4–78·4
SN/V	36·4	36·0–37·9	34·3–37·0	34·3–37·9		35·8–37·4	35·8–37·4	35		34·9–40·2	34·9–40·2
CP	15·6	16·2–17·6	15·3–17·4	15·3–17·6	18·5	15·3–17·5	15·3–18·5	19·1	17·0–17·4	14·8–18·7	14·8–19·1
D1d	25·3	24·7–26·7	25·7–26·1	24·7–26·7	26·8	24·8–27·0	24·8–27·0	25	20·0–25·4	23·5–29·2	20·0–29·2
Ad	23·9	23·8–25·5	24·0–24·5	23·8–25·5	24·1	23·7–24·7	23·7–24·7	23·5	22·7–23·1	21·4–28·0	21·4–28·0
Pw	15·8	15·5–17·7	16·3–16·9	15·5–17·7		15·9–16·1	15·9–16·1	15	15·3–16·5	15·3–18·4	15–18·4
Pl	18·3	17·9–18·9	17·3–18·9	17·3–18·9	20·6	19·8–22·1	19·8–22·1	16·1	18·8–19·2	18·8–21·5	16·1–21·5
D1w	11·9	12·5–13·8	13·3–14·8	11·9–14·8		11·4–11·9	11·4–11·9	13	14·3–18·2	9·9–16·6	9·9–18·2
Aw	9·6	9·2–10·8	11–12·1	9·2–12·1		9·0–9·1	9·0–9·1	11·3	11·4	7·2–14·1	7·2–14·1
D2b	11·2	10·7–13·2	10·4–11·2	10·4–13·2		11·6–12·1	11·6–12·1	10·3	10·4–10·9	9·8–12·9	9·8–12·9
Ab	15·5	14·3–15·5	13·2–14·4	13·2–15·5		14·5–15·4	14·5–15·4	14·3	13·2–13·7	13·2–16·4	13·2–16·4
As % H											
SN	20·7	18–23·3	17–21·2	17·0–23·3	19	20·3–21·3	19·0–21·3	20·5	18·0–21·3	17·0–23·6	17–23·6
LJL	22·8	21·2–24·2	23·7–28·7	21·2–28·7	21·1	24·2–25·2	24·2–25·2	22	22·8–26·8	20·9–28·6	20·9–28·6

TABLE I. Continued

	Mugil rubrioculus n. sp.				*Mugil* aff. *rubrioculus*			*Mugil curema*			
	Wide-jaw form							Wide-jaw form			
	Holotype	Other wide-jaw specimens	Narrow-jaw form	Total specimens	Wide-jaw form	Narrow-jaw form	Total specimens	Lectotype	Other wide-jaw specimens	Narrow-jaw form	Total specimens*
n	1	6	4	11	1	3	4	1	3	25	39
Mw	33·3	31·3–37·5	32·2–35·5	31·3–37·5	30·1	29·4–32·0	29·4–32·0	32	26·3–38·8	27·7–36·2	26·3–38·8
Pl	75·7	73·9–79·9	72·3–82·9	72·3–82·9	78·7	78·1–84·0	78·1–84·0	70·9	73·8–79·3	69·1–83·7	69·1–83·7
Pb	25·7	24·6–29·5	26·0–27·4	24·6–29·5		22·9–28·1	22·9–28·1	24	28·4	20·0–27·9	20–28·4
Vl	61	60·9–69·2	58·0–65·8	58·0–69·2		61·3–67·7	61·3–67·7	59·9	57·3	58·0–68·1	57·3–68·1
As % SN											
ULth	38·6	27·9–42·4	36·5–43·1	27·9–43·1		28·4–32·3	28·4–32·3	42·4	35·2	24·3–38·5	22·1–42·4
As % CP											
CPd	69·5	62·3–67·5	67·6–70·1	62·3–70·1	58·9	62·0–69·2	58·9–69·2	57·4	58–60·4	56·7–78	56·7–78·0
ALJ (Degrees)	104	102–112	84·9–98·4	84·9–112	100·8	83·8–89·7	83·8–100·8	104	103–108	78·8–96·9	78·8–108
Meristics											
LL	36	35–38	35–37	35–38	38–39	36–39	36–39	37	35–41	35–40	35–41
TR	12·5	11·5–12·5	11·5–13·5	11·5–13·5	12–13	10·5–12	10·5–13	11·5	11·5–13·5	11·5–13	11·5–13·5
CPs	18	18–19	18	18–19	18	18	18		17–18	17–18	17–19
D2s	23–24	22–24	23–24	22–24		22–23	22–23	23–24	24	21–25	21–25
Ps	9	9–11	9–10	9–11	9	9–10	9–10	9	9–10	8–11	8–11
D2	i,8	i,7–8	i,8–9	i,7–9		i,8	i,8	i,8	i,8	i,8–9	i,7–9
P	I,16	I,15–16	I,15–16	I,15–16	I,15	I,15	I,15	I,16	I,16–17	I,15–17	I,15–17
GR		65–66	76	65–76				63	62	43–82	43–82

*Including specimens of unknown jaw morphotype.

and adult fish sometimes apparently lacks teeth (or teeth possibly buried in lip tissue) or if teeth are present, they are fine, ciliform, unicuspid, not visible to naked eye, reasonably well spaced and in a single row. Teeth on lower jaw are very small, ciliform, in a single row or absent. Maxilla not extending beyond posteroventral corner of lachrymal. Corner of mouth level with anterior of eye.

Pharyngobranchial morphology. GR: 65–76; Pharyngobranchial organ with single, large valve that is longer (anteroposteriorly) than deep (dorso-ventrally).

Vertebral column and median fin osteology (ANSP 152244). VC: 11 + 13; S/DPP: 0010101(1211111).

Colouration. Live specimens are dusky on dorsal third of body, otherwise generally pale on remainder of flanks and ventral part of abdomen. No distinct longitudinal bands along flanks. Eye with well-developed circle of reddish-orange pigmentation over iris [Fig. 3(a)]. Small gold spot on dorsoposterior corner of opercle [Figs 2(a) and 3(a)]. Pectoral fin with small dark spot at dorsoanterior part of fin base and with uniform dusky pigmentation over fin rays and fin membrane [Fig. 2(a)]. First dorsal fin lightly speckled with fine dark spots. Second dorsal fin with denser coverage of fine dark spots than first dorsal fin; anterodorsal tip of second dorsal fin slightly darker than the other parts of the fin [Fig. 3(d)]. Pelvic fins are pale. Anal fin is pale, only weakly pigmented with sparse, fine spots along anterior six soft rays of fin [Fig. 3(b)]. Caudal fin is uniformly dusky [Fig. 2(a)].

In preserved specimens. body darkish dorsally and paler ventrally, lacking distinct bars. Operculum is dark. Pectoral fin may be slightly yellowish, lightly speckled; a very weak spot at origin of fin, about half the length of the base of the fin. First dorsal fin speckled dark along rays. Second dorsal fin may be slightly yellowish, speckled dark and fin noticeably darker at anterior dorsal margin. Pelvic fins are pale. Anal fin is yellowish at base of rays, with fine dark speckling at base of rays and along anterior margin of fin, otherwise pale. Caudal fin is yellowish near base of rays; otherwise speckled dark, especially around distal margin.

Karyotype. Mugil rubrioculus n.sp. exhibits a $2n = 48$ karyotype with entirely acrocentric chromosomes (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 and fluorescent *in situ* hybridization with the 18S ribosomal genes 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 nucleolus organiser region (NOR) bearing chromosomes in the chromosome complement. C-band positive segments, more pronounced on chromosome pair number 8, were observed in pericentromeric position in all chromosomes (M. Nirchio, C. Oliveira, L. Sola, A. R. Rossi, I. A. Ferreira, J. E. Pérez, J. I. Gaviria & I. Harrison, in prep.).

Distribution and ecology

Mugil rubrioculus n. sp. is known from shallow coastal waters of Western Atlantic and Caribbean, between 26°57′ N and 10°57′ N, collected thus far from Florida, Antigua, Belize and Margarita Island (Venezuela) (Fig. 4). Specimens from Margarita Island were collected over sandy and muddy substrates. Little information is known about its ecology.

DISCUSSION

Mugil rubrioculus n. sp. and *M. curema* are very similar; indeed, the lectotype of *M. curema* exhibits some features that make its distinction from *M. rubrioculus* n. sp. quite difficult (see below). Nevertheless, fresh specimens of *M. rubrioculus* n. sp. have a distinctively different colouration compared with *M. curema*, and *M. rubrioculus* n. sp. has a markedly different karyotype (see Diagnosis).

During the course of this study, two slightly different morphotypes of *M. rubrioculus* n. sp. were observed. The holotype and several of the other specimens (see Materials) have a wide angle to the lower jaw (102–112°), and the remaining specimens have a narrower angle (85–98°), with the jaw appearing more ogive in ventral view. The difference in the angle of the lower jaw seems quite distinct when individuals are compared, however, the karyotypes of the two morphotypes are the same and the specimens differ in very few other characters. The wide-jawed form, compared with the narrow-jawed form,

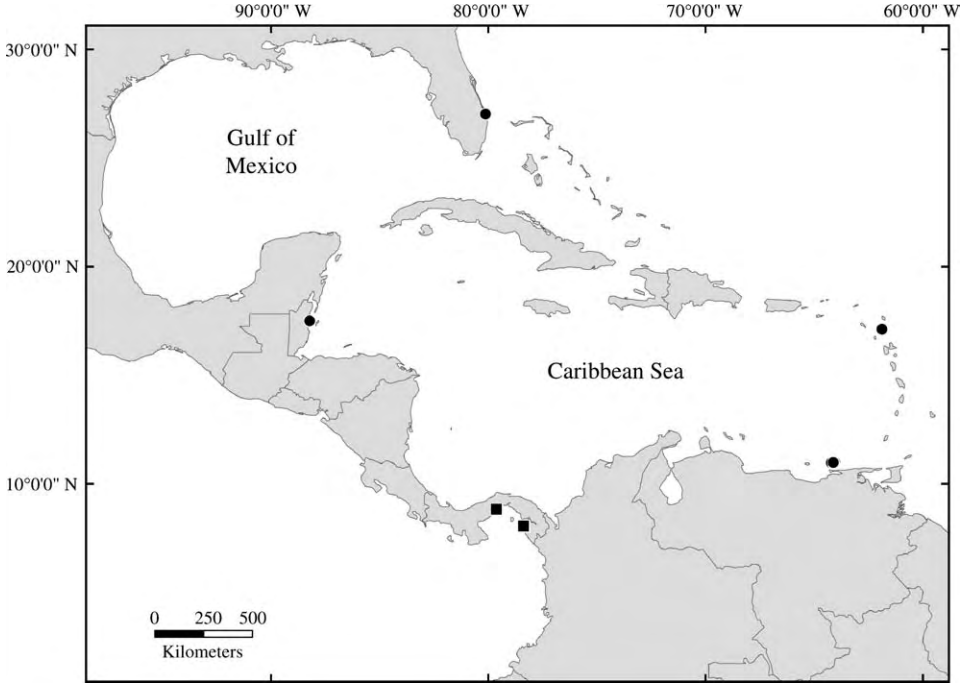

FIG. 4. Map showing locality records for *Mugil rubrioculus* n. sp. and *Mugil* aff. *rubrioculus* (see text for discussion) ●, *Mugil rubrioculus* n. sp.; ■, *Mugil* aff. *rubrioculus*.

has a proportionately thinner body (D1w 11·9–13·8% L_S *v.* 13·3–14·8% L_S; Aw 9·2–10·8% L_S *v.* 11·0–12·1% L_S), a proportionately longer base to the anal fin (Ab 14·3–15·5% L_S *v.* 13·2–14·4% L_S), usually a proportionately longer snout (SN 18·0–23·3% H *v.* 17–21·2% H) and sometimes a proportionately shallower caudal peduncle (CPd 62·3–69·5% CP *v.* 67·6–70·1% CP) (Table I). However, almost all of these differences are not significant, and therefore, the two morphotypes are not considered to be different species.

Specimens of *M. curema* can also be roughly grouped into a wide-jawed form (typified by the lectotype, MNHN A. 3638) and a narrow-jawed form. These two forms do not appear to be morphologically different except in jaw shape and perhaps also in caudal peduncle shape: in the wide-jawed form, the caudal peduncle is relatively shallower (57·4–60·4% CPd), whereas in the narrow-jawed form, the caudal peduncle is usually deeper (56·7–78% CPd).

The wide- and narrow-jaw morphotypes of *M. curema* are currently recognized as conspecific. Nevertheless, when making comparisons between *M. rubrioculus* n. sp. and *M. curema*, it can be very helpful to restrict comparisons between morphotypes of similar jaw type. These interspecific comparisons of individuals with similar jaw morphotypes provide a greater range of diagnostic characters for *M. rubrioculus* n. sp. and *M. curema*. If the wide-jawed forms of *M. rubrioculus* n. sp. and *M. curema* are compared, then there are several morphometric features that distinguish them. Compared with the wide-jawed morphotype of *M. curema*, the wide-jawed morphotype of *M. rubrioculus* n. sp. has a shorter and deeper caudal peduncle (CP 15·6–17·6% L_S in *M. rubrioculus* n. sp *v.* 17·0–19·1% L_S in *M. curema*; CPd 62·3–69·5% CP *v.* 57·4–60·4% CP); the body is slightly deeper (D1d 24·7–26·7% L_S in *M. rubrioculus* n. sp. *v.* 20·0–25·4% L_S in *M. curema*; Ad 23·8–25·5% L_S *v.* 22·7–23·5% L_S); the body is slightly narrower (D1w 11·9–13·8% L_S in *M. rubrioculus* n. sp. *v.* 13·0–18·2% L_S in *M. curema*; Aw 9·2–10·8% L_S *v.* 11·3–11·4% L_S); the base of the anal fin is longer (14·3–15·5% L_S in *M. rubrioculus* n. sp. *v.* 13·2–14·3% L_S in *M. curema*) and the pelvic fin is longer (60·9–69·2% H in *M. rubrioculus* n. sp. *v.* 57·3–59·9% H in *M. curema*). Compared with narrow-jawed *M. curema*, the narrow-jawed *M. rubrioculus* n. sp. has a shorter pectoral fin (17·3–18·9% L_S in *M. rubrioculus* n. sp. *v.* 18·8–21·5% L_S in *M. curema*).

Osteological analyses of *M. rubrioculus* n. sp. in comparison with *M. curema* have, thus far, been restricted to X-radiography. Observations for other species of *Mugil* (I.J.H.) have shown that there is relatively little variation of skeletal features within the genus. However, Kobelkowsky & Reséndez (1972) have reported some minor differences between *M. curema* and *M. cephalus*. Also, Cervigón (1993) has indicated that the cranium in '*Mugil gaimardianus*' (*M. rubrioculus* n. sp.) from Venezuela is slightly wider than in *M. curema*. Therefore, additional comparative analyses of cleared and stained specimens may prove useful.

The lectotype of *M. curema* (MNHN A. 3638) has some features that are very similar to those of the specimens of *M. rubrioculus* n. sp. In particular, there are relatively small teeth on the upper lip, the second dorsal fin has slightly darker pigmentation near its distal margin and the distal margin of the caudal fin is slightly dusky. However, these features have also been observed in other fresh specimens that have been positively identified as

M. curema by their karyotype ($2n = 28$) (Nirchio *et al.*, 2005). For example, small teeth are present on the upper lip of *M. curema* specimen ECAM 000181, and a darkish anterodistal tip to the second dorsal fin and dark margin to the caudal fin are present in *M. curema* specimen AMNH 238178. Therefore, these features cannot be taken as evidence of conspecificity between the lectotype of *M. curema* and *M. rubrioculus* n. sp.

Morphometric features of the lectotype of *M. curema* that are similar to *M. rubrioculus* n. sp. are as follows. The pectoral fin is relatively short in the lectotype (16·1% L_S), and in this respect, the lectotype is rather similar to *M. rubrioculus* n. sp., which tends to have shorter pectoral fins than *M. curema* (Table I and discussion above). However, the pectoral fin length in the lectotype is shorter even than in *M. rubrioculus* n. sp. The thickness of the upper lip is 42·4% L_S in the lectotype of *M. curema*, which is more typical of the slightly thicker lips seen in *M. rubrioculus* n. sp. compared with other specimens of *M. curema*. However, lip thickness may vary quite considerably with some species of mullets. The body width at the origin of the first dorsal fin (D1w) and the depth of the pectoral fin base (Pb) are lesser than in other wide-jawed morphotypes of *M. curema* and are comparable to wide-jawed morphotypes of *M. rubrioculus* n. sp. However, neither D1w nor Pb in the *M. curema* lectotype is outside the total range for all examined specimens on *M. curema* (wide- and narrow-jawed morphotypes). Moreover, the body width can be strongly influenced by sexual maturation (Ibanez-Aguirre *et al.*, 2006). The anal fin base (Ab) and the pelvic fins (Vl) are longer than in other wide-jawed morphotypes of *M. curema* and are comparable to wide-jawed morphotypes of *M. rubrioculus* n. sp. but neither Ab nor Vl in the *M. curema* lectotype is outside the total range for all examined specimens on *M. curema* (wide- and narrow-jawed morphotypes). The lectotype of *M. curema* exhibits some other morphometric features that are dissimilar to both *M. curema* and *M. rubrioculus* n. sp. The caudal peduncle is longer than in other *M. curema* or *M. rubrioculus* n. sp., and the body width at the origin of the pectoral fins (Pw) is slightly less than in other *M. curema* or *M. rubrioculus* n. sp. However, as noted above and by Ibanez-Aguirre *et al.* (2006), there seems to be quite large morphometric variation within Atlantic populations of *M. curema*. Therefore, none of the features discussed above reliably distinguish the lectotype from the other specimens of *M. curema*.

Mugil curema is widespread from the Atlantic coast of Africa to the Western Atlantic, Caribbean and Eastern Central Pacific (Harrison, 2002). Therefore, one would expect the different populations to show variation in their morphometric and genetic characters, as previously shown for *M. cephalus* (Crosetti *et al.*, 1993, 1994; Corti & Crosetti, 1996). More detailed geometric morphometric and molecular systematic analyses of *M. curema*, in comparison with *M. rubrioculus* n. sp., are also likely to provide important information concerning the phylogeography of this species.

Menezes (1983) had commented that Brazilian specimens with the coloured eye, which were referred as *M. gaimardianus*, had long pectoral fins with the tip of the fin reaching to or extending beyond the origin of the first dorsal fin. However, this feature is not evident in specimens of *M. rubrioculus* n. sp., suggesting that Menezes' specimens are not conspecific with *M. rubrioculus*

n. sp. However, four specimens from Panama (listed as *Mugil* aff. *rubrioculus* n. sp. above) have slightly longer pectoral fins compared with the other specimens (19·8–22·1% L_S *v.* 17·3–18·9% L_S in *M. rubrioculus* n. sp.; Table I) and in this respect are more similar to Menezes' nominal *M. gaimardianus* than the specimens of *M. rubrioculus* n. sp. The Panamanian specimens show some other differences to *M. rubrioculus* n. sp. One of these specimens (AMNH 238174) has a slightly different life colouration compared with the other specimens; the body was paler with a faint pinkish cast, the pectoral fins were pinkish-white and the fish had a pale pinkish-red iris (Fig. 5). In addition, the Panamanian specimens differ from other *M. rubrioculus* n. sp. in the following characters: sometimes more numerous scales in longitudinal series (36–39 *v.* 35–38 in *M. rubrioculus* n. sp.); a slightly longer head (25·1–23·3% L_S *v.* 22·7–25·3% L_S in *M. rubrioculus* n. sp.); thinner body (D1w 11·4–11·9% L_S *v.* 11·9–14·8% L_S in *M. rubrioculus* n. sp.; Aw 9–9·1% L_S *v.* 9·2–12·1% L_S in *M. rubrioculus* n. sp.). The morphometric differences between the Panamanian specimens and the other specimens of *M. rubrioculus* n. sp. are very small, however, and it is impossible to reliably distinguish the two populations as separate species. Further observations of fresh specimens and more detailed molecular phylogeographic analysis are necessary to resolve any taxonomic differences between these populations.

It was not possible to obtain type specimens of *Mugil longicauda* Guitart & Alvarez-Lajonchere, 1976 (holotype IOH 330; paratypes Cent. Invest. Mar. Univ. Habana 207 & 208). However, careful comparison with the original description for *M. longicauda* indicates several significant differences to *M. rubrioculus* n. sp. The teeth in *M. longicauda* are described as relatively long (*v.* fine and short in *M. rubrioculus* n. sp.), the head length is 17·8–20·2% L_S (*v.* 22·7–25·3% L_S in *M. rubrioculus* n. sp.), the caudal peduncle length is 17·8–18·9% L_S (*v.* 15·3–17·6% L_S in *M. rubrioculus* n. sp.) and the pectoral fin

FIG. 5. Fresh specimen of *Mugil* aff. *rubrioculus* n. sp. (AMNH 238174) from Golfo de San Miguel, Panama, L_S = 159 mm. Photo by Ross Robertson, STRI.

length is 90·9–95% H (*v.* 72·3–82·9% H in *M. rubrioculus* n. sp.). Examination of three syntypes of *Myxus calancalae* de Beaufort indicates that these specimens are *M. incilis* (A II,10; LL 42–45; CPs 20–21) (see Harrison, 2002 for data of *M. incilis*). Therefore, *Myxus calancalae* should be considered a junior synonym of *M. incilis* rather than *M. curema* as previously suggested by Thomson (1997). Examination of eight syntypes of *Querimana gyrans* Jordan & Gilbert shows similarity to *M. liza* and *M. trichodon*, but the combination of low scale counts in longitudinal series (28–31) and circumpeduncular series (16) suggests that these specimens are *M. trichodon* (see Harrison, 2002 for data of *M. trichodon and M. liza*). This agrees with Thomson's (1997) inclusion of *Querimana gyrans* as a junior synonym of *M. trichodon*. These studies confirm that there is no other existing available name for the species named in this study as *M. rubrioculus* n. sp.

KEY TO WESTERN CENTRAL ATLANTIC SPECIES OF *MUGIL*

The following key is based, in part, on Harrison's (2002) key to Mugilidae for the Western Central Atlantic.

1a. Anal fin with three spines (first spine very short) and eight soft rays in adults, or two spines and nine soft rays in specimens less than *c.* 50 mm L_S 2
1b. Anal fin with three spines (first spine very short) and nine soft rays in adults, or two spines and 10 soft rays in specimens less than *c.* 50 mm L_S 5
2a. Second dorsal and anal fins with small scales only on anterior basal parts; upper lip with small teeth, not visible to naked eye, or just visible as fine fringe; upper lip with outer row of unicuspid teeth and one to six inner rows of bicuspid teeth 3
2b. Second dorsal and anal fins with small scales over all parts of fin (except in specimens under *c.* 60 mm L_S, which may lack scales on distal part of fin); teeth on upper lip usually just visible to naked eye (except in small specimens); upper lip with outer row of moderately long, more or less recurved unicuspid teeth and inner row of unicuspid teeth present or absent; no bicuspid teeth 4
3a. 36 or more scales in longitudinal series; body depth at origin of first dorsal fin usually 24–28% L_S; body depth at origin of anal fin usually 20–24% L_S; head depth equal to or greater than head width at level of posterior of operculum; origin of second dorsal fin just posterior to vertical level of origin of anal fin ..... *Mugil cephalus*
3b. 36 or fewer (usually 29–34) scales in longitudinal series; body depth at origin of first dorsal fin usually 17–23% L_S; body depth at origin of anal fin usually 19–20% L_S; head depth less than head width at level of posterior of operculum; origin of second dorsal fin on vertical through anterior quarter to third of anal fin ..... *Mugil liza*
4a. One small spine (segmented at tip) and eight soft rays in second dorsal fin; usually 34–37 (rarely 33) scales in longitudinal series; 18 (rarely 17) scales in circumpeduncular series; upper lip with an outer row of close-set, long, unicuspid teeth, which have their tips recurved in towards the mouth; lower lip with a single row of close-set, usually long, unicuspid teeth with

recurved tips; depth of medial part of upper lip 5% or less of head length ..... *Mugil curvidens*

4b. One small spine (segmented at tip) and seven (or very rarely eight) soft rays in second dorsal fin; 28–34 scales in longitudinal series; 16 scales in circumpeduncular series; upper lip with outer row of moderately large and sturdy, unicuspid teeth, reasonably spaced from each other and usually with tips only weakly recurved; lower lip with single row of smaller unicuspid teeth, weakly recurved at tips; depth of medial part of upper lip 5–8% of head length ..... *Mugil trichodon*

5a. 43–47 scales in longitudinal series; 21–23 scales in circumpeduncular series ..... *Mugil incilis*

5b. 35–40 scales in longitudinal series; 17–19 scales in circumpeduncular series 6

6a. Body moderately deep (body depth at origin of first dorsal fin usually 24–30% L_S; body depth at origin of anal fin usually 21–27% L_S); pectoral fin with 15–17 soft rays, usually not quite reaching level of origin of first dorsal fin (pectoral fin 16–21% L_S, 70–83% H); 8–11 scales in longitudinal series anterior to tip of pectoral fin; upper lip with small to minute teeth 7

6b. Body elongate (body depth at origin of first dorsal fin usually 22–26% L_S; body depth at origin of anal fin usually 21–23% L_S); pectoral fin with 13 or 14 soft rays, long, reaching level of origin of first dorsal fin or extending just beyond this (pectoral fin 21–24% L_S, 80–96% H); 11–13 scales in longitudinal series anterior to tip of pectoral fin; upper lip with minute teeth ..... *Mugil hospes*

7a. Iris with reddish-orange colour; small goldish spot on opercle; flanks pale without distinct longitudinal bands; small dark spot dorsally on base of pectoral fin; anterodorsal tip of second dorsal fin slightly darker than other parts of fin; anal fin pale; caudal fin uniformly dusky; scales on flanks usually lack any minute, overlying, secondary scales, giving smooth texture to body; teeth on upper and lower lips usually very fine and short, or absent ..... *Mugil rubrioculus* n. sp.

7b. Iris without reddish-orange colour; moderate to large goldish spot on opercle; flanks dark with *c.* six longitudinal bands; large dark spot extending over most of base of pectoral fin; second dorsal fin usually uniformly dark; anal fin dark; caudal fin usually dark; scales on the flanks usually have minute, overlying, secondary scales; teeth on lips small but maybe minute as in *M. rubrioculus* ..... *Mugil curema*

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APPENDIX

COMPARATIVE MATERIAL

Mugil cephalus: NMW 67346, one specimen (holotype of *Mugil mexicanus* Steindachner), 220 mm L_S; Mexico, Acapulco; 1874. I. 1789. AMNH 14167, one specimen, 18·2 mm L_S; west coast of Mexico; Crocker; 2 February 1938. MNHN A. 3668, one specimen (paralectotype of *Mugil liza*), 152·6 mm L_S; Surinam; Leschenault & Doumerc; no date. SMNS 1995, one specimen, 366 mm L_S; Brazil, Santa Cruz, Rio Grande, 32°02′ S 52°05′ W; A. Agassiz; 1871. MNHN A. 3569, three specimens, 183–188 mm L_S; Brazil; Jobert; no date. MZUSP 55474, one specimen, 72·1 mm L_S; Rio de Janeiro, Cabo Frio, Arraial do Cabo, I.P.M.; 1973. MNHN 6307, one specimen (paralectotype of *M. liza*), 333 mm L_S; Buenos-Ayres; d'Orbigny; no date. BMNH 1878.5.16.19-20, two specimens (syntypes of *Mugil platanus* Günther), 365–380 mm L_S; Buenos Aires, River Plata; E. W.

White; no date. BMNH 1878.9.10.1-4, four specimens (syntypes of *Mugil platanus*), 215–305 mm L_S; Buenos Aires; River Plata; E. W. White; no date. BMNH 1881.7.2.21, one specimen, 555 mm L_S; Buenos Aires, River Plata; E. W. White; no date.

Mugil curema Valenciennes, 1836: MNHN A. 3638, one specimen, lectotype, 240 mm L_S (wide jaw); Brazil, Bahia; no date. MNHN A. 3653, one specimen, paralectotype, 205 mm L_S (narrow jaw); Santiago de Cuba; Choris; no date. MNHN A. 4655, one specimen, paralectotype, 345 mm L_S (wide jaw); Martinique; Plée; 1826. MNHN A. 4671, one specimen, paralectotype, 240 mm L_S; Martinique; Plée; no date. MNHN A. 3615, one specimen (paralectotype of *Mugil petrosus* Valenciennes), 133·7 mm L_S (narrow jaw); New York; Milbert; no date. AMNH 227723, one specimen, 103·1 mm L_S; U.S.A., New York, Hudson River, George's Island; C. L. Smith *et al.*; 4 October 1973. AMNH 2459, one specimen, 140·3 mm L_S (narrow jaw); U.S.A., Florida, Caximbao; no date. MNHN A. 3614, one specimen (paralectotype of *M. petrosus*), 139·3 mm L_S (narrow jaw); Vera-Cruz; no date. MNHN 1905-474, one specimen, 79·5 mm L_S (narrow jaw); Mexico; Field Columb. Mus.; no date. MNHN A. 3613, one specimen (lectotype of *Mugil petrosus*), 116·0 mm L_S (narrow jaw); Cuba; Desmarest; no date. MCZ 23888, one specimen, 274 mm L_S; Cuba; Poey; (no date). MCZ 23890, one specimen, 260 mm L_S; Cuba; Poey; no date. MCZ 31509, one specimen, 148·6 mm L_S; Cuba; Poey; no date. AMNH 3391, one specimen, 89·9 mm L_S (narrow jaw); Cuba, Havana market; J. T. Nichols; 26 February 1912. USNM 035179, one specimen, 107·1 mm L_S; Cuba, Havana; D. S. Jordan; no date. ECAM 000465, one specimen, 204 mm L_S (narrow jaw); Antigua, Gallon Beach, seine; O. Bailey; 3 April 1997. AMNH 226313, one specimen, 99·4 mm L_S (narrow jaw); Belize, coastal waters around Belize City; K. E. Carpeneter, M. DeGravelle & T. Orell; 6–9 July 1999. AMNH 226314, one specimen, 88·0 mm L_S (narrow jaw); Belize, St John's Campus, ditches running to sea; 9–16 July 1999. SMNS 3264, three specimens, 173–209 mm L_S (narrow jaw); Guatemala, mouth of Rio Michataya; F. Sarg; January 1885. ECAM 000181, one specimen, 194·9 mm L_S (wide jaw), karyotype $2n = 28$; Panama, La Chorrera district, Puerto Caimito, 8°49′53″ N 79°35′32″ W. BMNH 1903.5.15.280-9, one specimen, 30·5 mm; Panama; Jordan; no date. AMNH 238175, one specimen, 207 mm L_S (narrow jaw), karyotype $2n = 24$; Venezuela, Margarita Island, Macanao peninsula at Boca del Rio, 10°57′48″ N 64°10′42″ W; M. Nirchio; 11 September 2004. AMNH 238176, one specimen, 291 mm L_S (narrow jaw), karyotype $2n = 24$; Venezuela, Margarita Island, Macanao peninsula at Boca del Rio, 10°57′48″ N 64°10′42″ W; M. Nirchio; 25 January 2005. AMNH 238177, one specimen, 206 mm L_S (wide jaw), karyotype $2n = 24$; Venezuela, Margarita Island, Macanao peninsula, La Restinga, 10°58′59″ N 64°6′00′ W; M. Nirchio; 6 April 2004. ECAM 000179, one specimen, 168·5 mm L_S (narrow jaw), karyotype $2n = 28$; Brazil, Paraná State, Paranaguá Bay; no date. AMNH 238178, one specimen, 172 mm L_S (narrow jaw), karyotype $2n = 28$; Brazil, Paraná State, Paranaguá Bay; October 2002. MNHN 749, one specimen, 162·3 mm L_S (narrow jaw); Brazil; no date. AMNH 226156, one specimen, 115·5 mm L_S (narrow jaw); Brazil, Natal; E. C. Starks. SMNS 858, two specimens, 109·4–128·4 mm L_S (narrow jaw); Brazil, Bahia, 12°59′ S; 38°31′ W; Moniz d'Aragon; November 1861. USNM 043356, one specimen, 187·6 mm L_S; Brazil, Bahia, 'Albatross'; no date. AMNH 224469, one specimen, 95·5 mm L_S (narrow jaw); Golfo de Fonseca, La Union, East Pacific Zaca expedition; 20–27 December 1937. AMNH 224393, three specimens, 114·3–170 mm L_S (narrow jaw); Golfo de Nicoya, Isla Cedro; East Pacific Zaca expedition, NYZS, C. W. Beebe; 13 February 1938. AMNH 238179, two specimens, 41·6–49·3 mm L_S; J. Van Tassell.

Mugil cf. *curema*: USNM 013066, one specimen, 239 mm L_S; Cuba; no date. USNM 015157, two specimens, 107·4–115·5 mm L_S; Cuba; no date. AMNH 238180, one specimen, 156 mm L_S; James Van Tassell. MCZ 17539, two specimens, 119·5–165 mm L_S; West Indies, St Thomas, Hasslar Expedition; no date. AMNH 3784, one specimen, 150·9 mm L_S (narrow jaw); Brazil, Natal; E. C. Starks. SMNS 804, two specimens, 227 mm L_S (wide jaw) and 237 mm L_S (narrow jaw); Brazil, Bahia, 12°59′ S 38°31′ W; Moniz d'Aragon; December 1860.

Mugil curvidens Valenciennes, 1836: MNHN A.3626, five specimens, syntypes, 58·7–68·6 mm L_S; Brazil, Bahia; no collector and no date. MNHN A.3646, three specimens,

syntypes, 52·9–58·7 mm L_S; Ascension; Quoy & Gaimard; no date. MCZ 17548, one specimen, 180 mm L_S; Bermuda, North Sargasso Sea; no collector and no date. AMNH 21408, three specimens, 61·7–63·4 mm L_S; Bahamas, Great Inagua, Alfred Sound, Sheep Cay; C. L. Smith *et al.*; 30 June 1964. AMNH 21439, two specimens, 38·5–48·4 mm L_S; Bahamas, Great Inagua, mangroves near Muttonfish Point; C. L. Smith *et al.*; 1 July 1964. AMNH 3788, nine specimens, 62·4–114·0 mm L_S; Brazil, Natal; E. C. Starks; no date. SMNS 858, two specimens, 86·9–88·8 mm L_S; Brazil, Bahia, 12°59′ S 38°31′ W; Moniz d'Aragon; November 1861.

Mugil hospes Jordan & Culver, 1895: BMNH 1895.5.27.178, one specimen, syntype, 174 mm L_S; Mazatlan; Jordan; no date. BMNH 1975.10.7.1-2, two specimens, 157·2–157·5 mm L_S; Cuba; Alvarez-Lajonchere; no date. AMNH 226315, one specimen, 232 mm L_S; Belize, coastal waters around Belize City; K. E. Carpenter, M. DeGravelle & T. Orell; 6–9 July 1999. MNHN A. 4641, one specimen (paralectotype of *Mugil curema*), 200 mm L_S; Maracaibo; Plée; no date. MHNN 507 (in part), one specimen, 192 mm L_S; Brasil; Agassiz; no date. AMNH 224470, two specimens, 116·4–139·5 mm L_S; El Salvador, Golfo de Fonseca, La Union; East Pacific ZACA Expedition, NY Zoological Society; C. W. Beebe; 20–27 December 1937. AMNH 238181, one specimen, 166 mm L_S; Panama, Golfo de San Miguel; J. Van Tassel & R. Robertson; December 2002. BMNH 1938.11.18.45-6, one specimen, 205 mm L_S; Ecuador, Guyana River; Webb; no date. USNM 053479, one specimen, 183 mm L_S; Equador; P. O. Simons; no date.

Mugil incilis Hancock, 1830: AMNH 19097, one specimen, 185·8 mm L_S; Haiti; A. Curtiss; no date. AMNH 19397, one specimen, 72·3 mm L_S (same data as AMNH 19097). BMNH 1864.1.26.213-214, two specimens, 312–326 mm L_S; Panama Canal Zone (incorrectly noted as British Guiana on original label), Chagres River; purchased by Salvin; no date. ZMA 112.930, three specimens (syntypes of *Myxus calancalae* de Beaufort), 42·4–45·3 mm L_S; Venezuela, lower course of rîo Calancalae near San Antonio; P. Wagenaar-Hummelinck; 17 January 1937. BMNH 1932.11.10.55, one specimen, 162·5 mm L_S; British Guiana, New Amsterdam; Matthey; no date. AMNH 9318, two specimens, 124·6–131·7 mm L_S; British Guiana, Potaro River; no collector and no date. MNHN A. 3611, one specimen (paralectotype of *Mugil petrosus*), 193 mm L_S; Surinam, Leschenault; no date. MNHN A. 3612, one specimen (paralectotype of *M. petrosus*), 126·7 mm L_S; Surinam, Levaillant; no date. AMNH 18180, three specimens, 113·3–133 mm L_S; northern South America; no collector and no date.

Mugil liza Valenciennes, 1836: MNHN A. 4659, one specimen, lectotype, 476 mm L_S; Martinique; Plée; no date. MNHN A. 5763, one specimen, paralectotype, 630 mm L_S; Porto Rico (Harrison, 1993); Plée; no date. MNHN A. 4642, paralectotype, *c.* 225 mm L_S; Maracaibo; Plée; no date. MNHN A. 4656, paralectotype, 330 mm L_S (same data as MNHN A. 4642). MNHN A. 4657, paralectotype, 360 mm L_S (same data as MNHN A. 4642). MNHN A. 1050, paralectotype, 555 mm L_S; Cayenne; Frère; no date. MCZ 23885, one specimen, 377 mm L_S; Cuba; Poey; no date. AMNH 18618, one specimen, 173 mm L_S; Panama; no collector and no date. AMNH 226155, one specimen, 194 mm L_S; British Guyana, Potaro River; W. Warfield; summer 1912. AMNH 238182, one specimen, 131·0 mm L_S; Venezuela, Margarita Island, Macanao peninsula, La Restinga, 10°58′59″ N 64°6′00″ W; M. Nirchio; 30 January 2000. AMNH 238183, three specimens, 165–235 mm L_S; Venezuela, Margarita Island, Macanao peninsula at Boca del Rio, 10°57′48″ N 64°10′42″ W; M. Nirchio; March 2004. MZUSP 49087, one specimen, 136·6 mm L_S; Brasil, Rio de Janiero, Cabo Frio; A. Carvalho-Filho; January 1983. MZUSP 55474, one specimen, 110·2 mm L_S; Brasil, Rio de Janiero, Cabo Frio, Arraial do Cabo Frio, I.P.M.; 1973. AMNH 98136, one specimen, 270 mm L_S; Brazil, Lake Papary (see Nisia Floresta), 6°04′00″ N 35°08′00″ W.

Mugil trichodon Poey, 1875: AMNH 52448, one specimen, 175 mm L_S; Bermuda, Nonsuch Islands, Castle Harbour, Gurnet Rock Reefs; New York Zoological Society Bermuda Oceanographic Expedition, William Beebe; 1929–1932. AMNH 2482, four specimens, 99·3–165 mm L_S; U.S.A., Florida, Marco, 'Tekla' Expedition; 16 February 1910. AMNH 2903, one specimen, 197 mm L_S; U.S.A., Florida, Monroe Countyarco, 'Tekla' Expedition; 16 February 1910. AMNH 74040, three specimens, 51·6–70·4 mm

L_S; U.S.A., Florida, Sanibel Island, Point Ybel, grass flats west of lighthouse; F. H. Berry & family; 3 August 1964. AMNH 2473, one specimen, 190 mm L_S; U.S.A., Florida, Key West, Fabbri Tekla Expedition; J. T. Nichols; 30 January 1910. USNM 34966, eight specimens (syntypes of *Querimana gyrans* Jordan & Gilbert), 12·4–15·2 mm L_S; U.S.A., Florida, Key West; D. S. Jordan. BMNH 1884.7.7.185, one specimen, 206 mm L_S; Florida, Key West; Jordan; no date. AMNH 2839, one specimen, 59·1 mm L_S; Southern Florida; no collector and no date. BMNH 1967.6.16.280-290, one specimen, 25·3 mm L_S; Jamaica; Morant, presented by D. K. Caldwell; no date. AMNH 37153, one specimen, 92·5 mm L_S; Haiti, Port-au-Prince; Anthony Curtiss; 1949–1950. Uncatalogued specimens, St John's College collection, Belize City, three specimens, 70–77·9 mm L_S; Belize, coastal waters around Belize City; K. E. Carpenter, M. DeGravelle & T. Orrell; 6–9 July 1999. Uncatalogued specimen, St John's College collection, Belize City, one specimen, 82·5 mm L_S; Belize, coastal waters around Belize City; K. E. Carpenter, M. DeGravelle & T. Orrell; 6–9 July 1999. AMNH 238185, one specimen, 207 mm L_S; Venezuela, Margarita Island, Macanao peninsula at Boca del Rio, 10°57′48″ N 64°10′42″ W; M. Nirchio; 21 March 2004. AMNH 238184, one specimen, 206 mm L_S; Venezuela, Margarita Island, Macanao peninsula at Boca del Rio, 10°57′48″ N 64°10′42″ W; M. Nirchio; 4 April 2004.

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THE PAST AND THE FUTURE OF CYTOGENETICS OF MUGILIDAE: AN UPDATED OVERVIEW

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Abstract: Mugilidae (Teleostei) includes over 70 species, and approximately 25% of them, from all over the world, have been cytogenetically analysed. Although a review on Mugilidae cytogenetics has been recently published, new additional data provided a different perspective on the cytotaxonomic relationships previously inferred and stimulated a re-visitation of the chromosomal evolution in the family. Most of the mugilid species show the conservative 48 uni-armed karyotype, with small differences concerning the absence or the presence of short arms on a single subtelocentric chromosome pair, which allowed the identification of two different cytotypes, A and B, respectively. Within and between these two cytotypes differences could be detected in the location of 18S and 5S rRNA genes and in the composition of the constitutive heterochromatin. Recent data on the karyotype of a basal species in the family, *Agonostomus monticola*, found to have cytotype B, gave the opportunity to reconsider the relationships between the two cytotypes, and to infer the direction of chromosomal changes during evolution, suggesting the cytotype B might be the closest to the ancestor's karyotype. *Mugil curema* constitutes an exception in this picture, showing two cytotypes (C1 and C2), different both in terms of chromosome number, NORs location and heterochromatin distribution, but sharing a karyotype mainly or exclusively composed of bi-armed chromosomes. However, neither of the cytotypes (A or B) presently described in the family can be identified as the closest to the ancestor's karyotype that underwent Robertsonian fusion originating the biarmed chromosomes observed in the two cytotypes C, nor a direct cytotaxonomic relationship among them can be inferred. Such cytogenetic features offer a great opportunity both for cytotaxonomic and phylogenetic studies, as well as for structural, evolutionary and functional analyses of the genome, i.e., for a cytogenomic approach. This involves the isolation and cloning of additional satellite DNA families and the production of chromosome-specific painting probes, whose distribution in the karyotypes of different mugilid species will allow the investigation on the inter-specific homologies and on the structural changes that led to the divergence of mullets chromosome complements.

Key words: mullets, karyotype divergence, evolution

Resumen: La familia Mugilidae (Teleostei) incluye más de 70 especies de las cuales aproximadamente 25% de ellas, alrededor de todo el mundo, han sido analizadas citogenéticamente. Aunque recientemente fue publicada una revisión sobre la citogenética de los mugílidos, la obtención de nuevos datos han suministrado una perspectiva diferente sobre las relaciones citotaxonómicas en el grupo y estimulado una re-exploración de la evolución cromosómica en la familia. Muchas de las especies de mugílidos poseen un cariotipo conservado constituido por 48 cromosomas de un solo brazo, con pequeñas diferencias que estriban en la presencia o ausencia de brazos cortos sobre un único par subtelocéntrico, lo cual ha permitido identificar dos citotipos diferentes, A y B, respectivamente. Dentro y entre estos dos citotipos, pueden ser detectadas diferencias en la localización de los genes 18S rRNA y 5S rRNA y en la constitución de la heterocromatina constitutiva. El análisis reciente del cariotipo de *Agonostomus monticola*, la especie más basal en la familia, permitió establecer que ésta posee el citotipo B, y brinda la oportunidad de reconsiderar la relaciones entre los dos citotipos, así como también inferir la dirección de los cambios cromosómicos durante la evolución del grupo, sugiriendo que el citotipo B podría ser el más cercano a la condición cariotípica ancestral en los mugílidos. Dentro de este panorama, M*ugil curema* constituye una excepción, con dos citotipos (C1 and C2), diferentes tanto en términos de número de cromosomas, localización de las RONs y distribución de heterocromatina, pero que comparten la condición de estar constituidos principalmente o exclusivamente por cromosomas de dos brazos. No obstante, ninguno de los citotipos (A ó B) actualmente descritos en la familia pueden ser identificados como los más cercanos al cariotipo ancestral que experimentaron la fusión Robertsoniana que dio origen a los cromosomas de dos brazos observados en los dos citotipos C, ni tampoco es posible inferir una relación citotaxonómica directa entre ellos. Tales características citogenéticas ofrecen una gran oportunidad tanto para la realización de estudios citotaxonomicos y filogenéticos, como también para el análisis estructural, evolutivo y funcional del genoma, es decir, para una enfocar el análisis mediante una aproximación citogenómica. Esto involucra el aislamiento y clonación de familias de DNA satélite adicionales y la producción de sondas marcadoras cromosoma-específicas cuya distribución en los cariotipos de diferentes especies permitirá investigar las homologías inter-específicas y los cambios estructurales que han conducido a la divergencia de los complementos cromosómicos en el grupo.

Palabras clave: lisas, divergencia cariotípica, evolución

BACKGROUND

Shortly after a review on Mugilidae cytogenetics has been published (SOLA *et al.* 2007), it is useful to make a re-visitation of the chromosomal evolution in the family in the perspective of recent, published (NIRCHIO *et al.* 2007, 2008) and unpublished, data obtained on additional mullet species, which shed a new light on the cytotaxonomic relationships previously inferred for the studied species of Mugilidae.

The systematic position of the family is quite complex and controversial. Indeed, up to 1958 the family was considered to be a representative of a distinct order, Mugiliformes (BERTIN & ARAMBOURG, 1958), thereafter, and up to 1968, was placed in a suborder of Perciformes (GOSLINE, 1968). At present, the family is back in the order of Mugiliformes (THOMSON, 1997; NELSON, 2006) and is the only representative of the order. Also within the family numerous systematic revisions have been done at both the generic and specific level, due to the considerable morphological conservativeness of mullets. In addition to this, the interspecific differences of meristic and morphometric characters are often of the same extent of the intraspecific ones and this also contributes to rise numerous taxonomic and nomenclatural problems. The most recent revisions (THOMSON, 1997; NELSON, 2006) account for a number of valid species ranging from 62 to 72, grouped in 14 to 17 genera, distributed in all temperate and tropical regions of the world. Most of the species inhabit coastal water and accomplish trophic migrations in brackish and, occasionally, freshwater environments as they are eurytherm and euryhaline.

Among the characters different from the morphological ones, the cytogenetic features may provide important insight into systematics. The so far published cytogenetic studies cover a total number of at least 17 species (grouped in seven genera), probably more, given that the karyological data have probably disclosed the existence of a *M. curema* species complex (NIRCHIO *et al.* 2005a). Cytogenetic data mainly refer to species sampled from Mediterranean (six species) or Caribbean and South American Atlantic (seven species) areas. Although the studied species represent approximately only 25% of living Mugilidae, the available cytogenetic data have already disclosed several really interesting issues which stimulate to extend karyological studies on species of Mugilidae still no yet described.

Cytogenetic data published up to 2000 (reviewed in SOLA *et al.* 2007), were indicating a high karyological conservativeness in the family. Indeed, 13 out of the 14 until then studied species show the 48 uniarmed karyotype, proposed by OHNO (1974) as the karyotype of the ancestor of all Teleostei and more recently considered restricted only to the ancestor of Clupeomorpha and Euteleostei by BRUM & GALETTI (1997). Whatever, this is certainly to be considered a conservative karyotype as it is displayed by a high number of un-related species. However, there was already one notable exception, constituted by *Mugil curema*, as specimens from Louisiana (LE GRANDE & FITSIMONS 1976) were found to have a karyotype mainly composed by biarmed chromosomes, with a diploid number of 28 and a conserved fundamental number (FN) of 48. Obviously, the authors interpreted this karyotype as a result of extensive Robertsonian fusions from an all uniarmed chromosome complement. Interestingly, more than 20 years later specimens of *M. curema* from Isla de Margarita (NIRCHIO & CEQUEA 1998) have been found with 2n=24 and an exclusively biarmed karyotype.

CURRENTLY

The present state of the art of the cytogenetic knowledge in Mugilidae reflects the fact that after 2000, studies (reviewed in SOLA *et al.* 2007) were carried out by the application of differential staining techniques and fluorescence *in situ* hybridization (FISH) with several types of DNA probes and that the investigations were extended to additional samples and species of mullets from Central America. Moreover, the latter studies integrated cytogenetic and morphological data.

Specifically talking about the species with the conservative 48 uniarmed chromosomes karyotype, actually two cytotypes (Fig. 1) can be identified among them. Indeed, there is a group of species which has only acrocentric chromosomes, i.e., all chromosomes with a terminal centromere (cytotype A), and there is a second group of species which, among the acrocentric chromosomes, has one pair of subtelocentric chromosomes, i.e., one pair of chromosomes with short arms (cytotype B). After the application of differential staining techniques and FISH, both cytotypes were found to be differentiated for finer cytogenetic features, such as the different location of genes for 18S rRNA and for 5S rRNA and for the composition of the constitutive heterochromatin.

In more detail, the cytotype A, with 48 acrocentric chromosomes, is displayed by ten species belonging to four genera from all over the world (Fig.1, references in SOLA *et al.* 2007). *M. cephalus* from Mediterranean and *Mugil liza* from Venezuela have been investigated with all the above mentioned staining techniques and show an overlapping karyotype for the investigated cytogenetic features, as summarized in the idiograms of Fig. 1. All the techniques (silver- and Chromomycin A_3-staining, FISH with a 18S rDNA probe) generally used to investigate on the location of major ribosomal genes were consistent in identifying only one chromosome pair to bear the Nucleolus Organizer Regions (NORs). Thus, in both species NORs are located in the terminal region of the largest chromosome pair, chromosome pair number 1. Similarly, in both species, FISH with a 5S rDNA probe provides fluorescent signals in interstitial position of the smallest chromosome pair, chromosome pair number 24. A similar location for NORs was suggested by Ag-staining in *M. platanus* and *Rhinomugil corsula*.

The cytotype A, with the major (and minor) ribosomal genes locations observed in *M. liza* and *M. cephalus*, has been so far regarded as plesiomorphic in any cytotaxonomic consideration in the family (SOLA *et al* 2007). In fact, it has to be emphasized that cytotype A is shared by all the *Mugil* species investigated (with the exception of *M. curema*) and this genus is phylogenetically more basal (Thomson 1997) compared to the other genera (*Rhinomugil*, *Valamugil*, *Liza*, *Chelon*, *Oedalechilus*) karyologically studied until the early 2000.

Under this perspective, *M. trichodon* was the first species to be described (NIRCHIO *et al.* 2005b) with an interstitial NOR location on a medium-sized chromosome, location which was a novelty in the genus, and in the family, in general. This same species has been recently investigated for 5S rDNA, and these preliminary and still unpublished data suggest the presence of two different chromosomal location for the minor ribosomal genes, both in heterozygotic condition in the only analysed specimen (Fig. 1). Thus, the location of major and minor ribosomal genes in *M. trichodon* suggests its derived position and the occurrence of chromosome rearrangements.

Shortly after (NIRCHIO *et al.* 2007) a second species from Isla de Margarita, *Mugil rubrioculus* (HARRISON *et al.* 2007), formerly *M. gaimardianus*, has been found with interstitial NORs, located on a different chromosome pair from *M. trichodon*. Also in this species unpublished data are available on the 5S rDNA location and the minor ribosomal genes show once again a location, on a chromosome pair classified as number 13, different from the one observed in *M. trichodon, M. cephalus* and *M. liza*, as well as in any other species of the family (Fig. 1).

Thus, comparing the idiograms (Fig. 1) of *M. trichodon* and *M. rubrioculus* to the one of *M. cephalus* and *M. liza*, supposed to be the closest to the ancestral one, it is hard to find out the cytotaxonomical relationships and several chromosomal rearrangements should be invoked to explain the different location of these markers. Therefore, the original assumption of a high karyological conservativeness in Mugilidae weakens.

As far as the second group of species with 46 acrocentrics and two subtelocentrics, i.e., with Cytotype B, is concerned, former data (reviewed in SOLA *et al.* 2007) regarded only five Mediterranean species, *Liza ramada*, *L. aurata*, *Liza saliens*, *Chelon labrosus* and *Oedalechilus labeo* (Fig. 1). In most of them (four species), the subtelocentric pair is the smallest one of the chromosome complement, chromosome pair number 24, in the fifth species, *O. labeo*, the subtelocentric chromosome pair is larger and classified as chromosome pair number 9. In all species the subtelocentrics have been found to have NORs located on their short arms, by both Ag-staining, CMA_3 and FISH with 18S rDNA.

Considering the chromosome location of 5S rDNA, an interesting picture emerges. In *Liza ramada, L. aurata*, *C. labrosus* and *O. labeo* the minor ribosomal genes are located in interstitial position of a medium-sized chromosome pair which is different from the NOR-bearing chromosome pair. Pursuing a parsimonious criterion, the 5S rDNA-bearing chromosome pair was considered homeologous in all of them and classified as chromosome pair number 8 in all species. In *L. saliens*, in addition to the site on pair number 8, a second site for 5S rDNA was detected, located in terminal position of chromosome pair number 1. Therefore, comparing the idiogram (Fig. 1) of *L. saliens* to the cytotype A shown by *M. cephalus* and *M. liza*, proposed to be the closest to the ancestral one, an alternative and reverse location of major and minor ribosomal genes is observed, so that an event of reciprocal translocation with respect to a common ancestral karyotype might be hypothesized.

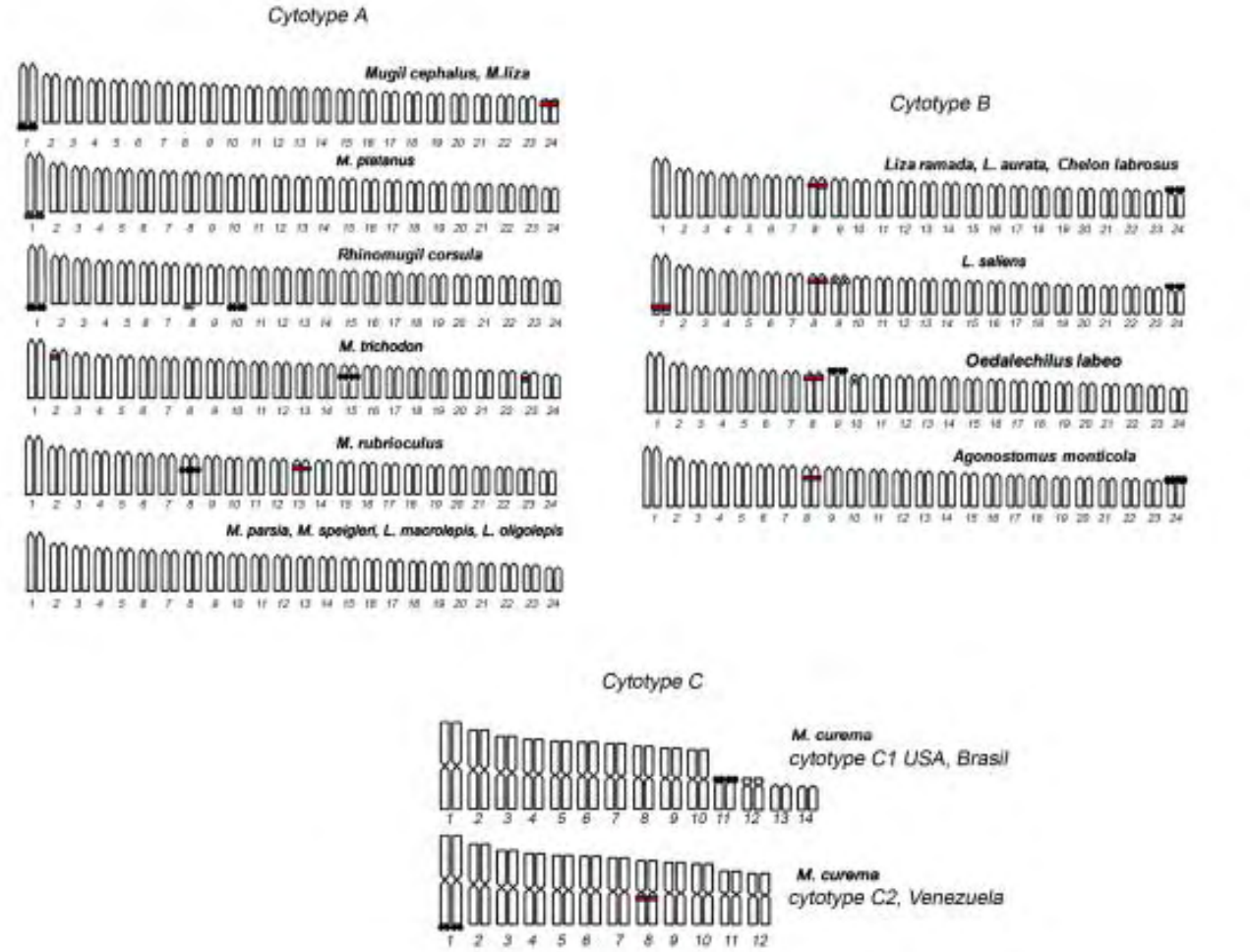

Fig. 1. Idiograms summarizing the karyotypes and the major and minor ribosomal genes locations reported in species of Mugilidae with different cytotypes. For *Liza macrolepis* and *L. oligolepis*, *Mugil parsia*, *M. speigleri* (these two latter later respectively assigned to the genera *Liza* and *Valamugil* - THOMSON, 1997), only the Giemsa stained karyotype is available. For *Mugil platanus, Rhinomugil corsula* and *M. curema* cytotype C1, data on NORs are based on Ag-staining only. *M. curema* cytotype C1 is shown by specimens from USA and from Brazil, cytotype C2 by specimens from Venezuela. *Solid circles* represent constant and *open circles* represent additional and variable locations of 18S rDNA. *Grey bars* represent locations of 5S rDNA.

Within this cytotaxonomic picture, the cytogenetic features recently described by NIRCHIO *et al.*(2007) in a species from Venezuela, *Agonostomus monticola*, were found to be different from the expected ones. Indeed, this species belongs to a genus which is considered to be the most primitive among living mugilid (THOMSON, 1997), and it was expected to show the chromosome constitution which has, to date, been considered plesiomorphic. On the contrary, though the species shows the 48 all-uniarmed karyotype (Fig. 1), its smallest chromosome pair is subtelocentric, bearing 18S rRNA genes on its short arms. Considering also the 5S rDNA location, in interstitial position of a medium-sized chromosome pair, *A. monticola* shows an overall apparent similarity in the karyotype constitution, major and minor rDNA loci numbers and locations, to *Chelon labrosus* and three *Liza* species, *Liza ramada*, *L. aurata*, *L. saliens*, (see SOLA *et al.* 2007, for references), that is, the karyotype of *A. monticola* can be assigned to cytotype B.

The latter cytotype B is therefore shared by a genus, *Agonostomus,* which is the most basal in the family, and

by two other genera which are, on the other hand, considered to be the most advanced in the family (Thomson, 1997). This indicates that the cytotype B might be regarded as the plesiomorphic condition for the karyotype in the family. Consequently, the cytotype A, shared by most of the species of a genus, *Mugil,* in an intermediate systematic position, and which has been so far considered to be the closest to the ancestor's karyotype, should now to be re-considered as derived. This factor should be taken into consideration in any cytotaxonomic reconstruction in the family.

The analysis of the constitutive heterochromatin location has been so far applied to a limited number of the species with the 48 uniarmed karyotype, four with cytotype A and six with cytotype B (Sola *et al.* 2007, Nirchio *et al.* 2007). Clear chromosome markers, useful for cytotaxonomic considerations, have not been identified, as a generalized pericentromeric location has been observed in all of them. However, preliminary data on the heterochromatin composition in the six Mediterranean species (Sola *et al.* 2007) have suggested the existence of different types of satellite DNA whose analysis might provide a deeper insight into the understanding of the mechanisms of chromosome divergence in Mugilidae.

As far as *Mugil curema* is concerned, as mentioned before, two cytotypes had been described for specimens from USA (Cytotype C1, 2n=28, FN=48; Le Grande & Fitsimons 1976) and from Venezuela (Cytotype C2, 2n=24, FN=48; Nirchio & Cequea 1998) (Fig. 1). Recent morphological and cytogenetic data obtained on additional samples and the application of differential staining techniques and FISH revealed additional information on the extent of the chromosome differentiation.

As far as the new samples examined, specimens from a farther southern region of the a real of the species, from Paranà, Brazil (Nirchio *et al.* 2005a), have been investigated and found to have the same cytotype C1 shown by specimens from Louisiana (Le Grande & Fitsimons 1976). Thus, based on geographical considerations and on the coastal habits of the species, it is noteworthy that the farthest away populations (from Louisiana and Brazil) share a similar chromosome complement, which is different from the one found in the population collected at a locale (Venezuela) which is geographically intermediate. In addition to this, three specimens from Isla Margarita (Venezuela), collected over a 10 years period, have been found to be characterized by the cytotype C1, i.e., a cytotype different from the most common cytotype C2 in the area (unpublished data). It is worth to emphasize that individuals with an intermediate karyotype between Cytotype 1 and Cytotype 2 (i.e., 2n=26) have never been reported from any collecting locale.

The same specimens with cytotype C1 from Paraná (Nirchio *et al.* 2005a) have been investigated through Ag-staining and NORs have been localized on the short arms of the largest subtelocentric chromosome pair (Fig. 1). On the other hand, in specimens with cytotype C2 from Isla de Margarita (Rossi *et al*. 2005), both Ag-staining, CMA_3 and FISH with 18S rDNA show signals, corresponding to NORs, at the terminal region of the largest metacentric chromosome pair (Fig. 1). Thus, NORs do have a different location in the two cytotypes and this identifies a further degree of differentiation among them.

The two cytotypes appear to be further differentiated based on the heterochromatin distribution. Indeed, specimens with cytotype C1, from Brazil display only pericentromeric C-positive regions, whereas specimens with cytotype C2 from Venezuela, in addition to these, show heterochromatic blocks also at the terminal regions of most chromosomes, as well as in an interstitial location in some of them (Nirchio *et al.* 2005a).

In specimens with cytotype C2 (Rossi *et al*. 2005), also the 5S rRNA genes have been localized and found in a subcentromeric position of a medium-sized chromosome pair (Fig. 1). These same specimens have been investigated also for the location of the telomeric sequences, which might have provided insight into Robertsonian rearrangements in the species. However, the telomeric probe hybridized only at the ends of all chromosomes, no pericentromeric or interstitial signals have been obtained. It is worthy to emphasize that the absence of additional interstitial telomeric signals is considered to be a stabilizing factor for fusions.

Thus, going back to the cytotype A of *Mugil cephalus* for comparison, the original hypothesis made by Le Grande & Fitsimons (1976), of a direct derivation of the karyotype of *M. curema* through Robertsonian fusions from an ancestral group with a chromosome complement similar to that of *Mugil cephalus*, is not really supported. Indeed, neither of the cytotypes C, compared to cytotype A, has cytogenetic features compatible to the original

hypothesis: cytotype C1 because of its different location of NORs, cytotype C2 because of its different location of 5S rRNA genes, clearly not located on the smallest chromosome pair of the chromosome complement. Nor a direct cytotaxonomic relationship among the two cytotypes C can be inferred.

FROM NOW ON

Obviously the question rises whether data are disclosing an intraspecific polymorphism or the existence of two - or three - different species. All these cytogenetic features prompted to the morphological re-examination of Venezuelan and Brazilian samples with the two different cytotypes, and preliminary data suggest that, indeed, we are dealing with two different species (NIRCHIO *et al.* 2005a). Similarly, the three specimens with cytotype C1 from Isla de Margarita were found to be morphologically different from both the sympatric specimens with cytotype C2 and specimens from Brazil with the same cytotype C1 (unpublished data). Thus, the whole amount of cytogenetic and morphological data strongly support the existence of a species complex in *Mugil curema*. The cytogenetic analysis of additional samples as well as additional analyses with other molecular markers, i.e., nuclear or mitochondrial sequences, are needed to clarify the magnitude of the genetic divergence and, thus, the systematic position of populations with different cytotypes. This will provide the basis for the formal description of possible cryptic species.

In conclusion, the available data, on the one hand, suggest to carry out karyological studies on the remaining undescribed species of Mugilidae, in order to provide a more general picture of karyoevolutive trends in the family. On the other, the current picture stimulates to go beyond the current available cytogenetic markers, and this will bring the study of chromosomal evolution in Mugilidae into the future. Indeed, the cytogenetic features of the family so far available appear to offer a great opportunity both for cytotaxonomic and phylogenetic studies, as well as for structural, evolutionary and functional analyses of the genome, i.e. for a cytogenomic approach.

In this context, on the one hand, the isolation and cloning of additional satellite DNA families are being carried out, to study their chromosomal location on the Mediterranean and Caribbean species. On the other, the production of chromosome-specific painting probe is been pursuing. This implies the isolation, by laser micro-dissection, followed by DOP-PCR amplification, of an entire marker chromosome to be used as a probe in cross-hybridization FISH experiments between different species, belonging or not to the same genus and/or to the same or a different geographic area. This will allow the investigation on the inter-specific homologies, on the degree of synteny conservation and on the structural changes that led to divergence of mullets chromosome complements.

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Cytogenetic studies in three species of *Lutjanus* (Perciformes: Lutjanidae: Lutjaninae) from the Isla Margarita, Venezuela

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In the present study, three species of Lutjaninae, *Lutjanus analis*, *L. griseus* and *L. synagris*, were analyzed by conventional Giemsa staining, C-banding and silver staining, to reveal active Nucleolus Organizer Regions (NORs). Fluorescent *in situ* hybridization (FISH) was also applied to establish the number and location of the ribosomal gene clusters (18S and 5S rRNA genes). Counts of diploid metaphasic cells revealed a diploid modal chromosome complement composed of 48 acrocentric chromosomes in both *L. analis* and *L. griseus*. Two cytotypes were observed in *L. synagris*: cytotype I, with 2n=48 acrocentric chromosomes, found in 19 specimens, and cytotype II, with 46 acrocentric chromosomes and one large metacentric, found in two specimens. The large metacentric, which possibly originated from a Robertsonian rearrangement, was not found to be sex-related. In the three species, constitutive heterochromatin is located in the centromeres of all chromosomes. NORs were detected on the short arms of a single chromosome pair, number 24 in *L. analis* and number 6 in both cytotypes of *L. synagris.* In *L. griseus*, a polymorphism of the NORs number was detected, by both Ag-staining and FISH, as females show a maximum of three NORs, and males a maximum of six NORs. In all species, minor ribosomal genes were found located on a single chromosome pair. The obtained data, along with those previously reported for other five Lutjanidae species, show that a general chromosome homogeneity occurs within the family, but that derived karyotypes based on Robertsonian rearrangements as well as multiple and variable NORs sites can also be found.

No presente estudo três espécies de Lutjaninae, *Lutjanus analis*, *L. griseus* e *L. synagris* foram analisadas através da coloração convencional com Giemsa, banda C e coloração com nitrato de prata para identificar as Regiões Organizadoras de Nucléolo (NORs) ativas. Hibridação fluorescente *in situ* (FISH) foi também aplicada para estabelecimento do número e localização dos agrupamentos de genes ribossômicos (18S e 5S rRNA). A contagem de células metafásicas revelou um número diplóide modal de 48 cromossomos acrocêntricos em *L. analis* e *L. griseus*. Dois citótipos foram observados em *L. synagris*: citótipo I com 2n=48 cromossomos acrocêntricos, encontrado em 19 espécimes, e citótipo II com 46 cromossomos acrocêntricos e um grande metacêntrico, encontrado em dois espécimes. O grande metacêntrico, que possivelmente se originou por um rearranjo Robertsoniano, não está relacionado com o sexo. Nas três espécies a heterocromatina constitutiva está localizada nas regiões centroméricas de todos os cromossomos. NORs foram detectadas no braço curto de um único par cromossômico, número 24 em *L. analis* e número 6 em ambos os citótipos de *L. synagris.* Em *L. griseus*, um polimorfismo de número de NORs foi observado, após coloração com prata e por FISH, as fêmeas apresentaram um máximo de três NORs e os machos um máximo de seis NORs. Em todas as espécies os genes ribossômicos 5S foram encontrados em um único par cromossômico. Os dados obtidos, somados aos demais previamente publicados para cinco outras espécies de Lutjanidae, mostram que na família há uma homogeneidade cromossômica, porém também são encontrados cariótipos derivados, originados por rearranjos Robertsonianos, assim como pela ocorrência de sítios múltiplos e variados de NORs.

Key words: Karyotype, Ribosomal genes, NOR polymorphism, C-banding, Robertsonian rearrangement.

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Introduction

The Lutjanidae (snappers) is a group composed of 17 genera and 105 species of mostly reef-associated marine fishes, which are distributed in all the tropical and subtropical seas of the world (Nelson, 2006). The family is divided in four subfamilies. Three smaller subfamilies include the Paradichthyinae, with two monotypic genera (*Symphorus* and *Symphorichthys*), the Etelinae, with five genera (*Aphareus*, *Aprion*, *Etelis*, *Pristipomoides* and *Rhandallichthys*) and 19 species, and the Apsilinae, with four genera (*Apsilus*, *Lipocheilus*, *Paracesio* and *Parapristipomoides*) and 12 species (Nelson, 2006). The subfamily Lutjaninae is the largest, with three monotypic genera (*Hoplopagrus*, *Ocyurus* and *Rhomboplites*), the genera *Macolor* and *Pinjalo* with two species each, and the genus *Lutjanus*, which is the most speciose, with 64 species. In Venezuela, Cervigón (1993) recognizes six genera of Lutjanidae (*Etelis*, *Pristipomoides*, *Apsilus*, *Ocyurus*, *Rhomboplites* and *Lutjanus*) including 15 species, 10 of which belong to the genus *Lutjanus* (*L. analis*, *L. apodus, L. aya, L. bucanella, L. cyanopterus*, *L. griseus, L. jocu, L. mahogoni, L. purpureus, L. synagris* and *L. vivanus*).

In spite of their high number and their ecological and economic importance, cytogenetic studies on Lutjanidae are scarce. In fact, among the 105 recognized species of Lutjanidae, barely five species have been karyotyped to date: *Lutjanus argentimaculatus* (Raghunath & Prasad, 1980), *L. kasmira* (Choudhury *et al*., 1979; Ueno & Takai, 2008), *L. sanguineus* (Rishi, 1973), *L. russelli* (Ueno & Ojima 1992), and *L. quinquelineatus* (Ueno & Takai, 2008). For most of them, only the chromosome number and morphology have been reported and there is no data regarding the chromosomal distribution and composition of the constitutive heterochromatin or numbers and locations of the major and minor ribosomal genes, which have proved to be useful markers in the investigation of the phylogenetic relationships among fish species within a family (Sola *et al*., 2007).

In the present study, three species of Lutjaninae, *Lutjanus analis*, *L. griseus* and *L. synagris* were analyzed by conventional Giemsa staining and C-banding, and by Fluorescent *in situ* hybridization with 18S rDNA and 5S rDNA, in order to obtain a fine karyotype characterization, and, thus, chromosome markers which can provide useful information concerning relationships within the family.

Materials and Methods

Eight sexually immature (unsexed) specimens of *L. analis*, seven specimens of *L. griseus* (3 males, 3 females, 1 unsexed) and 21 specimens of *L. synagris* (9 males, 10 females, 2 unsexed) were captured with a fishing trap in the locality of Guayacancito, on Margarita Island, Venezuela. Voucher specimens (Table 1) were deposited at the Ichthyology Collection of the Escuela de Ciencias Aplicadas del Mar (ECAM), Universidad de Oriente.

Twenty four hours before chromosome preparations, the fishes were injected intramuscularly with a yeast glucose solution (Lee & Elder, 1980) for mitosis stimulation. Chromosomes were obtained from kidney cells according to Foresti *et al.* (1993). C-bands were obtained according to the method described by Sumner (1972), modified by testing different time of exposition to barium hydroxide, from 1 to 180 seconds, in order to enhance the contrast of constitutive heterochromatin on chromosomes. For detection of the active Nucleolus Organizer Regions (NORs), slides were stained with silver nitrate using the method of Howell & Black (1980).

The 5S and 18S rDNA sites were identified by FISH according to the method of Pinkel *et al.* (1986). A sequence of 1800 base pairs of the 18S rRNA gene of *Oreochromis niloticus* (Nile tilapia), cloned in pGEM-T plasmid, was used as a probe to localize sites for 45S rDNA. PCR products containing 5S rDNA repeats from each species were used as probes for the chromosome mapping of 5S rDNA. DNA was extracted from muscle (Sambrook & Russel, 2001) and the 5S rDNA repeats were generated by Polymerase Chain Reaction (PCR) with the primers 5SA (5'TAC GCC CGA TCT CGT CCG ATC3') and 5SB (5'CAG GCT GGT ATG GCC GTA AGC3') according to Martins & Galetti (1999).

The 18S rDNA and 5S rDNA probes were labeled by nick translation with biotin-14-dATP, following the manufacturer's (Bionick™ Labelling System-Gibco.BRL) instructions. Signals were detected and amplified by a three-round application of Avidin-FITC/biotinilated Anti-avidin. Chromosomes were counter-stained with Propidium Iodide (50µg/ml) diluted in Antifade.

The mitotic figures were photographed using a Motic B400 microscope equipped with a Moticam 5000C digital camera. The fundamental number (NF) of arms was determined considering acrocentrics (A) as having one chromosome arm and metacentrics (M) as having two chromosome arms. FISH metaphases were photographed with a Olympus BX61 photomicroscope equipped with a DP70 digital camera.

Results

The counts of diploid metaphasic cells (Table 1) revealed a modal chromosome complement composed of 2n=48 acrocentric chromosomes (NF=48) in both *L. analis* and *L. griseus* and in 19 out of the 21 examined specimens of *L. synagris* (cytotype I). The two remaining specimens, one male and one unsexed, of *L. synagris* show a modal count of 2n=47 (NF=48), made up of one large metacentric and 46 acrocentric chromosomes. This karyomorph was named cytotype II. The karyotypes obtained by arranging the chromosomes in order of decreasing size are shown in Fig. 1. The negligible differences in chromosome sizes make it impossible to identify homologous pairs with any certainty, with the exception of a chromosome pair, classified as number 6, in *L. analis* (Fig. 1a), which shows a secondary constriction when chromosomes are elongated, and chromosome pair number 24 in all the three species, clearly the smallest of the chromosome complements.

Fig. 1. Giemsa-stained karyotypes of *Lutjanus analis* (a), *L. griseus* (b), *L. synagris* cytotype I (c) and *L. synagris* cytotype II (d).

In all the three *Lutjanus* species examined, C-banding (Fig. 2) revealed that the heterochromatin is restricted to the centromeres of all chromosomes, including the large metacentric in cytotype II of *L. synagris*, though some of them show weaker signals.

The analysis of the nucleolus organizer regions with the Ag-NOR staining technique detected a maximum of two Ag-positive paracentromeric signals in *L. analis* (Fig. 3b) and in both cytotypes of *L. synagris* (Fig. 3d for cytotype I, data not shown for cytotype II). In *L. analis* the Ag-signals (Fig. 3b) are located on the secondary constriction, often evident in Giemsa-stained metaphases (Fig. 3a), of chromosome pair number 6. In *L. synagris* the Ag-signals (Fig. 3d) are located on the smallest chromosome pair number 24 of the complement. In *L. griseus*, a variable number of paracentromeric Ag-positive signals per metaphase was observed, up to two in females (Fig. 4a, b) and up to four in males (Fig. 4c, d). By its morphology and size and for the presence of a secondary constriction (Fig. 4a, b), one of the NOR-bearing chromosome pair in this is likely to be homoeologous to the chromo-

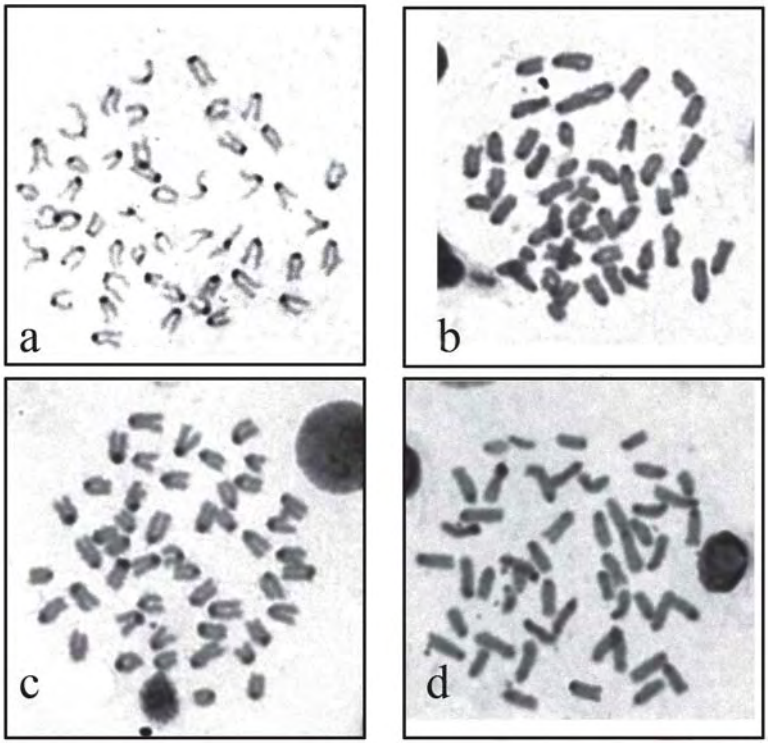

Fig. 2. C-banded metaphases of *Lutjanus analis* (a), *L. griseus* (b), *L. synagris* cytotype I (c) and *L. synagris* cytotype II (d).

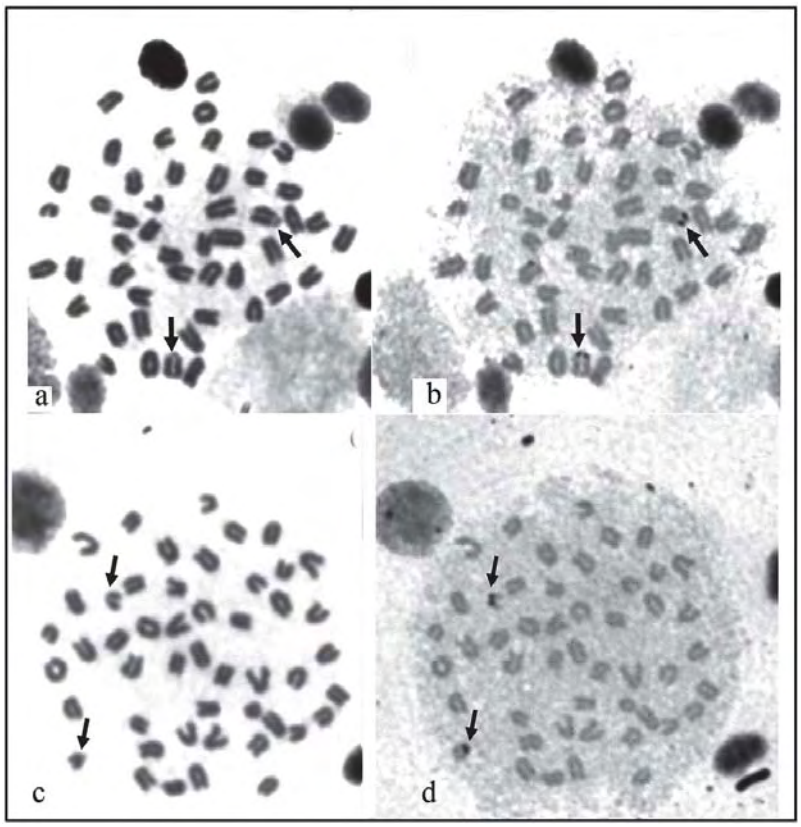

Fig. 3. Metaphases of *Lutjanus analis* (a; b), *L. synagris* cytotype I (c; d) sequentially stained with Giemsa (left) and $AgNO_3$ (right). Arrows indicate the NOR bearing chromosomes.

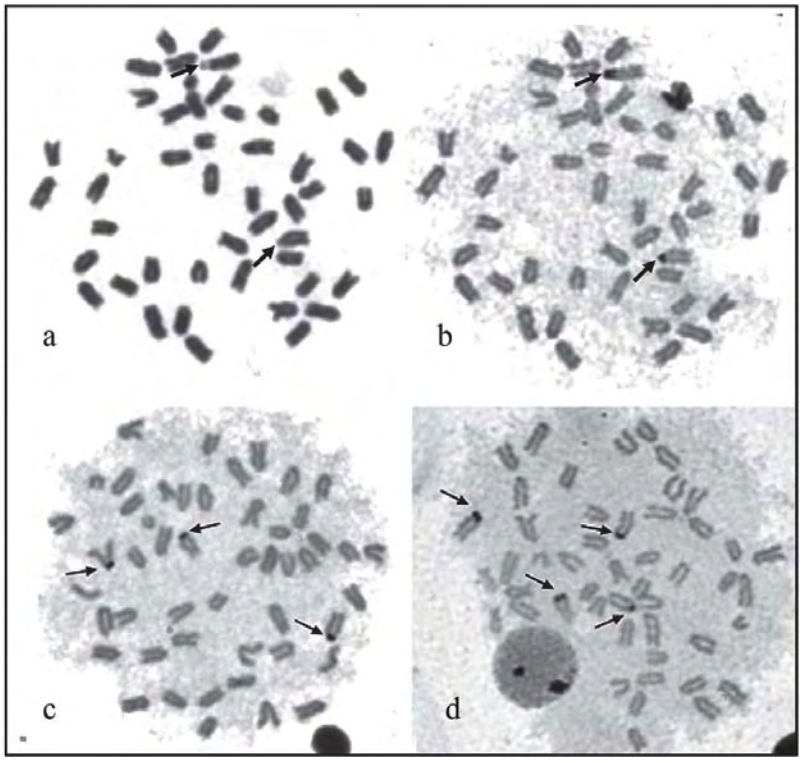

Fig. 4. Metaphases of *Lutjanus griseus*, with two (b), three (c) or four (d) Ag-NORs (arrows). In (a) the same metaphase as (b) previously stained with Giemsa.

some pair number 6 identified in *L. analis* (Fig. 3a, b). The remaining signals are located on medium-sized chromosomes.

After FISH with 18S rDNA, in *L. analis* and in *L. synagris* two fluorescence hybridization signals were seen at the same chromosome locations detected with Ag-staining, i.e., in paracentromeric positions of chromosome pair number 6 in *L. analis* (Fig. 5a) and of chromosome pair number 24 in both cytotypes of *L. synagris* (Fig. 5c, e), confirming that in these species a single pair of NOR-bearing chromosomes is present. In *L. griseus* (Fig. 6) hybridization with 18S rDNA confirms the NORs sites detected by Ag-staining, but also reveals further positive sites, so that up to three NORs were observed in females (Fig. 6a) and up to six in males (Fig. 6b). All FISH positive chromosomes are medium-sized, thus major ribosomal gene clusters location on chromosome pair 24 can be excluded.

After FISH with 5S rDNA, in all species - *L. analis* (Fig. 5b), both cytotypes of *L. synagris* (Fig. 5d, f) and *L. griseus* (Fig. 6c) - one cluster of 5S rRNA genes was found close to the centromere of a medium-sized acrocentric chromosome pair, which is apparently the same in the three species.

Discussion

The cytogenetic features here reported for the examined specimens of *L. analis*, *L. griseus* and *L. synagris* revealed that the three species have the 48-acrocentric karyotype which is shared by most of the Lutjaninae species previously analyzed, such as *Lutjanus argentimaculatus* (Raghunath & Prasad, 1980), *L. kasmira* (Choudhury *et al*., 1979; Ueno & Takai, 2008), *L. sanguineus* (Rishi, 1973) and *L. russelli* (Ueno & Ojima, 1992). The only exception is *L. quinquelineatus* which has been reported to possess 2n=48 A in females and 2n = 47 (1M+46A) in males (Ueno & Takai, 2008).

In fish, Robertsonian rearrangements, which, by fusions (or fissions) of two uniarmed chromosomes into one biarmed chromosome (or vice-versa), cause changes in chromosome number, but leave the fundamental number of arms unchanged, are infrequent. However, examples of Robertsonian polymorphism in marine fishes have been reported for species of Gobiidae (*Gobius paganellus*, Giles *et al*., 1985; *Neogobius eurycephalus*, Ene, 2003), Sparidae (*Diplodus annularis*, Vitturi *et al*., 1996), Pomacentridae (*Chromis insolata* and *C. flavicauda*, Molina & Galetti, 2002), Cottidae (*Myoxocephalus scorpius*, Yershov, 2005), Gadidae and Pleuronectidae (*Gadus morhua* and *Pleuronectes platessa,* Fana & Fox, 1991). In this context, the presence of cytotype II, with a large unpaired metacentric chromosome, found in one male and one unsexed specimen of *L. synagris*, is quite interesting. In fact, when chromosome differences are restricted to one sex, the presence of sex chromosomes is strongly suggested (Devlin & Nagahama, 2002). As a matter of fact, the presence of 2n=48 acrocentric chromosomes in females and 2n=47 (1 M+46A) in males of *L. quinquelineatus* was interpreted by Ueno & Takai (2008) as the result of a single Robertsonian fusion which formed a neo-Y chromosome, establishing a multiple sex chromosome system of $X_1X_1X_2X_2/X_1X_2Y$ type. In the case reported here for *L. synagris*, the reduced 2n=47 cytotype II characterizes only two (one male, one immature) of the 21 specimens analyzed, but, among these latter, the remaining eight males present the same 48 acrocentric chromosome cytotype I shown by all ten females and one immature specimens. Thus, the two cytotypes co-exist in different males and the presence of the large unpaired metacentric chromosome does not seem to be associated to sex determination. Due to the frequency of cytotype II, approximately 9.5%, this rearrangement suggests the existence of a chromosomal polymorphism in the studied population of *L. synagris*. Nevertheless, the possibility that the observed fusion in *L. synagris* might originate a neo-Y chromosome and that an incipient stage of multiple sex chromosome differentiation, as the one reported in *L. quinquelineatus* (Ueno & Takai, 2008), has been identified, can not be discarded.

In all the three *Lutjanus* species examined, the C-positive heterochromatin distribution is restricted to the centromeres

Table 1. Number of scored cell and percentages of different diploid counts in specimens of the three species of *Lutjanus* examined. Undetermined sex (?), male (M), and female (F).

Species (n)	Voucher number	Sex	Number of scored cells	Frequency 46	47	48	49
L. analis (8)	ECAM-365	?	45	8.9	11.1	73.3	6.7
	ECAM-366	?	26	3.8	11.5	76.9	7.7
	ECAM-422	?	24	0.0	8.3	87.5	4.2
	ECAM-425	?	34	0.0	5.9	85.3	8.8
	ECAM-426	?	28	7.1	3.6	85.7	3.6
	ECAM-427	?	40	2.5	7.5	85.0	5.0
	ECAM-405	?	38	0.0	7.9	81.6	10.5
	ECAM-428	?	15	0.0	13.3	80.0	6.7
	Subtotal		250	2.8	8.6	81.9	6.7
L. griseus (7)	ECAM-399	F	67	4.5	7.5	82.1	6.0
	ECAM-408	?	23	4.3	8.7	82.6	4.3
	ECAM-410	M	11	0.0	18.2	81.8	0.0
	ECAM-409	F	30	0.0	13.3	76.7	10.0
	ECAM-002	F	36	5.6	11.1	80.6	2.8
	ECAM-413	M	26	3.8	15.4	80.8	0.0
	ECAM-429	M	76	3.9	10.5	78.9	6.6
	Subtotal		269	3.2	12.1	80.5	4.2
L. synagris (21)	ECAM-364	?	70	4.3	4.3	85.7	5.7
	ECAM-367	?	56	5.4	83.9	7.1	3.6
	ECAM-423	M	16	6.3	12.5	75.0	6.3
	ECAM-424	F	59	3.4	10.2	81.4	5.1
	ECAM-430	F	49	6.1	10.2	77.6	6.1
	ECAM-438	F	83	3.6	4.8	83.1	8.4
	ECAM-439	F	26	0.0	3.8	88.5	7.7
	ECAM-451	M	47	8.5	76.6	10.6	4.3
	ECAM-452	F	64	3.1	6.3	84.4	6.3
	ECAM-453	F	33	3.0	9.1	84.8	3.0
	ECAM-454	F	25	16.0	8.0	72.0	4.0
	ECAM-455	M	50	6.0	14.0	74.0	6.0
	ECAM-456	F	38	5.3	7.9	81.6	5.3
	ECAM-457	F	51	5.9	7.8	78.4	7.8
	ECAM-458	M	18	5.6	5.6	77.8	11.1
	ECAM-459	M	21	0.0	9.5	85.7	4.8
	ECAM-460	M	13	0.0	7.7	92.3	0.0
	ECAM-461	M	40	0.0	10.0	87.5	2.5
	ECAM-462	F	37	5.4	8.1	86.5	0.0
	ECAM-463	M	43	4.7	4.7	83.7	7.0
	ECAM-464	M	62	1.6	8.1	87.1	3.2
	Subtotal		901	4.5	14.9	75.5	5.1
	TOTAL		1440	3.5	11.9	79.2	5.45

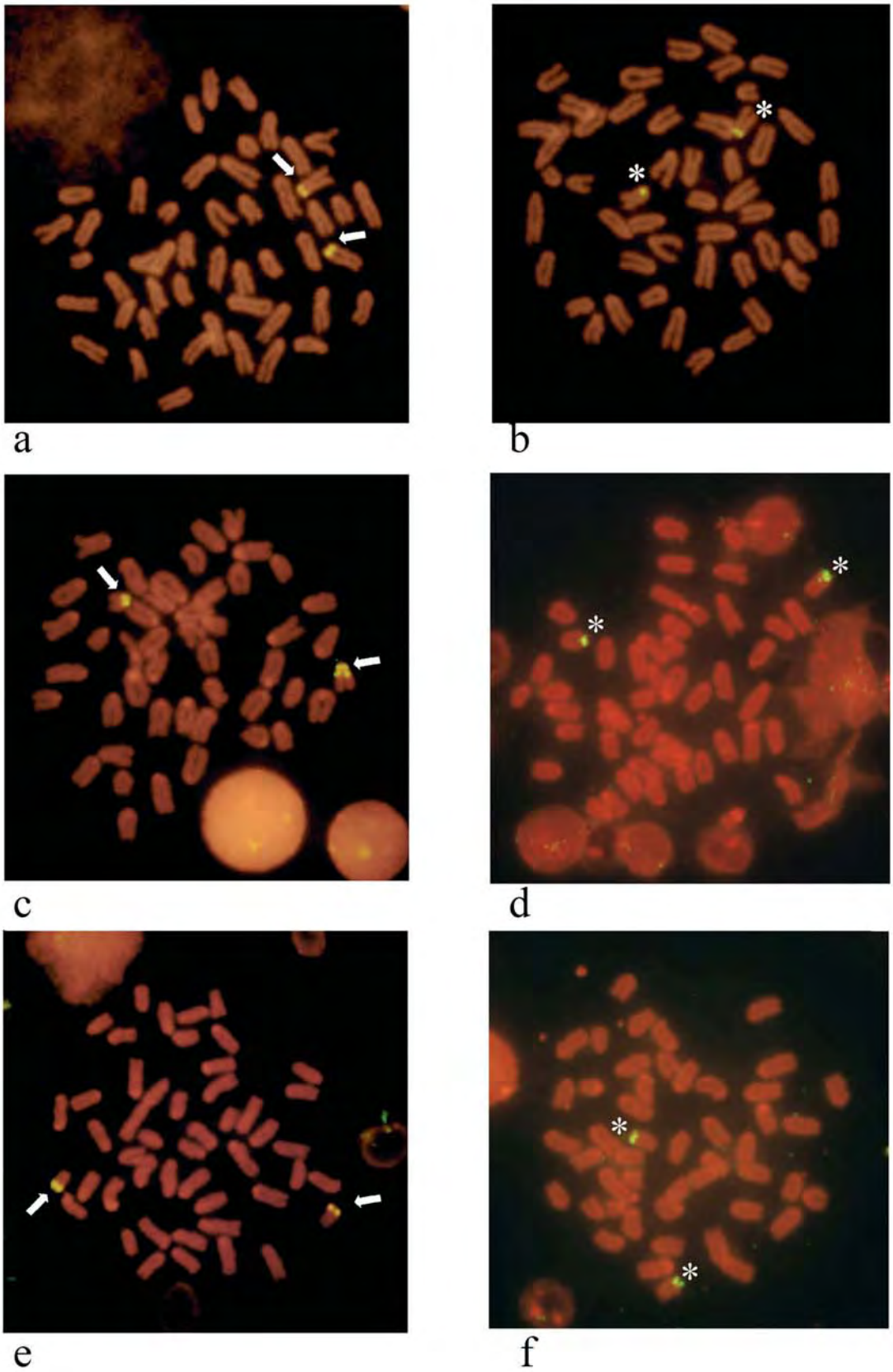

Fig. 5. Metaphases of *Lutjanus analis* (a, b), *L. synagris* cytotype I (c, d) and *L. synagris* cytotype II (e, f) after FISH with 18S rDNA (left) and with 5S rDNA (right). Arrows indicate the NOR bearing chromosomes. Asterisks indicate the 5S rDNA bearing chromosomes.

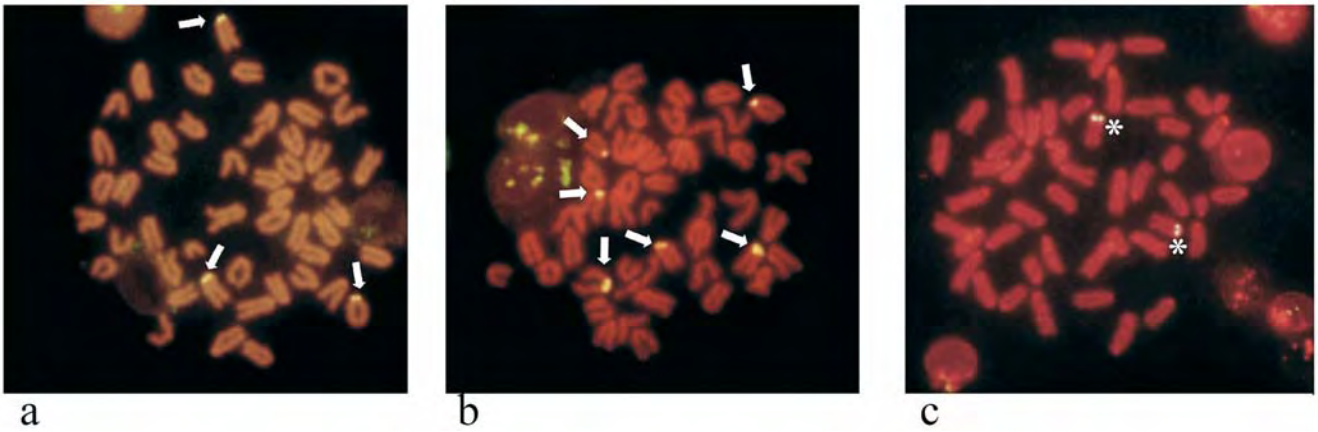

Fig. 6. Metaphases of *Lutjanus griseus* after FISH with 18S rDNA (arrows) in a female (a) and in a male (b) and (c) with 5S rDNA (asterisks).

of all chromosomes, a pattern similar to the one reported for the Pacific *L. kasmira* and *L. quinquelineatus* (Ueno & Takai, 2008), the only two, among the five karyologically investigated Lutjaninae species, for which data are available. Although the heterochromatin distribution does not identify species-specific chromosome markers, it is worth noting that considerably different exposition times to BaOH, during the C-banding procedure, were applied, from 4 seconds in *L. analis*, to 30 seconds in *L. synagris* and up to 180 seconds in *L. griseus*, a fact which might reflect differences in chromatin condensation levels and/or composition among the species of Lutjaninae here studied.

As far as NORs are concerned, different aspects can be considered. The first one is related to the methodology. In fish, NORs are usually indirectly visualized by silver staining (Nirchio & Oliveira, 2006), which localizes the transcribing major ribosomal genes because silver precipitates in correspondence to a rRNA protein complex synthesized only when NORs are active in the preceding interphase (Hubbel 1985, Sánchez-Pina *et al*. 1984, Jimenez *et al*. 1988). However, recent evidence in some fish species (Gromicho *et al.*, 2005) have cast doubt on the accuracy and reliability of Ag in NORs detection, both because other regions, besides NORs, can be stained with Ag or because most of the 28S rDNA sites, as revealed by FISH, were not detected. In the studied species of Lutjaninae, all the Ag-positive sites were also FISH-positive, so the technique is NOR-specific, but FISH revealed the presence of additional and inactive NORs in only one of them, *L. griseus*, confirming that this species has a higher NOR variability, both in their number and location, compared to the other congeneric species. As far as this variability is concerned, though a sex-associated trend is identifiable, as males show a higher number of structural NORs, compared to females, a larger sample is needed to verify the extent and the basis of this variability.

A further aspect is related to the cytotaxonomic considerations. A single pair of NOR-bearing chromosomes has been suggested to represent the primitive karyotypical condition in most vertebrate species (Hsu *et al.*, 1975; Schmidt, 1978). In most of the teleost fish displaying the primitive karyotype with 48 acrocentric chromosomes, two terminal NORs near the centromere can be found (Vitturi *et al.*, 1995). Therefore, on one hand, a single NOR bearing chromosome pair, with ribosomal sites interstitially located, as observed in *L. analis* and *L. synagris* (present study) and in *L. kasmira* and *L. quinquelineatus* (Ueno & Takai, 2008), could be considered a plesiomorphic condition for *Lutjanus,* whereas the presence of multiple NOR bearing chromosomes, found in *L. griseus*, would be an apomorphic feature. These data would indicate that *L. analis* and *L. synagris* are more closely related, and that the occurrence of duplication and translocation of ribosomal genes in *L. griseus* might reflect an important mechanism involved in the diversification of this taxon. On the other, when considering NORs location, a different perspective highlights. Indeed, the single NOR-bearing chromosome pair observed in *L. analis* (chromosome pair 6), as well as one of the NOR-bearing chromosome pairs observed in *L. griseus,* when pursuing a parsimonious criterion, appears to be homeologous to the one identified both in *L. kasmira* and *L. quinquelineatus* (Ueno & Takai, 2008), and, thus, it could be regarded as the primitive condition in the genus. As a consequence, no homeology could be identified with the NOR-bearing chromosome pair of *L. synagris*. Similar ambiguous results were obtained from molecular and morphological data. Indeed, in a phylogenetic study of Lutjanidae, based on isozyme and morphological data, Chow & Walsh (1992) suggested that *L. analis* and *L. synagris* form a clade, which is a sister group of *L. griseus.* However, subsequent molecular phylogenetic studies, based on mitochondrial DNA (Sarver *et al*., 1996) have not provided a similar clear-cut subdivision.

FISH experiment with 5S rDNA, revealed their location on a chromosome which is clearly distinct from the one (number 24) bearing major rDNA genes in *L. synagris*, but, though the difference in size is smaller, also from the NOR bearing chromosome pair number 6 of *L. analis* and *L. griseus*. The 5S rRNA gene sites are usually located in separate areas from 45S rRNA genes of the genome in higher eukaryotes (Martins & Galetti, 1999). This is probably because a separate location may permit them to independently evolve, since the divergent functional dynamics of these sequences require physical distancing (Martins & Galetti, 2000).

In conclusion, data here obtained for *Lutjanus analis*, *L. griseus* and *L. synagris*, along with those previously reported for *L. kasmira* and *L. quinquelineatus* (Ueno & Takai, 2008), show that a general chromosome homogeneity occurs within the family, but that derived karyotypes based on Robertsonian rearrangements as well as multiple and variable NORs sites can also be found. Phylogenetic relationships among species remained unresolved and further data on the other species of *Lutjanus* are needed to obtain a more general picture of the karyoevolutive trends in the family.

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Cytogenetic characterization of *Rhomboplites aurorubens* and *Ocyurus chrysurus*, two monotypic Lutjaninae from Cubagua Island, Venezuela, with a review on cytogenetics of Lutjanidae (Teleostei: Perciformes)

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Abstract

The family Lutjanidae, commonly known as snappers, includes 105 species, grouped in four subfamilies. In spite of the high number of species and of its worldwide distribution, the family is poorly investigated and phylogenetic relationships among some of its genera and species are still debated. Only a small number of the species have been analysed karyologically. This study reports the first description of the karyotype of *Rhomboplites aurorubens* as well as data on the distribution of the constitutive heterochromatin and on the location of the 18S rRNA and the 5S rRNA genes. Specimens of *Ocyurus chrysurus* from Venezuela were also investigated for the same cytogenetic features. Both species have the 48 uniarmed karyotype, but *R. aurorubens* has a single subtelocentric chromosome pair, the smallest of the chromosome complement, among the other acrocentric chromosomes. The C-positive heterochromatin is limited to the pericentromeric regions of all chromosomes. Both species show a single chromosome pair bearing the Nucleolus Organizer Regions, but NORs are differently located, in paracentromeric position in a chromosome of large size in *O. chrysurus* and on the short arms of the smallest chromosomes in *R. aurorubens*. In *O. chrysurus*, the 5S rDNA gene cluster is located on a medium size chromosome pair, whereas in *R. aurorubens* is syntenic with the 18S rDNA gene cluster on chromosome pair number 24. The obtained cytogenetic data, along with previous cytogenetic, morphological and molecular data in the family, reinforce the proposal to synonymize genus *Ocyurus* with *Lutjanus*. A review on Lutjanidae cytogenetics is also reported

INTRODUCTION

Snappers (Perciformes, Lutjanidae) are reef-associated marine fishes worldwide distributed along the tropical and sub-tropical regions. The family includes 105 species grouped in four subfamilies (Paradichthyinae, Etelinae, Lutjaninae and Apsilinae) and 17 genera (Nelson, 2006). Lutjaninae is the largest subfamily and includes approximately 70 species, grouped in six genera: *Lutjanus*, with 64 species, *Macolor* and *Pinjalo*, with two species each, and three monotypic genera, *Hoplopagrus*, *Ocyurus* and *Rhomboplites* (Nelson, 2006). In spite of the high number of species and of its worldwide distribution, the family is poorly investigated, and contradictory results are obtained on the phylogenetic relationships and the taxonomic status of some of its genera and species. As an example, the validity of the genus *Ocyurus* has been extensively discussed (Anderson, 1967; Davis *&* Birdsong, 1973; Domeier *&* Clarke, 1992; Chow *&* Walsh, 1992; Loftus, 1992), leading some authors to propose the synonymization of *Ocyurus* with the genus *Lutjanus* (Loftus, 1992; Clarke *et al*., 1997).

Until quite recently, the number of snapper species whose karyotype had been described was limited to four (Rishi, 1973; Choudhury *et al*., 1979; Raghunat & Prasad, 1980; Ueno & Ojima, 1992) and for three of them only the Giemsa features had been reported. However, in 2008 three different studies added new data, mainly on species of genus *Lutjanus* (Nirchio *et al*., 2008; Rocha & Molina, 2008; Ueno & Takai, 2008), reporting karyotypes as well as several finer cytogenetic features. All these studies have shown that a general chromosome homogeneity occurs within the family, but that derived karyotypes can also be found, whose phylogenetic interpretation remains unclear. Therefore, further data on other snapper species are needed to obtain a more general picture of the karyoevolutive trends in the family.

This study continues a cytogenetic survey of Venezuelan snappers (Nirchio *et al*., 2008, Table 1) by extending the investigation to the two monotypic genera, *Ocyurus chrysurus,* the yellowtail snapper*,* and *Rhomboplites aurorubens*, the vermilion snapper, which, along with the *Lutjanus* species, represent the three genera of Lutjaninae, with a total of 12 species, existing in Venezuela (Cervigón, 1993). The two monotypic species have an almost overlapping western Atlantic distribution, extending southwards from Massachusetts, USA, and Bermuda to southeastern Brazil, including the Gulf of Mexico and Caribbean Sea (Allen, 1985; Froese & Pauly, 2008). This study reports the first description of the karyotype of *R. aurorubens* and reports, in both species, data on the distribution of the constitutive heterochromatin and the locations of the 18S rRNA and the 5S rRNA genes obtained by conventional (Giemsa staining, C-banding, silver staining) and molecular techniques (Fluorescent *In Situ* Hybridization - FISH). A review of the available karyological literature of Lutjanidae is also presented.

MATERIALS AND METHODS

Cytogenetic analyses were performed on 9 specimens of *Rhomboplites aurorubens* and on four unsexed specimens of *Ocyrus chrysurus* captured in

Cubagua Island, Venezuela. Voucher specimens were deposited at the Ichthyology collection of the Escuela de Ciencias Aplicadas del Mar (ECAM), Universidad de Oriente.

Twenty four hours prior to chromosome preparations, the fishes were injected intramuscularly with a yeast glucose solution (Lee & Elder, 1980) for mitosis stimulation. Chromosomes were obtained from kidney cells according to Foresti *et al*. (1993). C-bands were obtained according to the method described by Sumner (1972). For detection of the active Nucleolus Organizer Regions (NORs), the chromosome sites where major ribosomal genes -18S, 5.8S and 28S- are clustered, slides were stained with silver nitrate using the method of Howell & Black (1980).

The 18S and 5S rDNA sites were also identified by Fluorescent *In Situ* Hybridization (FISH), according to the method of Pinkel *et al*. (1986). A sequence of 1,800 base pairs of the 18S rRNA gene of *Oreochromis niloticus* (Nile tilapia), cloned in pGEM-T plasmid, was used as a probe to localize sites of 45S rDNA. Polymerase Chain Reaction (PCR) products containing 5S rDNA repeats from *O. chrysurus* were used as probe for the chromosome mapping of 5S rDNA. DNA was extracted from muscle (Sambrook & Russel 2001) and the 5S rDNA repeats were generated by PCR with the primers 5SA (5'TAC GCC CGA TCT CGT CCG ATC3') and 5SB (5'CAG GCT GGT ATG GCC GTA AGC3') according to Martins & Galetti (1999).

The 18S rDNA and 5S rDNA probes were labeled by PCR with biotin-14-dATP for the single-FISH experiments. In the case of *R. aurorubens*, a double-FISH with both probes was carried out by labeling the 18S rDNA with 11-dUTP digoxigenin and 5S rDNA with biotin-14-dATP. Biotin signals were detected and amplified by a two-round application of Avidin-FITC/biotinilated Anti-avidin. Digoxigenin signals were not amplified and were detect by Anti-digoxigenin/rhodamine. Chromosomes were counter-stained with Propidium Iodide (50µg/ml) or DAPI diluted in Antifade.

The mitotic figures were photographed using a Motic B400 microscope equipped with a Moticam 5000C digital camera. FISH metaphases were photographed with a photomicroscope Olympus BX61 equipped with a DP70 digital camera.

RESULTS

The counts of diploid metaphasic cells revealed a modal diploid number of 2n=48 uniarmed chromosomes (NF=48) in both species. However, in *R. aurorubens* (Fig. 1a) a single subtelocentric chromosome pair, the smallest of the chromosome complement, is present among the acrocentrics, while in *O. chrysurus* (Fig. 1b) all chromosomes are acrocentric. The chromosomes exhibit very small differences in size, thus, the precise classification of chromosomes in homologous pairs is not possible, with the exception of a large chromosome pair (numbered 6) in *O. chrysurus*, which shows a conspicuous secondary constriction, and chromosome

pair number 24 in both species, clearly the smallest of the chromosome complement.

In both species C-banding showed C-positive blocks of heterochromatin at the pericentromeric regions of all chromosomes (Fig. 2a,b), quite conspicuous in one of the largest chromosome pairs of *R. aurorubens* (Fig. 2a). In this latter species also the short arms of the subtelocentric chromosome pair 24 are C-positive (Fig. 2a).

The analysis of the nucleolus organizer regions with the Ag-NOR technique sequential to Giemsa staining (Fig. 3), detected a maximum of two Ag-positive signals in both species. In *R. aurorubens* the Ag-positive signals are located along chromosome pair 24 short arms (Fig. 3b), which are often heteromorphic and may appear heteropycnotic in Giemsa (Fig. 3a). In *O. chrysurus* the Ag-positive signals are located proximally to centromeres (Fig. 3d), on the secondary constriction evident on chromosomes 6 in Giemsa-stained metaphases (Fig. 3c).

FISH with the 18S rDNA probe confirmed the unique location of NORs on the short arms of chromosome pair 24 in *R. aurorubens* (Fig. 4a) and on paracentromeric positions of chromosome pair number 6 in *O. chrysurus* (Fig. 4c). FISH with 5S rDNA produced one hybridization signal close to the centromere of the smallest subtelocentric chromosome pair in *R. aurorubens* (Fig. 4b) and of a medium-sized acrocentric chromosome pair in *O. chrysurus* (Fig. 4d). Thus, the double FISH (Fig. 4a-b) shows that both ribosomal gene clusters are located on the same chromosome pair in *R. aurorubens*.

DISCUSSION

By adding the chromosome complement of *R. aurorubens*, reported in this study, to the Lutjanidae database, the number of the species of the family so far cytogenetically analysed rises to 13 (Table 1), out of the approximately 105 recognized species (Nelson, 2006).

The 48 all-acrocentrics karyotype here reported for the examined specimens of *O. chrysurus* is consistent with data reported by Rocha & Molina (2008) for specimens from the Brazilian north-eastern coast. This karyotype is shared by 10 out of the 13 species so far analyzed (Table 1). The second species reported in this paper, *R. aurorubens* shows instead a karyotype characterized by the presence of a subtelocentric chromosome pair, which is a novelty among the so far studied species of the family. The remaining two species, *Lutjanus quinquelineatus* (Ueno & Takai, 2008) and *L. synagris* (Nirchio *et al*., 2008), show Robertsonian rearrangements (Table 1), so that one metacentric chromosome, among the remaining 46 acrocentric chromosomes, is present in males of *L. quinquelineatus* (Ueno & Takai, 2008), and in two out the 21 examined specimens of *L. synagris* (Nirchio *et al*., 2008). Thus, in the former species, the Robertsonian rearrangement is related to the presence of a chromosomal sex determination mechanism, while in the latter it is apparently unrelated to sex and reflects an intra-specific

chromosome polymorphism. It is worth to notice that specimens of *L. synagris* from Brazil (Rocha & Molina, 2008) do not show any biarmed chromosome.

The pattern of the heterochromatin distribution observed in *O. chrysurus* and *R. aurorubens* confirms that a limited presence of heterochromatic blocks at the centromeres of all chromosomes is a general picture for Lutjanidae, as this pattern is shared by all the 11 species investigated in this sense (Table 1), including those species for which more than one population has been studied, such as *L. analis*, *L. synagris* (Nirchio *et al*., 2008; Rocha & Molina, 2008) and *O. chrysurus* (Rocha & Molina, 2008; this paper). This evidence suggests that heterochromatinization processes have not played an important role in the karyotypic evolution of Lutjanidae.

Regarding the number and location of the major ribosomal genes, in *O. chrysurus* and *R. aurorubens*, the silver staining, which generally detects those NORs which are active in the preceding interphase (Hubbel, 1985; Jimènez *et al*., 1988; Sánchez-Pina *et al*., 1984), produced overlapping results to those obtained by FISH with 18S rDNA, i.e., two NOR sites were detected in both species which show, however, different locations, on different chromosome pairs. It is useful to frame the obtained results within the whole data on nucleolar organizer regions in the family, mostly obtained by silver staining, in 11 species (Table 1). Most (nine) of the species show a single chromosome pair coding for major ribosomal genes. The remaining two species, *L. griseus* and *L. jocu*, show other variable NOR sites in addition to the main species-specific NOR-bearing chromosome pair.

The precise classification of chromosome pairs is difficult due to the small differences in chromosome size and to the absence of banding. However, in spite of this, at least three different main karyomorphs can be identified with certainty, summarized in Fig. 5. A group of seven species shows a large NOR-bearing chromosome pair and NORs are located in a paracentromeric position. Different authors, in different studies (Table 1) classified this chromosome pair as number 2, 5 or 6. However, in all species, in the Giemsa-stained metaphases this chromosome pair shows a large secondary constriction, corresponding to the NORs site, which may affect chromosome size and therefore its classification. Thus, pursuing a parsimonious criterion this pair might be considered homeologous in this group of species (Fig. 5a). Two species, *L. synagris* and *R. aurorubens,* show NORs on the smallest chromosome pair, but their location is clearly different, representing therefore the two remaining karyomorphs. In *L. synagris* (Fig. 5b)*,* NORs are located in a paracentromeric position of chromosome pair 24 (Nirchio et *al*., 2008; numbered 23 by Rocha & Molina, 2008), in *R. aurorubens* (Fig. 5c) NORs are located on the short arms of this same chromosome pair, which is, differently from the other Lutjanidae species, the only one made by subtelocentrics in the chromosome complement.

Considering that the karyomorph of Fig. 5a is the most common, it could be tentatively assumed that this might reflect the plesiomorphic condition in the family from which karyotypes with additional NORs (*L. griseus*, Nirchio et *al*., 2008; *L.*

jocu Rocha & Molina, 2008) or with different locations of NORs (*L. synagris*, Fig. 5b*,* Nirchio et *al*., 2008; *R. aurorubens*, Fig. 5c, present paper) derived.

As far as the 5S rDNA sites are concerned, by adding the results here reported for *O. chrysurus* and *R. aurorubens*, data are available for only five species (Table 1). In *O. chrysurus* the minor ribosomal genes show a number and location corresponding to those observed in the other three species, *Lutjanus analis, L. synagris* and *L. griseus* (Nirchio et al., 2008), so far investigated, i.e., a single site in paracentromeric position of a medium-sized acrocentric chromosome pair, which might be homeologous in all of them, numbered as 9 in Table 1 and Fig. 5 (a,b). *R. aurorubens* similarly shows a single pair of 5S rDNA bearing chromosomes, however, this pair is certainly different, being the smallest of the chromosome complement, where major ribosomal genes are co-located (Fig. 5c). The syntenic organization of 45S and 5S rDNA loci is quite uncommon in vertebrates and in fish in particular (Martins 2007).

By framing these cytogenetic data within a systematic and phylogenetic context, some considerations concerning the validity of genus *Ocyurus* and the relationships among the Lutjaninae species can be made.

There is a long term debate regarding the validity of the genus *Ocyurus.* According to Domeier & Clarke (1992), the morphological characters in *Ocyurus*, which allows the separation of this genus from *Lutjanus*, are merely adaptations to a pelagic lifestyle. On the other hand, the numbers of morphological and meristic similarities among these genera are far greater. In addition, Domeier & Clarke (1992) and Loftus (1992), based on the evidence of natural and laboratory hybrids between *L. synagris* (and likely *L. griseus*) and *O. chrysurus*, claimed *Ocyurus* can not represent a distinct evolutionary lineage from *Lutjanus*. Finally, according to Clarke *et al*. (1997), also the resemblance of the larval forms provides further evidence for the synonymization of the two genera.

Similar evidences, suggesting the synonymization of *Ocyurus* with *Lutjanus*, were obtained using molecular markers. In fact, in a phylogenetic study based on mitochondrial 12S rRNA and cytochrome b genes sequences of 14 species of snappers occurring in western Atlantic, Sarver *et al*. (1996) emphasized that the single most-parsimonious tree obtained from analysis of weighted characters placed *Ocyurus* in a clade with the red snapper group (*Lutjanus campechanus* and *L. vivanus*); whereas the strict consensus of the three most-parsimonious trees from analysis of unweighted characters placed it in a polytomy with several species of *Lutjanus* and the monotypic genus *Rhomboplites*. In addition to these, recent mitochondrial data (16S rDNA and cytochrome b) by Miller & Cribb (2007), aimed at investigating phylogenetic relationships of Indo-Pacific snappers (27 species), with the inclusion of three western Atlantic snappers, showed that the genus *Lutjanus* is paraphyletic.

In this context, *O. chrysurus* share the cytogenetic features shown by most of the species of *Lutjanus* from both Indo-Pacific and western Atlantic Oceans while *R. aurorubens* shows the most derived features compared to them. Therefore,

cytogenetic data here reported, while supporting the classification of *Rhomboplites* as a monotypic genus, do not rule out the inclusion of *Ocyurus chrysurus* into the genus *Lutjanus.*

Considering the more general picture of karyotype evolution in Perciformes, the presence of 48 chromosomes has been regarded as a primitive condition for this fish group (Accioly & Molina 2008, Galetti Jr. *et al*., 2000, 2006). It can be stated that there can be found both a extreme karyotype conservativeness, as for example in the Haemulidae, as well as tendencies for karyotype differentiation, such as in Gobiidae (Molina 2007). The marine familiy of Lutjanidae, including the species studied in this work, show a higher level of chromosomal stability, compared to closely related taxa like Sparidae (Johnson 1980, Orrell & Carpenter 2004, Miller & Cribb 2007), which shows higher levels of chromosomal diversification. Nevertheless, in spite of their morphologically conservative karyotype, when investigated for the finer cytogenetic features, Lutjaninae species were found to have undergone to a certain degree of chromosome divergence

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Figure Captions

Fig. 1. Giemsa-stained karyotypes of *Rhomboplites aurorubens* (a) and *Ocyurus chrysurus* (b)*. Bar = 10 µm.*

Fig. 2. C-banded metaphases of *Rhomboplites aurorubens* (a) and *Ocyurus chrysurus* (b)

Fig. 3. Metaphases of *Rhomboplites aurorubens* (a; b) and *Ocyurus chrysurus* (c; d) sequentially stained with Giemsa (left) and $AgNO_3$ (right). Arrows indicate the NOR-bearing chromosomes.

Fig. 4. Metaphases of *Rhomboplites aurorubens s* (a; b) and *Ocyurus chrysurus* (c; d) after FISH with 18S rDNA (left) and with 5S rDNA (right). Arrows indicate the NOR bearing chromosomes. Arrowheads indicate the 5S rDNA bearing chromosomes

Fig. 5. Idiograms reporting the major and minor ribosomal genes locations summarizing the three main karyomorphs identified in the so far investigated species of Lutjanidae. *Solid circles* represent locations of 18S rDNA. *Grey bars* represent locations of 5S rDNA.

Fig. 1

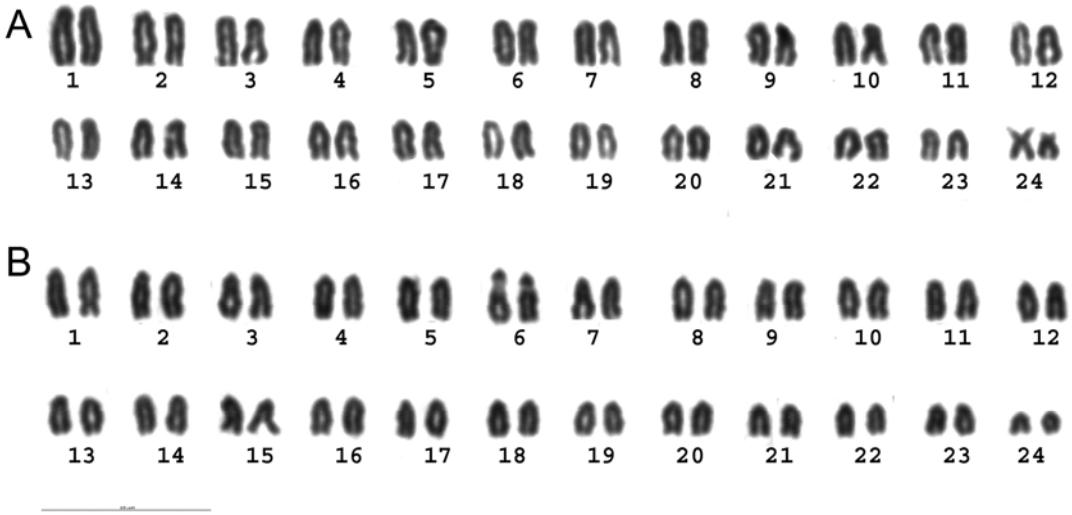

A
1 2 3 4 5 6 7 8 9 10 11 12
13 14 15 16 17 18 19 20 21 22 23 24
B
1 2 3 4 5 6 7 8 9 10 11 12
13 14 15 16 17 18 19 20 21 22 23 24

FIG. 2

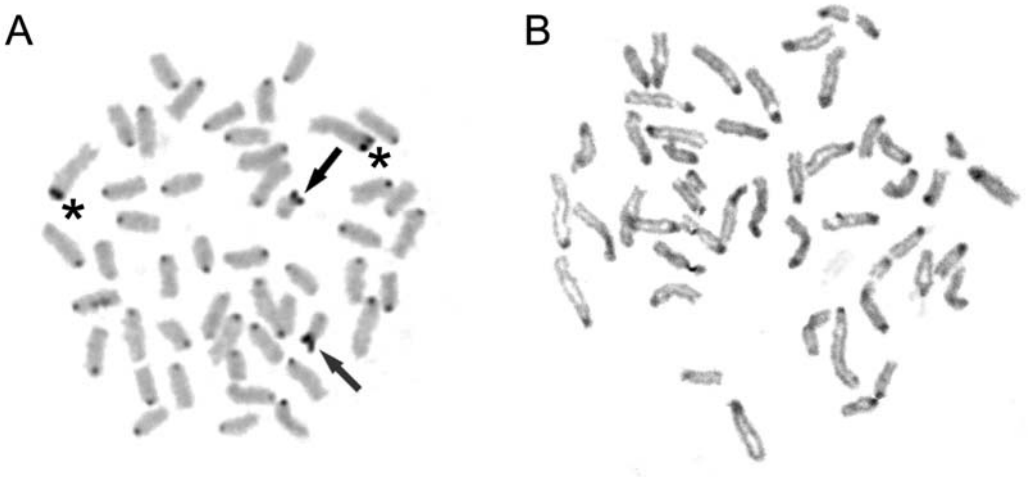

A
B

Fig. 3

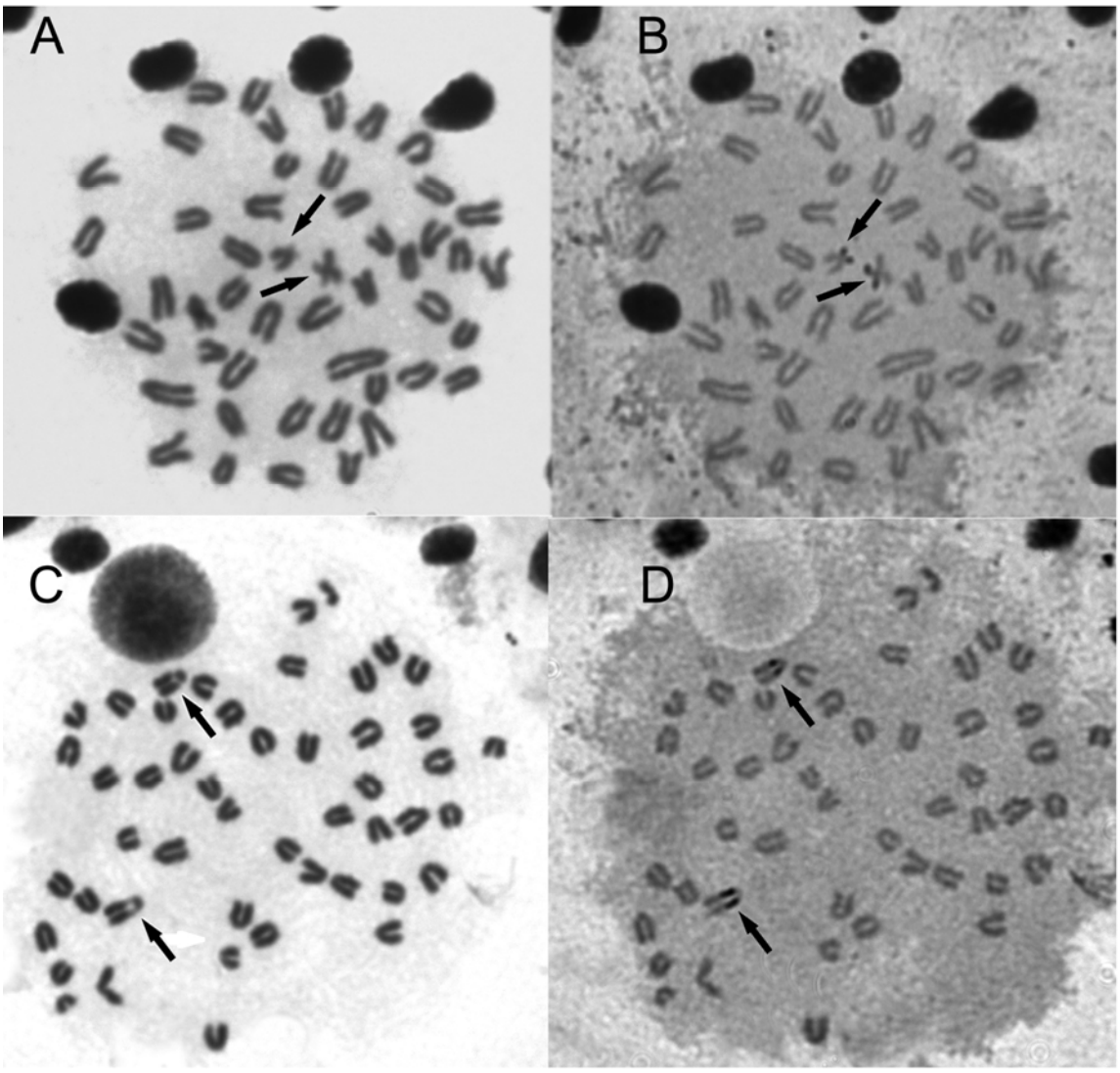


FIG. 4

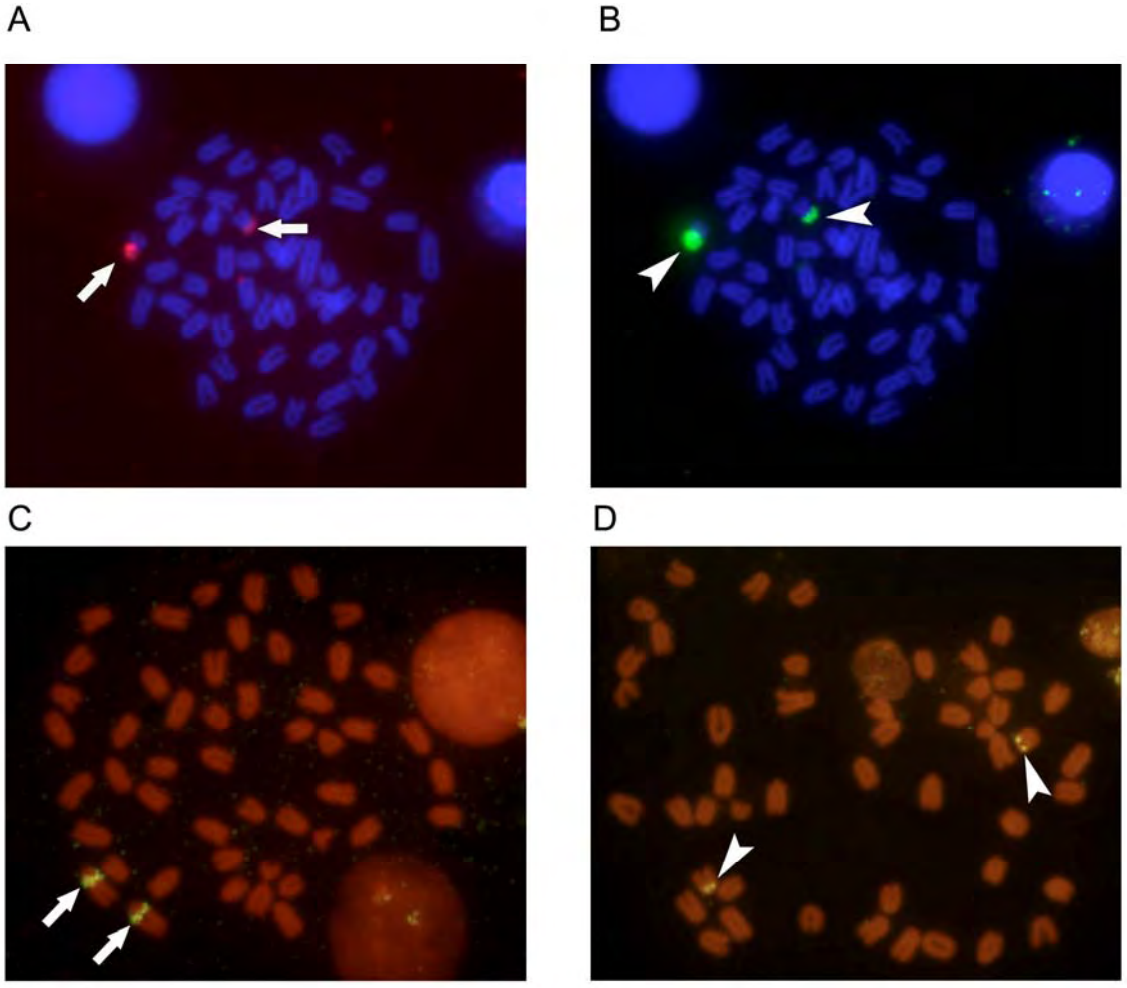
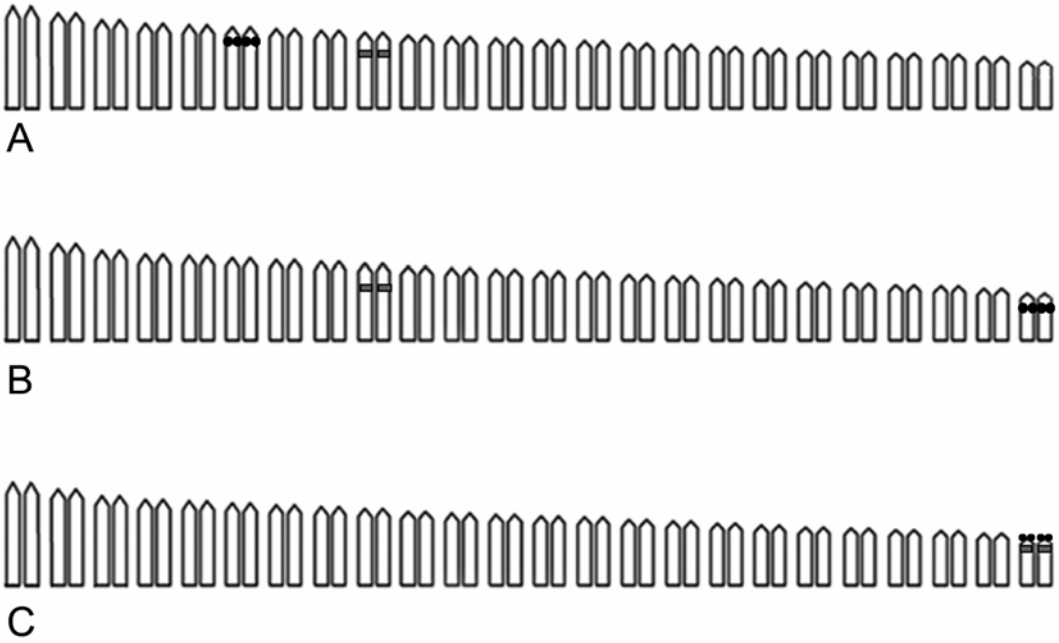


FIG.5



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ORIGINAL PAPER

Cytogenetic analysis of three species of the genus *Haemulon* (Teleostei: Haemulinae) from Margarita Island, Venezuela

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Abstract This paper describes the karyotype analysis of *Haemulon aurolineatum*, *Haemulon bonariensis* and *Haemulon plumierii*, by Giemsa staining, C-banding, Ag-staining and fluorescent in situ hybridization (FISH), to locate the 18S and 5S rRNA genes. Diploid modal count in the three species was $2n = 48$ acrocentric elements. Except for pair 24, which exhibited an unmistakable secondary constriction in all three species, it was not possible to classify them as homologous to each other because differences in chromosome size were too slight between adjacent pairs within a size-graded series. Ag-NOR clusters were located in pair 24 in the three species with signal located on the secondary constriction of these chromosomes. C-banding demonstrated that the three species share the same distribution pattern of the constitutive heterochromatin with centromeric heterochromatic blocks in the 23 chromosome pairs and a pericentromeric block in pair 24 which is coincident with the NORs. FISH experiments showed that 18S rDNA sequences were located coincident with the Ag-NOR site in the three species; however, differences in both the number and chromosome distribution of 5S-rDNA cluster were detected among them. Our data suggest that chromosome evolution of *Haemulon* has been preserved from major changes in the karyotypic macrostructure, whereas microstructural changes have occurred.

Keywords C-banding · FISH · NOR · 5S rDNA · 18S rDNA · Perciformes · Haemulidae

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Introduction

The family Haemulidae (grunts) comprises coral-reef fishes found in abundance within the greater Caribbean area (Randall 1996; Lindeman 2002). In tropical regions, grunts are commercially (Appeldoorn and Lindeman 1985) and ecologically (Meyer and Schultz 1985) important. Haemulidae contains 17 genera and approximately 150 species (Nelson 1994) grouped into two different sub-families: Haemulinae and Plectorhynchinae, the latter not represented in the Americas (Cervigón 1993).

The genus *Haemulon* belongs to Haemulinae and comprises fishes of small size and lengthened body, laterally compressed, which in most cases exhibit fringes or lines of vivid colors in contrast with the general color of the bottom. Fourteen species have been recognized in Venezuela (Cervigón 1993). Despite their importance to coral-reef ecology, little is known about their life history and genetics.

Cytogenetic data on Haemulidae are scarce and less than 10% of the 150 recognized species have been karyotyped (Lima and Molina 2004). This paper describes the karyotype of *Haemulon aurolineatum*, *Haemulon bonariense*, and *Haemulon plumierii* by means of Giemsa staining, C-banding, silver staining

and Fluorescent in situ hybridization (FISH) to locate the ribosomal genes (18S rRNA and 5S rRNA).

Materials and methods

A sample consisting of 12 specimens (8 females and 4 males) of *H. aurolineatum*, 18 specimens (7 females and 11 males) of *H. bonariense* and six specimens (4 females and 2 males) of *H. plumierii* were analyzed. All specimens were collected around Margarita Island, Venezuela, and voucher specimens were deposited in the fish collection of the Escuela de Ciencias Aplicadas del Mar, Universidad de Oriente.

Chromosome preparation followed the technique described by Foresti et al. (1993). For the conventional karyotype, the preparations were stained for 20 min with 5% Giemsa in phosphate buffer pH 6.8. Detection of the Nucleolus Organizer Regions (NORs) was done following the silver ($AgNO_3$) staining method of Howell and Black (1980). C-bands were obtained according to the method described by Sumner (1972).

The 5S and 18S rDNA sites were identified using FISH, according to the method described by Pinkel et al. (1986). A segment of 1,800 base pairs of the 18S-rRNA gene of *Oreochromis niloticus* (Nile tilapia) cloned in pGEM-T plasmid was used as a probe to locate the sites for the 45S rDNA. PCR products containing 5S rDNA repeats from *H. aurolineatum* were used as probes for the chromosome mapping of 5S rDNA. The DNA was extracted from muscle (Sambrook and Russel 2001), and the 5S rDNA repeats generated by Polymerase Chain Reaction (PCR) with the primers 5SA (5′TAC GCC CGA TCT CGT CCG ATC3′) and 5SB (5′CAG GCT GGT ATG GCC GTA AGC3′), according to Martins and Galetti (1999).

The 18S rDNA and 5S rDNA sequences were labeled by nick translation with biotin-14-dATP, following the manufacturer's (Bionick™ Labelling System-Gibco.BRL) instructions. The 18S rDNA and 5S rDNA probes were located in the chromosomes by Avidin-*N*-fluorescein Isothiocyanate (FITC) conjugate and the signal was enhanced by using biotinylated goat Antiavidin antibodies following a second round of Avidin-FITC detection. Chromosomes were counterstained with propidium iodide diluted in Antifade Vector (50 μg/ml).

Giemsa, Ag-stained and C-banded mitotic chromosomes were photographed using a digital camera and the images were digitally processed with Adobe Photoshop v. 7.0 software. The karyogram was constructed with chromosomes organized in order of decreasing size and the chromosomes classified according to Levan et al. (1964). Metaphases analyzed through FISH were examined with a Zeiss Axiophot photomicroscope and Kodak Gold Ultra 400 ASA film was used to take the pictures.

Results and discussion

The chromosome diploid modal count in the three species studied was $2n = 48$ acrocentric elements, obtained from 70.30%, 74.07%, and 76.10% of all the cells examined in *H. aurolineatum*, *H. bonariense* and *H. plumierii*, respectively. Although low, the frequency of cells with hypomodal and hypermodal chromosome numbers in the three species probably resulted from chromosome loss, overlap, miscounting, and additional chromosomes from another spread.

The representative karyotype for each species obtained by arranging the chromosomes in order of decreasing size is shown in Fig. 1. With the exception of chromosome pair 24, which presented a clear secondary constriction in all three species, it was not possible to accurately classify chromosomes as homologous pairs, since differences in chromosome size were too slight between adjacent pairs within a size-graded series. No differences were observed between male and female karyotypes. Species of Haemulidae karyotyped so far are showed in Table 1. All these species share $2n = 48$ acrocentric chromosomes with the exception of *H. sciurus* (Reagan et al. 1968), which has been reported as possessing a bimodal karyotype suggesting the possibility of a chromosome polymorphism with two cytotypes in the population ($2n = 46$ and $2n = 48$).

Although several authors (Ohno 1974; Garcia et al. 1987; Vitturi et al. 1991) suggest that the 48 uniarmed-chromosome type represents the ancestral complement in fish of diploid origin, Brum and Galetti (1997) theorized that this characteristic should not be assumed as a primitive feature, since most of the basal vertebrates like Agnatha, Condrichthyes, and Sarcopteygii possess higher diploid ($2n$) and fundamental numbers (FN) like Chondrostei, Gynglimodi, Halecomorpha, Osteoglossomorpha and Elopomorpha. Based on this observation, the basic chromosome number in Teleostei would be approximately $2n = 60$, with few metacentric chromosomes. Thus, the diploid number $2n = 48$ could have arisen, at the macrostructural level, through fusions and deletions from the ancestral karyotype and extended as a synapomorphic character in the Clupeomorpha and Euteleostei, which could have conserved this karyotype mainly in the marine species belonging to the Atherinomorpha and Percomorpha (Brum and Galetti 1997).

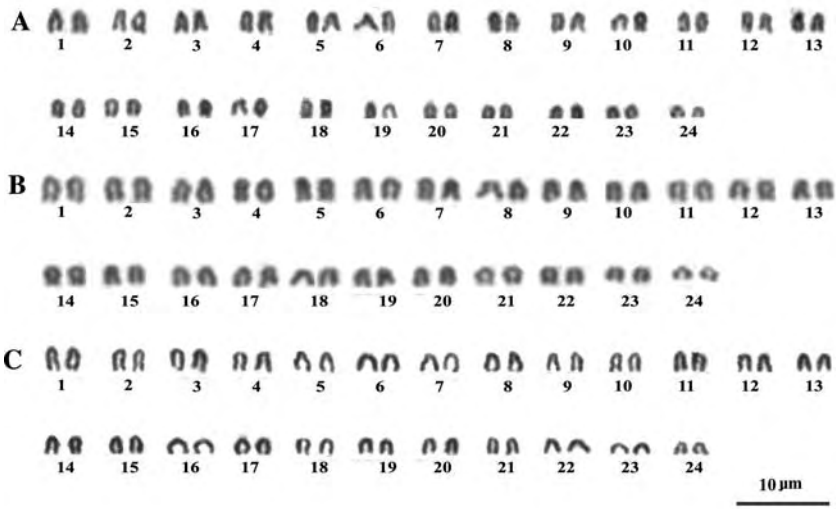

Fig. 1 Karyotypes of *Haemulon aurolineatum* (**A**), *H. bonariense* (**B**), and *H. plumierii* (**C**) after Giemsa staining

C-banding (Fig. 2A–C) revealed that the three species share the same distribution of constitutive heterochromatin with centromeric heterochromatic blocks in the 24 chromosome pairs with the exception of *H aurolineatum*, which presented a conspicuous heterochromatic block coincident with the NOR sites on the secondary constriction of the chromosome pair 24 (Fig. 2A). This distribution of heterochromatin restricted to centromere is also shown by *H. flavolineatum* from Margarita Island, Venezuela (Ron and Nirchio 2005). On the other hand, pericentromeric and telomeric heterochromatic blocks have been reported in samples of *H. parra*, *H. striatum* and *H. plumierii* from Brazil (Lima and Molina 2004), suggesting that constitutive heterochromatin distribution could be applied as cytogenetic markers for the discrimination of species or populations in the Haemulidae family.

Silver staining of the selected metaphase spreads revealed that the NOR clusters are located in pair 24 in all three species studied. Ag-NOR signals were located interstitially on the secondary constriction of these chromosomes (Fig. 2D–F). In the genus *Anisotremus* the NORs are located on chromosome pair 18 (Accioly and Molina 2004). Lima and Molina (2004) were unable to accurately identify the NOR-bearing chromosomes in *H. parra*, *H. plumierii* and *H. striatum* from the western Atlantic because of the minute variation in size among chromosomes. In *H. flavolineatum* from Venezuela the NORs are located on chromosome pair 24 (Ron and Nirchio 2005).

FISH with rDNA-probing is a useful technique to characterize some species. It allows researchers to know whether differences in Ag-NOR number are related only to the differential activities of these re-

Table 1 Diploid number (2*n*), chromosome arm number (FN), Karyotype formula, number and position of Nucleolus Organizer Regions (NORs) and constitutive heterochromatin distribution (C-Bands) in different Haemulidae species

Species	2*n*	FN	Karyotype formula	NORs	C-bands	References
Anisotremus moricandi	48	48	48a	1 pair (I)	–	Accioly and Molina (2004)
Anisotremus surinamensis	48	48	48a	1 pair (I)	–	Accioly and Molina (2004)
Anisotremus virginicus	48	48	48a	1 pair (I)	–	Accioly and Molina (2004)
Haemulon aurolineatum	48	48	48a	–	–	Duran et al. (1990)
Haemulon flavolineatum	48	48	48a	1 pair (I)	C	Ron and Nirchio (2005)
Haemulon parra	48	48	48a	1 pair (I)	P and T	Lima and Molina (2004)
Haemulon plumierii	48	48	48a	1 pair (I)	P and T	Lima and Molina (2004)
Haemulon sciurus	46	48	2 sm/st, 44a	–	–	Reagan et al. (1968)
Haemulon sciurus	48	48	48a	–	–	Reagan et al. (1968)
Haemulon striatum	48	48	48a	1 pair (I)	P and T	Lima and Molina (2004)
Orthopristis ruber	48	50	2 sm, 36st, 10a	–	C and T	Brum (1996)
Orthopristis ruber	48	48	48a	–	–	Brum (1996)
Pomadasys corvinaeformis	48	48	48a	1 pair (I)	C and P	Accioly and Molina (2004)

sm = submetacentric; st = subtelocentric; a = acrocentric; C = centromeric; T = telomeric; P = pericentromeric; I = interstitial

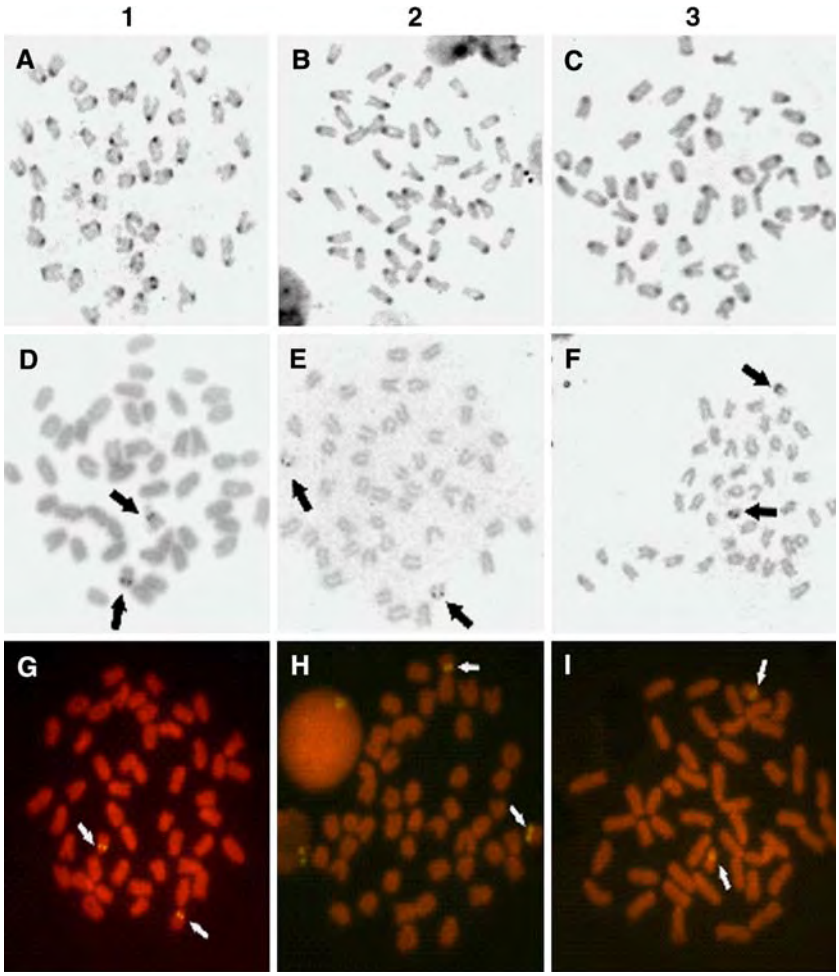

Fig. 2 Metaphase plates after C-banding (**A–C**), Ag-NOR (**D–F**); and FISH with 18S rDNA probe (**G–I**). Species are arranged in columns: *Haemulon aurolineatum* (1), *H. bonariense* (2), and *H. plumierii* (3). Arrows indicate localization of positive signals (see ''Results and discussion'')

gions or to different numbers of NORs in the genome of diverse species (Gornung et al. 1997; Nirchio et al. 2003, 2004; Gromicho et al. 2005). The FISH experiments performed here show that 18S rDNA signals coincide with signals seen in the Ag-NOR-bearing chromosomes (Fig. 2G–I), indicating that the three species here studied do not possess additional NOR sites and that all NOR-cistrons are active.

When 5S rDNA sequences were evidenced through FISH (Fig. 3a–c) it was observed that *H. aurolineatum* has two chromosome pairs marked by FISH. One pair presents two 5S rDNA clusters close to the centromeric

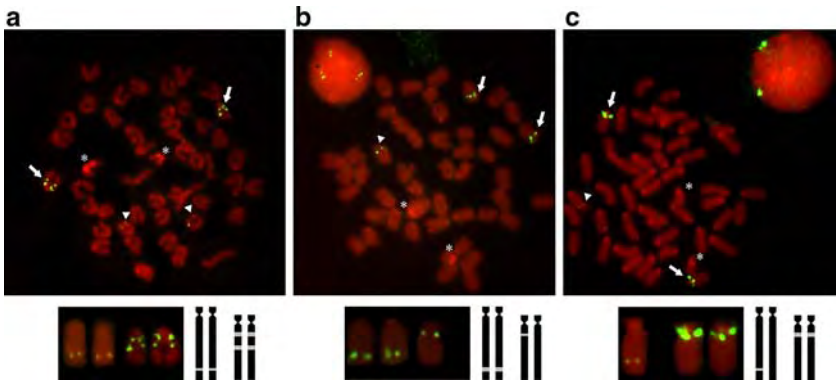

Fig. 3 Metaphase plates showing the 5S rDNA position. Arrows and arrowheads indicate location of strong and tenuous positive signals, respectively. Asterisks indicate NOR regions evidenced under the propidium iodide staining after FISH with the 5S rDNA probe. Details of 5S-bearing chromosomes are shown in the partial karyotype analysis and ideogram below each metaphase for *Haemulon aurolineatum* (**a**), *H. bonariensis* (**b**), and *H. plumierii* (**c**)

area and another pair has a single signal more tenuous than the double marks and is located next to the telomere of the long arm (Fig. 3a). On the other hand, *Haemulon bonariensis* presents conspicuous sites in the terminal position of the long arm of one chromosome pair and a faint site in a second chromosome (Fig. 3b). *H. plumierii* showed one chromosome pair with a strong signal close to the centromeric area and a second faint signal in the terminal position of a single chromosome (Fig. 3c). Sequential Ag-NOR staining of 5S rDNA/FISH probed showed that the 5S and 18S rDNAs are clustered in different chromosomes in the three Haemulidae analyzed (data not shown). Chromosomal sites of the NOR were also detected after the propidium iodide staining of the 5S rDNA/FISH probed metaphases (Fig. 3). The divergent locations of 18S and 5S rDNA loci seem to be the most common situation observed in fish (Martins and Wasko 2004), and by far the most frequent distribution pattern observed in vertebrates (Lucchini et al. 1993; Suzuki et al. 1996).

According to Sola et al. (1981), similar karyotypes are considered indicators of a relatively recent separation. Thus, the cytogenetic steadiness herein observed in the genus *Haemulon* and the substantial degree of similarity revealed by Cequea and Pérez (1971) through electrophoretic studies on haemoglobin and plasma protein in *H. steindachneri*, *H. bonariensis*, *H. flavolineatum*, *H. aurolineatum*, *H. chrysargyreum* and *H. plumierii* suggests the monophyletic origin of the genus *Haemulon* and indicates this group as one of recent diversification. Indeed, although the conserved diploid number, the presence and location of secondary constrictions on chromosome 24, the 18S rDNA cluster location and activity and the C-band pattern do not have a potential cytotaxonomic value for discriminating among any of the species of *Haemulon* herein studied, the number of chromosomes bearing the 5S-rDNA sequences and the distribution of 5S-rDNA clusters allowed the clear differentiation of *H. aurolineatum* from the other species we analyzed. Thus, although the chromosome macrostructure of *Haemulon* has been preserved from major changes, our data on 5S rDNA suggest that microstructural changes may have played an important role in the karyotype evolution of these fishes.

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CAPITULO VI: Tetraodontiformes

Bol. Inst. Oceanogr. Venezuela, Univ. Oriente 46 (2): 117-123 (2007); 1 Fig., 1 Tab.

FIRST DESCRIPTION OF THE KARYOTYPE AND AG-NORS LOCALIZATION OF *STEPHANOLEPIS SETIFER* (BENNETT, 1831) (TETRAODONTIFORMES: MONACANTHIDAE) WITH REMARKS ON SEX CHROMOSOME DIFFERENTIATION

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ABSTRACT: A cytogenetic study of *Stephanolepis setifer* by conventional Giemsa stain and localization of Nucleolus Organizer Regions (NORs) by silver staining is reported. Females possess a diploid chromosome number of 2n=34 chromosomes, all of which are acrocentric, Males exhibit a karyotype with 2n=33 chromosomes, consisting of 32 acrocentric chromosomes including two unpaired acrocentric chromosomes, and one medium-size metacentric chromosome. The results suggest the presence of a multiple sex chromosome system of X1X1X2X2/X1X2Y. The X1 and X2 chromosome sizes suggest that the metacentric chromosome is derived from Robertsonian fusion between the original Y chromosome and an autosome which probably corresponded to one element of the pair 16 and another of the pair 17, resulting in a neo-Y sex chromosome. Silver staining of metaphase spreads revealed a single pair of Ag-NORs interstitially located on the chromosome pair numbered 7. The comparison of our results with previous reports in Tetraodontiformes species suggest that its evolutionary divergence has been accompanied by macrostructural karyotype changes as far as microstructural alteration in NORs cluster.

Key words: cytogenetic, multiple sex chromosomes, Ag-NORs

RESUMEN: Se presenta el estudio citogenético de *Stephanolepis setifer* mediante tinción convencional con colorante de Giemsa y localización de las Regiones Organizadoras del Nucléolo (RONs) mediante impregnación con nitrato de plata. La especie posee un número diploide 2n= 34 cromosomas totalmente acrocéntricos en hembras, mientras que los machos exhibieron un cariotipo con 2n= 33, constituido por 32 acrocéntricos entre los cuales dos elementos no pudieron ser apareados con homólogos y un metacéntrico de tamaño mediano que sugiere la presencia de un sistema de cromosomas sexuales múltiple del tipo X1X1X2X2/ X1X2Y. A partir del tamaño de los cromosomas X1 y X2 es posible sugerir que el cromosoma metacéntrico podría haber derivado de la fusión Robertsoniana entre el cromosoma Y original y un autosoma los cuales probablemente corresponden a un elemento de del par 16 y otro del par 17, resultando en un cromosoma sexual neo-Y. La tinción con plata de células metafásicas revelaron un par de Ag-RONs localizados intersticialmente en le par cromosómico número 7. La comparación de nuestros resultados con reportes previos en otras especies de Tetraodontiformes sugiere que la divergencia evolutiva en este grupo ha estado acompañada por cambios macroestructurales así como también de modificaciones microestructurales de las RONs.

Palabras clave: citogenética, cromosomas sexuales múltiples, Ag-RONs

INTRODUCTION

The order Tetraodontiformes is represented by 101 genera and 357 species; most are marine, although 14 of them occur only in freshwater and another eight may be found in freshwater (NELSON 2006). Within the tetraodontiformes, the Monacanthidae includes about 32 genera and 102 species (NELSON, 2006; ACERO & POLANCO, 2006). The pygmy filefish, *Stephanolepis setifer*, is a monacanthid which occurs in the Western Atlantic from North Carolina to Brazil, including Bermuda, the Gulf of Mexico, and the Caribbean Sea (MATSUURA, 2002).

Cytogenetic studies of tetraodontiformes have, thus far, covered only about 17% of the species (ca. 60 species) but have characterized the group as karyotypically very diverse (Table 1). With the aim of contributing to the cytogenetic knowledge of Tetraodontiformes, in the present study we report the karyotype and the localization of Ag-NORs localization for *Stephanolepis setifer,* with remarks on sex chromosome differentiation in the species.

MATERIALS AND METHODS

Four males and six females of *Stephanolepis setifer* collected with inshore fish traps in Cubagua Island, Venezuela, were analyzed. Their sex was established by external sexual dimorphism (second soft dorsal-fin ray greatly prolonged in mature males -Fig. 1 -) and confirmed by the presence of well differentiated testes or ovaries during dissection of the fishes. Voucher specimens are deposited in the Fish Collection of the Escuela de Ciencias Aplicadas del Mar at Universidad de Oriente.

Chromosome preparations were obtained from cephalic kidney cells by conventional air-drying techniques (BERTOLLO *et al.,* 1983) using stimulation of mitotic activity (LEE and ELDER, 1980). Silver-stained Nucleolus Organizer Regions (Ag-NORs) were obtained as described by HOWELL & BLACK (1980). The best metaphases were photographed using a Motic camera and images were digitally processed using the Adobe Photoshop CS2 software. The chromosomes were classified according to the arm ratio (LEVAN *et al.,* 1964). Fundamental number (NF) was determined considering subtelocentrics (ST) and acrocentrics (A) as having one chromosome arm and metacentrics (M) and submetacentric (SM) as having two chromosome arms.

RESULTS AND DISCUSSION

Tetraodontiform fishes display diploid numbers and karyotype formulae that include exclusively acrocentric chromosomes as well as very differentiated and distinct karyotypes with chromosome numbers ranging from 2n=28 to 2n=48, and NF values from 33 to 78 (Table 1).

In female *S. setifer* the karyotype has 2n=34 chromosomes, all of which are acrocentric. Males exhibit a karyotype with 2n=33 chromosomes, consisting of 32 acrocentric chromosomes including two unpaired acrocentrics, and one medium-size metacentric chromosome. This result suggests the presence of a multiple sex chromosome system of X1X1X2X2/ X1X2Y type in *S. setifer* (Fig. 1). This multiple sex chromosome system requires the attachment of the Y chromosome to an autosome by translocation or Robertsonian fusion, which results in males and females having different diploid chromosome numbers and different numbers of metacentric and acrocentric chromosomes (e.g. UYENO & MILLER, 1971; THORGAARD, 1978). The X1 and X2 chromosome sizes in *S. setifer* suggest that the metacentric chromosome is derived from Robertsonian fusion between the original Y chromosome and an autosome which probably corresponded to one element of the pair 16 and another of the pair 17, resulting in a neo-Y sex chromosome.

Identifying which sex is heterogametic and which is homogametic provides information about the sex-determination mechanism present in the species (DEVLIN & NAGAHAMA, 2002). Five different systems of sexual chromosome determination (ZZ/ZW, XX/XY, XX/X0, X1X1X2X2/X1X2Y, XX/XY1Y2 and ZZ/ZW1W2) have been identified in neotropical fish (NIRCHIO & OLIVEIRA, 2006; ALVES *et al.,* 2006). The main evolutionary events that determine sexual chromosome systems include additions or deletions of heterochromatic blocks (e.g. PHILLIPS & IHSSEN, 1985; ANDREATA *et al.,* 1992; CANO *et al.,* 1996; STEIN *et al.,* 2001), reductions in chromosome size (e.g. PARK & KANG, 1979), increments in chromosome size (e.g. GALETTI *et al.,* 1981), and chromosome rearrangements (e.g. UYENO & MILLER, 1971; THORGAARD, 1978; BERTOLLO *et al.,* 1983; de ALMEIDA TOLEDO *et al.,* 1984; PEZOLD, 1984; VITTURI *et al.,* 1991).

Among Monacanthidae, the genera *Cantherhines*, *Navodon*, *Paramonachantus*, *Rudarius* and *Oxymonacanthus* have been reported as possessing exclusively subtelocentric or acrocentric chromosome complements with the same diploid number in both genders , but for the species so far cytogenetically analyzed of the genus *Stephanolepis*, except for *S. japonicus*, a multiple sex chromosomes of the X1X1X2X2/ X1X2Y type as the one here reported for *S. setifer* have been reported also in *S. cirrhifer*, and *S. hispidus* (Table 1). Since the occurrence of cytologically differentiated sex chromosomes in marine fishes appears to be rare (GALETTI *et al*., 2000), the presence of differentiated sex chromosomes only in *Stephanolephis* suggested that this mechanism might be a derived feature of the genus. Additional studies in different tetraodontiform species will be important to know if those sex chromosomes originated only once in the order or, alternatively, this was a common feature for the group.

NOR-silver staining is one of the methods used to locate 18S and 28S rRNA cistrons in chromosomes (HOWELL & BLACK 1980) and requires transcriptional activity of the ribosomal genes during the preceding interphase (see HUBBEL 1985, SÁNCHEZ-PINA *et al*. 1984, JIMENEZ *et al*. 1988).

Table 1. Table 1. Chromosome number in tetraodontiform species. a: acrocentric, st: subtelocentric, sm= submetacentric, m=metacentric.

Family	Species	2N	Karyotype formula	NF	Reference
TRIACANTHIDAE	*Triacanthus brevronsis*	48	48 a	48	Choudhury *et al.* 1982
	T. striglifer	48	48 a	48	Rishi *et al.* 1973
	Arothron hispidus	42	36 sm + 6 st/a	78	Natarajan & Subrahamanjan, 1974
	A. immaculatus	42	14 m + 16sm + 12 st/a	72	Arai & Nagaiwa, 1976
	A. immaculatus	42	12 m + 16sm + 16 st/a	68	Choudurry *et al.* 1982
	A. leopardus	40	14 m + 14 sm + 12 st/a	68	Arai & Nagaiwa, 1976
	A. nigropunctatus	38	14 m +20 sm + 4 st/a	72	Arai & Nagaiwa, 1976
	A. reticularis	42	12 m + 14 sm +16 st/a	68	Choudury *et al.* 1982
	Canthigaster coronate	28	8 m/sm + 20 st/a	36	Arai, 1983
	C. rivulata	34	4m + 6sm + 10st/a +14a	54	Arai & Nagaiwa, 1976
	Chelonodon patoca	40	14m + 16sm + 10st/a	70	Arai & Nagaiwa, 1976
	Fugu chrysops	44	6m + 14sm + 24 st/a	64	Arai & Nagaiwa, 1976
	F. niphobles	44	20m/sm + 24st/a	64	Arai & Katsuyama, 1973
	F. pardalis	44	-	-	Arai, 1983
	F. poecilonotus	44	-	-	Arai, 1983
	Lagocephalus laevigatus	46	-	-	Arai, 1983
TETRAODONTIDAE	*L. lunaris*	44	10m + 14sm + 20st/a	68	Choudury *et al.* 1982
	Monotetra palambagensis	42	-	-	Hinegardner & Rosen, 1972
	Sphoeroides greeleyi	46	24sm/sm + 22st/a	70	Brum & Mota, 2002
	S. tyleri	46	12 m/sm + 34st/a	58	Brum *et al*, 1996
	S. spengleri	46	20m/sm + 26st/a	66	Brum *et al*. 1996
	S. testudineus	46	20m/sm + 26st/a	66	Noleto *et al*. 2006
	Takifugu niphobeles	44	4m/sm + 16sm + 24st/a	64	Miyaki *et al.* 1995
	T. pardalis	44	6m/sm + 16sm + 22st/a	66	Miyaki *et al.* 1995
	T. poecilonotus	44	12m + 10st + 22st/a	66	Miyaki *et al.* 1995
	T. radiatus	44	8m + 14st + 22st/a	66	Miyaki *et al*. 1995
	T. rubripes	44	10m + 12st + 22st/a	66	Miyaki *et al.* 1995
	T. xanthopterus	44	8m + 14st + 22st/a	66	Miyaki *et al*. 1995
	Tetraodon cutcutia	42	16m + 12st + 10a	70	Khuda-Bukhsh & Barat, 1987
	Tetraodon fluviatilis	42	2m + 4sm + 2st + 34a	50	Mandrioli *et al*. 2000
	Tetraodon nigroviridis	42	20m/sm + 22st	62	Fischer *et al.* 2000
	Diodon blekeeri	46	-	58	Arai & Nagaiwa, 1976
	D. holocanthus	46	20m/sm + 26st/a	66	Sá-Gabriel & Molina, 2001
DIODONTIDAE	*Chilomycterus spinosus*	52	16m/sm+36st/a	68	Brum, M.J.I. *et al*. 1996
	Chilomicterus antennatus	52	6m + 46st/a	58	Sá-Gabriel & Molina, 2005
	Ciclicthys spinosus	50	4m + 18sm + 12st + 16a	76	Noleto *et al.* 2006

Continuación Tabla 1

Family	Species	2N	Karyotype	NF	Reference
OSTRACIDAE	*Lactoria diaphana*	36	-	48	Arai, 1983
	Ostracion cubicus	50	4sm + 46st/a	54	Arai & Nagaiwa, 1976
	O. immaculatus	50	4sm + 46st/a	54	Arai, 1983
BALISTIDAE	*Balistapus undulatus*	42	42st/a	42	Takai & Ojima, 1987
	Balistes vetula	44		44	Sá-Gabriel & Molina, 2005
	Balistoides conspicullum	44	44st/a	44	Takai & Ojima, 1987
	B. viridescens	44	2m + 2sm + 40st/a	48	Takai & Ojima, 1987
	Melichthys niger	40	-	40	Sá-Gabriel & Molina, 2005
	Melichthys vidua	40	40st/a	40	Kitayama & Ojima, 1984
	Odonus niger	42	42st/a	42	Kitayama & Ojima, 1984
	Parika scaber	40	40st/a	40	Murofushi *et al.* 1989
	Pseudobalistes flavimarginatus	44	2sm + 42st/a	46	Arai & Nagaiwa, 1976
	Rhineacanthus aculeatus	44	44st/a	44	Arai & Nagaiwa, 1976
	R. echarpe	44	44st/a	44	Kitayama & Ojima, 1984
	R. verrucosus	44	44st/a	44	Arai & Nagaiwa, 1976
	Rucanus arcodas	36	36st/a	36	Arai & Nagaiwa, 1976
	Suflamen chysopterus	46	46st/a	46	Arai & Nagaiwa, 1976
	S. traenatus	46	46st/a	46	Takai & Ojima, 1987
	Cantherhines macroceus	40	40st/a	40	Arai & Nagawaia, 1976
	Navodon modestus	40	40st/a	40	Murofushi & Yosida, 1979
MONACANTHIDAE	*Stephanolepis cirrifher (M)*	33	33st/a		Murofushi *et al.* 1980
	Stephanolepis cirrifher (F)	34	34 st/a		Murofushi *et al.* 1980
	Stephanolepis hispidus (M)	33	33a + 1sm		Sá-Gabriel & Molina, 2005
	Stephanolepis hispidus (F)	34	34 a		Sá-Gabriel & Molina, 2005
	Stephanolepis setifer (M)	33	32a + 1m	34	This paper
	Stephanolepis setifer (F)	34	34 a	34	This paper
	Stephanolepis japonicus	34	34st/a	34	Murofushi & Yosida, 1979
	Paramonachantus japonicus	34	34st/a	34	Murofushi & Yosida, 1979
	Rudarius ercodes	36	36st/a	36	Arai & Nagaiwa, 1976
	Oxymonacanthus longirostris	36	36st/a	36	Arai & Nagaiwa, 1976

Silver staining of metaphase spreads revealed a single pair of Ag-NORs interstitially located on the chromosome pair number 7 (Figure 1). As far as ribosomic cistrons are concerned, silver impregnation performed on some Tetraodotiformes species has revealed that NOR localization in this group is also a cytogenetically variable feature. Indeed, a survey of Brazilian tetraodontiforms (Bezerra, 2007) showed a single NOR located interstitially on chromosome pair number 2 in *Melichthys niger* (Balistidae), pericentromerically located on pair 7 in *Cantherhines macrocerus* and on pair 5 in *C. pullus* (Monacanthidae), terminally located on the short arm of the metacentric chromosome pair number 2 in *Sphoeroides testudineus* (Tetraodontidae), and on the tips of the short arms of the subtelocetric pair number 7 in *Chilomycterus antennatus* (Diodontidae).

The high diversity of karyotypes indicates that the evolutionary divergence in tetraodontiforms has been frequently accompanied by macrostructural rearrangements that originated different diploid number. On the other hand, the occurrence of Ag-NORs in different

x

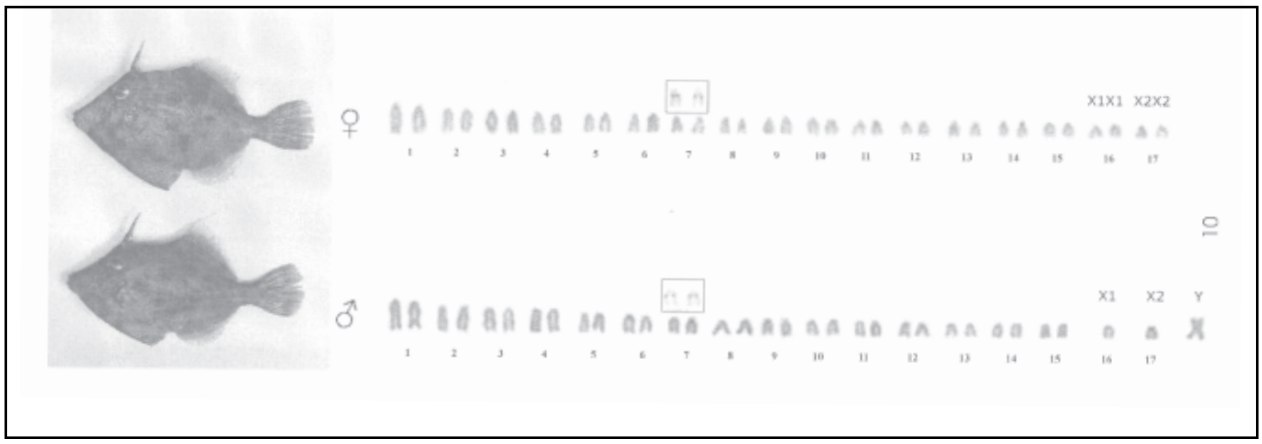

Fig. 1. Karyotype of *Stephanolepis setifer.* NOR-bearing chromosomes appear in the box.

chromosomes and positions also shows that microstructural changes were fixed in karyotype evolution in tetraodontiforms.

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CONSIDERACIONES Y CONCLUSIONES FINALES

La predominancia en los peces de un cariotipo con 48 elementos acrocéntricos ha conducido a aceptar la generalización de que ese complemento cromosómico sería la característica más primitiva para los teleósteos (Ohno *et al*., 1969; Ebeling *et al*., 1971; Uyeno, 1972; Kirpichnikov, 1973; Ohno, 1974; Arai & Nagaiwa, 1976; Gold, 1979; Vitturi *et al*., 1995). En favor de esa hipótesis, a partir del análisis de la secuencia del genoma de *Tetraodon negroviridis* (Tetarodontiformes) se ha inferido que el cariotipo ancestral de los vertebrados podría haber estado constituido por 12 cromosomas y que, por lo tanto, el valor modal haploide n=24 en los Teleostei sería consistente con la presunción de que la duplicación total del genoma, a partir de un complemento con 12 cromosomas, habría dado origen a un complemento ancestral constituido por 48 cromosomas en los Actinopterygii después de su separación del linaje de los Tetrápodos hace 450 millones de años (Jaillon *et al*., 2004).

Mank y Avise (2006) intentaron reconstruir el cariotipo ancestral en los Actinopterygii mapeando el número de cromosomas sobre la topología generada por el estudio filogenético de Mank *et al*. (2005) para los Actinopterygii y encontraron que entre 7 y 20 eventos de poliploidización habrían ocurrido en todos los Órdenes, concentrados en las ramas terminales del árbol filogenético, específicamente a nivel de género y especie, excepto en el grupo de los Acypenseriformes, en los que todos los taxa existentes parecen haber derivado a partir de un ancestro poliploide y, aunque no pudieron reconstruir con precisión el cariotipo ancestral debido a la variabilidad que existe en el número de cromosomas entre los Condrostei, indicaron, con base en el análisis de parsimonia, que la condición ancestral fue probablemente menor de 48 elementos cromosómicos.

Sin embargo, la noción que sostiene que 48 cromosomas acrocéntricos sería la condición plesiomórfica en los peces teleósteos, se enfrenta a varias dificultades:

a) la suposición de que esa es una condición ancestral deriva enteramente de la elevada frecuencia y amplia distribución de ese cariotipo en los peces teleósteos vivientes;
b) en los Perciformes, aproximadamente el 60% de las especies estudiadas a nivel global posee un cariotipo con 48 elementos acrocéntricos (Galetti *et al*., 2000), pero este es uno de los grupos más derivados y presentan la mayor diversidad específica entre los Actinopterygii (Nelson, 2006).
c) en los Clupeiformes, grupo considerado como basal entre los Teleósteos, predomina un cariotipo con 48 cromosomas acrocéntricos (Doucette & Fitzsimons, 1988), pero entre los Actinopterigios no teleósteos aun más primitivos que los Clupeiformes, como los Polypteriformes, Acipenseriformes y Lepisosteiformes, el número de cromosomas es usualmente mayor, variando entre 120 – 260 para Acipenseridos (Fontana *et al*., 1998); 56-68 para lepisosteidos y 120 para *Polyodon spathula* (ver referencias en Sola *et al*., 1981), además un gran número de elementos cromosómicos poseen un segundo brazo, lo cual es una característica compartida por todos los actinopterigios no teleósteos para los cuales se dispone de información, razón por la cual se ha sugerido que la semejanza cariotípica entre Clupeiformes y Perciformes, separados por 30 órdenes, probablemente es más una cuestión de convergencia que de proximidad a un antepasado común (Doucette e Fitzsimons, 1988).

La idea de que el cariotipo primitivo de los teleósteos tenía aproximadamente 60 elementos y que la reducción en el número de cromosomas a 48 ocurrió independientemente en más de una línea filogenética mediante fusiones y deleciones, ya había sido considerada previamente por Dingerkus (1979). Dieciocho años más tarde, Brum & Galetti (1997) también argumentaron que la observación del número diploide y número fundamental en clados de teleósteos basales y derivados, indicaría que la condición 2n=48 no debería ser asumida como ancestral en los peces, sino que, por el contrario, el número básico de cromosomas en los Teleostei debería ser aproximadamente 2n=60, con pocos elementos metacéntricos y que 2n=48 a sería más bien una sinapomorfía

extendida entre las ramas más apicales de los teleósteos existentes (Clupeomorpha y Euteleostei) habiéndose conservado principalmente en las especies marinas de Atherinimorpha y Percomorpha.

La grafica del número diploide de cromosomas (2n) y el Número fundamental (NF) contra la ordenación filogenética propuesta por Nelson (2006) para los Actinopterygii (Figuras 4) proporciona un panorama del comportamiento global de los datos y revela, en ambos casos, una relación inversamente proporcional que se encuentra reforzada por el análisis de Correlación por Rangos de Spearman (Tabla 7) con una probabilidad altamente significativa (más allá del 99% de confianza), desde valores cercanos a 2n=60 en los ordenes más ancestrales aquí estudiados, hasta valores de 2n=48 en los grupos más recientes, principalmente en el grupo de los Perciformes. Por lo tanto, la tendencia observada hacia la reducción del número diploide y del Número Fundamental en la filogenia de los Actynopterygii conduce a reforzar la noción de que un cariotipo con aproximadamente 60 cromosomas con varios elementos de dos brazos sería la condición plesiomórfica en vez de considerar 2n=48A como condición ancestral.

Si bien nuestros datos sustentan esa tendencia, con la finalidad de salvar la objeción que podría surgir por fundamentar las conclusiones aquí expuestas sobre la base de solo 92 especies, se sometió a prueba la hipótesis empleando los datos del número diploide de cromosomas para 638 especies (excluidos los registros de especies poliploides) contenidas en 30 órdenes de Actinopterigios que fueron obtenidos de la base de datos **Animal Genome Size Database** (Gregory, 2009), y resultó que los coeficiente de correlación por Rangos de Spearman entre la posición filogenética y Valor C (R=-0,385285593; P=2,61245E-24) y entre la posición filogenética y número diploide (R= -0,443; P=1,97061 x 10^{-32}), revelaron nuevamente una relación inversa altamente significativa (Fig. 5).

Considerando el número de cromosomas como un indicador del tamaño del genoma, los análisis gráficos y estadísticos antes indicados ponen de manifiesto una tendencia hacia la simplificación del cariotipo en la filogenia de los peces

radiados, tanto en el número de cromosomas, como en el tamaño del genoma (contenido de DNA por núcleo).

Aunque los peces radiados exhiben una variación del tamaño del genoma escepcionalmente alta (Hinegardner, 1976; Venkatesh 2003), los resultados de estudios moleculares obtenidos por a partir del análisis de un conjunto de datos no redundantes para 1.043 especies de Actinopterygii contenidas en 190 familias revelan que el genoma haploide (medido en pares de bases) de *Acypenser brevirostrum* (Acypenseriformes), perteneciente a uno de los grupos más basales es 20 veces mayor que el de *Arothron meleagris* (Tetraodontiformes), que pertenece al grupo filogenéticamente más derivado (Yi & Streelman, 2005) lo que indica una clara tendencia hacia la reducción del tamaño del genoma en los grupos mas derivados.

Aunque los casos documentados de reducción del genoma en peces son escasos, los estudios realizados en Tetraodontiformes indican que tal disminución parece estar asociada a la pérdida de secuencias repetitivas y/u otras secuencias de DNA no codificador (Neafsey & Palumbi, 2003). La eliminación de DNA no funcional pudiera resultar ventajosa, contribuyendo con la disminución del número de cromosomas y causaría un menor gasto energético a la hora de la replicación del material genético durante la multiplicación celular, todo lo cual permitiría manejar los eventos de división celular con más eficiencia y con menores probabilidades de error durante la disyunción.

Con base en a): el hecho demostrado de que los peces dulceacuícolas poseen mayor número de cromosomas que los marinos; b): la existencia de una tendencia hacia la reducción del número de cromosomas en los peces actinopterigios desde valores que rondan 2n=60, con gran número de cromosomas de dos brazos y c): que el cariotipo más simple y diseminado 2n=48 a predomina en los peces marinos, podría especularse que el linaje de los peces actynopterigios pudo haberse originado en ambientes salobres o dulceacuícolas.

En este sentido Gupta & Gupta (2006) sostienen que fue en el agua dulce en donde surgieron los cordados a partir de los cuales evolucionaron los ostracodermos, argumentando que:

1. Los primeros vertebrados (Ostracodermos) fueron enteramente habitantes de aguas continentales en tiempos del Ordovícico.
2. Los Ostracodermos permanecieron esencialmente como formas dulceacuícolas hasta el fin de su historia en el Devónico, aunque hubo alguna tendencia hacia formas de vida marinas en los Pteraspidiformes (=Heterostraci).
3. Los Placodermos fueron de origen dulcecauícola, pero, mientras los Antiarchidos permanecieron enteramente dulceacuícolas, los Arthrodiros del Devónico inferior migraron hacia los mares.
4. Los peces similares a los tiburones se originaron en aguas dulces, donde los típicos Acanthodianos (grupo más antiguo de tiburones conocidos) permanecieron hasta el fin de su historia, pero de nuevo una fuerte tendencia hacia el mar tomó lugar en el comienzo del Devónico. Los océanos fueron el centro de su historia evolutiva desde ese tiempo hasta ahora.
5. Los peces óseos superiores tuvieron su origen en aguas dulces en las cuales su centro evolutivo permaneció hasta el Devónico. Los Actinopterigios mostraron una propensión temprana hacia los mares en casos aislados. Los peces pulmonados desarrollaron una corta rama de vida marina en tiempos del Devónico, pero persistieron como grupo dulceacuícola.

Para explicar el origen de los primeros vertebrados, Griffith (1985) propuso la posibilidad de un escenario en el que algunos pre-vertebrados cordados similares a los cefalocordados que se alimentaban por filtración (eg. *Amphioxus*), debieron invadir los estuarios para usarlos como territorio de alimentación, libres de competidores y depredadores, adentrándose progresivamente en aguas continentales en donde evolucionaron desarrollando gradualmente novedades que

condujeron a establecer características de los vertebrados como huesos dérmicos, regulación osmótica e iónica, capacidad metabólica mejorada y sistema nerviosos central y sensorial sofisticado (Griffith, 1994) que poseen los peces actuales.

Hace casi cuatro décadas Ohno (1970) presentó, la hipótesis según la cual la duplicación de genes y, aun más, del genoma total, podría ser uno de los principales mecanismos responsables de la diversificación durante la evolución, sugiriendo que una copia de un gen retendría la función original y que la copia adicional podría evolucionar hacia una nueva variante. En los últimos años, las tecnologías avanzadas de secuenciación de ADN y los avances en bioinformática, han permitido realizar análisis de genomas completos que han reforzado cada vez más la visión inicial de Ohno.

Una de las evidencias más convincente, aunque no exclusiva, en favor de la duplicación del genoma en los vertebrados ha sido suministrada por el estudio del complejo de genes conocidos como genes HOX, que regulan la estructura y organización corporal durante la embriogénesis de los metazoarios. En amphioxus, cordado que se presume es el representante viviente mas parecido al ancestro común de los vertebrados, los estudios indican que aquellos poseen un solo cluster de genes HOX (Garcia-Fernández & Holland, 1994) mientras que en los linajes de vertebrados más complejos existen copias múltiples de estos cluster (Stellwag, 1999).

Por otro lado, los estudios citogenéticos en cefalocordados existentes (*Amphioxus*; *Branchiostoma*) han revelado que éstos poseen números diploides de 2n=32, 2n=36 (Zhang *et al*., 2004, Wang *et al*, 2003) y 2n=40 (Zhang *et al*., 2009). Además, los datos moleculares indican que los cefalocordados poseen genomas con copias únicas de genes (Garcia-Fernandez & Holland, 1994), mientras que los peces poseen copias múltiples de genes lo cual sustenta la hipótesis de ocurrencia de eventos de duplicación total de genoma hace 350 millones de años (Jaillon *et al*., 2004; Christoffels *et al*., 2004) momento en que se cree ocurrió esta duplicación total del genoma que condujo a la diversificación de los vertebrados.

Por lo tanto, bajo la premisas arriba indicadas, la posibilidad de un escenario en el que un antecesor similar a los actuales *Amphioxus* haya podido entrar a los estuarios desde el mar y de allí a las aguas continentales, dando origen, luego de un evento de duplicación total del genoma, a un ancestro con un complemento diploide entre 64 y 80 cromosomas a partir de los cuales habrían surgido los peces actuales, sería entonces compatible con la propuesta inicial de Dingerkus (1979) la cual estaría reforzada por la tendencia que arrojan nuestros datos en relación a la evolución del cariotipo en los actinopterigios.

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TABLAS

Tabla 1. Número de Familias, géneros y Número de especies analizadas (D: dulceacuícolas; M: Marinos) para 18 Ordenes de Actynopterygios

ORDEN	FAMILIAS	GENEROS	NUMERO DE ESPECIES		
			D	M	Total
Anguilliformes	1	1		2	2
Atheriniformes	1	1		1	1
Aulopiformes	1	1		1	1
Batrachoidiformes	1	1		6	6
Beloniformes	2	3		4	4
Beryciformes	1	1		1	1
Characiformes	3	9	13		13
Cyprinodontiformes	2	2	1	1	2
Elopiformes	1	1		1	1
Gymnotiformes	1	1	1		1
Lophiiformes	1	1		1	1
Mugiliformes	1	2	1	6	7
Perciformes	14	24	2	34	36
Pleuronectiformes	1	2		2	2
Scorpaeniformes	1	1		2	2
Siluriformes	4	7	5	2	7
Synbranchiformes	1	2	2		2
Tetraodontiformes	2	2		3	3
18	**39**	**62**	**25**	**67**	**92**

Tabla 2. Número diplóide (2n), Fórmula cariotípica, Número fundamental (NF) para xx especies de peces Neotropicales marinas (M) y dulceacuícolas (F). Se indica la ancestralidad (posición según Nelson, 2006)

Medio	Ancestr.	Orden	Familia	Especie	2n	Fórmula	NF	Determinación Sexual	AgNOR
F	30	Characiformes	Characidae	*Brycon amazonicus*	50	22M+14SM+7ST	100		1
F	30	Characiformes	Characidae	*Brycon falcatus*	50	18M+16SM+16ST	100		
F	30	Characiformes	Characidae	*Colossoma macropomum*	54	54M	108		2
F	30	Characiformes	Characidae	*Mylossoma duriventris*	54	54M/53M+1A	108		1
F	30	Characiformes	Characidae	*Piaractus brachypomus*	54	54M	108		2
F	30	Characiformes	Characidae	*Triportheus orinoscensis*	52	20M+14SM+12ST+6A	98	ZZ/ZW	2
F	30	Characiformes	Characidae	*Triportheus venezuelensis*	52	20M+16SM+ 8ST	104	ZZ/ZW	2
F	30	Characiformes	Prochilodontidae	*Prochilodus mariae*	54	40M+14SM	108		1
F	30	Characiformes	Prochilodontidae	*Semaprochilodus kneri*	54	40M+14SM	108		1
F	30	Characiformes	Prochilodontidae	*Semaprochilodus laticeps*	54	40M+14SM	108		1
F	30	Characiformes	Serrasalminae	*Pygocentrus cariba*	60	18M+30SM+2ST+ 10A	110		2
F	30	Characiformes	Serrasalminae	*Serrasalmus altuvei*	60	26M+22ST+12A	108		2
F	30	Characiformes	Serrasalminae	*Serrasalmus rhombeus*	60	10M+16SM+4ST+10A	110		2
F	31	Siluriformes	Callichthyidae	*Hoplosternum littorale*	60	6M+2SM+2ST+50A	70		1
F	31	Siluriformes	Loricariidae	*Glyptoperichthys gibbiceps*	52	20M+24SM+8ST	104		1
F	31	Siluriformes	Loricariidae	*Liposarcus multiradiatus*	52	22M+18SM+12ST	104		2
F	31	Siluriformes	Pimelodidae	*Pimelodus blockii*	56	18M+16SM+10ST+12A	100		1
F	31	Siluriformes	Pimelodidae	*Pseudoplatistoma orinocoense*	56	24 M+14 SM+6St +12A	100		
F	32	Gymnotiformes	Rhamphichthyidae	*Ramphichthys sp*	50	22M+18SM+6ST+4A	96		1
F	48	Mugiliformes	Mugilidae	*Agonostomus montícola*	48	2ST+46A	52		1
F	51	Cyprinodontiformes	Rivulidae	*Rivulus hartii*	44	2M+4SM+8ST+30A	54		1
F	56	Synbranchiformes	Synbranchidae	*Ophisternon aenigmaticum*	44 45 46	5M+1ST+38A 6M+1ST+38A 6M+2ST+38A	50 52 54		1
F	56	Synbranchiformes	Synbranchidae	*Synbranchus marmoratus*	44	6M+38A	50		1
F	58	Perciformes	Cichlidae	*Cichla orinocensis*	48	48A	48		1
F	58	Perciformes	Cichlidae	*Geophagus surinamensis*	48	2M+2SM+12ST+32A	64		1
M	23	Elopiformes	Elopidae	*Elops saurus*	50	6M+4T+40A	60		1
M	24	Anguilliformes	Muraenidae	*Gimnotorax moringa*	44	12M+32A	56		1
M	24	Anguilliformes	Muraenidae	*Gimnotorax ocellatus*	42	34M/SM+8A	76		1
M	31	Siluriformes	Ariidae	*Cathorops spixii*	52	14m+20sm+18ST	104		1
M	31	Siluriformes	Ariidae	*Sciades herzbergii*	54	14M+20SM+18ST+2A	106		1

Tabla 2. Número diplóide (2n), Fórmula cariotípica, Número fundamental (NF) para xx especies de peces Neotropicales marinas (M) y dulceacuícolas (F). Se indica la ancestralidad (posición según Nelson, 2006)									
Medio	Ancestr.	Orden	Familia	Especie	2n	Fórmula	NF	Determinación Sexual	AgNOR
M	38	Aulopiformes	Synodontidae	*Synodus foetens*	46	28M/SM+18A	74		1
M	46	Batrachoidiformes	Batrachoididae	*Amphichthys cryptocentrus*	46	4M+2SM+10ST+30T	64		1
M	46	Batrachoidiformes	Batrachoididae	*Batrachoides manglae*	46	6M+8SM+16ST+16A	76		1
M	46	Batrachoidiformes	Batrachoididae	*Batrachoides pacifici*	46	6M+8SM+4ST+28A	64		1
M	46	Batrachoidiformes	Batrachoididae	*Halobatrachus didatctylus*	46	8M+12ST+26A	66		1
M	46	Batrachoidiformes	Batrachoididae	*Porichthys plectrodon*	44	8M+10SM+6ST+20A	68		1
M	46	Batrachoidiformes	Batrachoididae	*Thalassophryne maculosa*	46	12M+6SM+20ST+8A	84		2
M	47	Lophiiformes	Antennariidae	*Antennarius multiocelatus*	48	12M+16ST-20A	76		
M	48	Mugiliformes	Mugilidae	*Mugil curema*	24	22M+2SM	48		1
M	48	Mugiliformes	Mugilidae	*Mugil incilis*	48	48A	48		1
M	48	Mugiliformes	Mugilidae	*Mugil liza*	48	48A	48		1
M	48	Mugiliformes	Mugilidae	*Mugil rubrioculus*	48	48A	48		1
M	48	Mugiliformes	Mugilidae	*Mugil sp.*	28	20M+4ST+4A	52		1
M	48	Mugiliformes	Mugilidae	*Mugil trichodon*	48	48A	48		1
M	49	Atheriniformes	Atherinidae	*Odontestes regia*	48	2SM+32ST+14A	82		1
M	50	Beloniformes	Belonidae	*Strongylura marina*	48	2M+46A	50		1
M	50	Beloniformes	Belonidae	*Strongylura timucu*	48	12M+36A	60		1
M	50	Beloniformes	Belonidae	*Tylosurus crocodrilus*	48	8M+12SM+28A	68		1
M	50	Beloniformes	Hemiramphidae	*Hyporhamphus unifasciatus*	38	2M+36A	40		1
M	51	Cyprinodontiformes	Cyprinodontidae	*Cyprinodon dearborni*	48	2M+38SM+8A	88		1
M	53	Beryciformes	Holocentridae	*Holocentrus adscensionis*	50	2M+6ST+42A	58		1
M	57	Scorpaeniformes	Scorpaenidae	*Scorpaena brasiliensis*	46	2SM+44ST-A	48		
M	57	Scorpaeniformes	Scorpaenidae	*Scorpaena isthmensis*	38	8M+10ST+20A	56		
M	58	Perciformes	Acanthuridae	*Acanthurus bahianus*	34	14M+2ST+18A	50		1
M	58	Perciformes	Carangidae	*Selene vómer*	46	2ST+46A	50		1
M	58	Perciformes	Carangidae	*Trachinotus falcatus*	48	2M+2St+44A	52		1
M	58	Perciformes	Centropomidae	*Centropomus undecimalis*	48	48A	48		1
M	58	Perciformes	Gerreidae	*Euscinostomus argenteus*	48	48A	48		1
M	58	Perciformes	Haemulidae	*Haemulon aerolineatus*	48	48A	48		1
M	58	Perciformes	Haemulidae	*Haemulon bonariense*	48	48A	48		1
M	58	Perciformes	Haemulidae	*Haemulon flavolineatum*	48	48A	48		1

Tabla 2. Número diplóide (2n), Fórmula cariotípica, Número fundamental (NF) para xx especies de peces Neotropicales marinas (M) y dulceacuícolas (F). Se indica la ancestralidad (posición según Nelson, 2006)

Medio	Ancestr.	Orden	Familia	Especie	2n	Fórmula	NF	Determinación Sexual	AgNOR
M	58	Perciformes	Haemulidae	*Haemulon plumierii*	48	48A	48		1
M	58	Perciformes	Haemulidae	*Haemulon sciurus*	48	48A	48		1
M	58	Perciformes	Haemulidae	*Haemulon steindachneri*	46	46A	48		1
M	58	Perciformes	Haemulidae	*Haemulon striatum*	48	48A	48		1
M	58	Perciformes	Haemulidae	*Orthopristis ruber*	48	48A	48		1
M	58	Perciformes	Labridae	*Halichoeres bivittatus*	48	48A	48		1
M	58	Perciformes	Labridae	*Lachnolaimus maximus*	48	6M+16ST+26A	70		1
M	58	Perciformes	Lutjaninae	*Lutjanus analis*	48	48A	48		1
M	58	Perciformes	Lutjaninae	*Lutjanus apodus*	48	48A	48		1
M	58	Perciformes	Lutjaninae	*Lutjanus griseus*	48	48A	48		2
M	58	Perciformes	Lutjaninae	*Lutjanus synagris*	47 / 48	1M+46A/48A	48		1
M	58	Perciformes	Lutjaninae	*Ocyurus chrysurus*	48	48A	48		1
M	58	Perciformes	Lutjaninae	*Rhomboplites aurorubens*	48	46A+2ST	50		1
M	58	Perciformes	Opistognathidae	*Opistognathus macrognathus*	40	1M+6SM+2ST+28A	46		1
M	58	Perciformes	Pomacanthidae	*Pomacanthus arcuatus*	48	48A	48		1
M	58	Perciformes	Pomacanthidae	*Pomacanthus paru*	48	2ST+46A	50		1
M	58	Perciformes	Scaridae	*Nicholsina usta*	48	8M+10SM+6ST+24A	72		1
M	58	Perciformes	Scaridae	*Sparisoma aurofrenatum*	46	12M+12SM+12ST+10A	82		1
M	58	Perciformes	Scaridae	*Sparisoma chrysopterum*	46	6M+12SM+10ST+18A	74		1
M	58	Perciformes	Sciaenidae	*Bairdiella ronchus*	48	48A	48		
M	58	Perciformes	Sciaenidae	*Bairdiella sanctaluciae*	48	48A	48		
M	58	Perciformes	Sciaenidae	*Ophioscion punctatissimus*	48	48A	48		1
M	58	Perciformes	Sciaenidae	*Stellifer sp.*	48	48A	48		1
M	58	Perciformes	Serranidae	*Diplectrum formosum*	48	2M+46A	50		1
M	58	Perciformes	Serranidae	*Paralabrax dewegeri*	48	48A	48		1
M	58	Perciformes	Sparidae	*Archosargus rhomboidalis*	48	14M-SM+34A	62		
M	59	Pleuronectiformes	Paralichthyidae	*Citharichthys spilopterus*	26	14M+4SM+4ST+4A	48		
M	59	Pleuronectiformes	Paralichthyidae	*Etropus crossotus*	36	11M+14ST+11A	61		1
M	60	Tetraodontiformes	Monachantidae	*Stephanolepis setifer*	33 34	1M+32A 34A	34	X1X1X2X2 X1X2Y	1
M	60	Tetraodontiformes	Tetraodontidae	*Sphoeroides testudineus*	46	24M+22ST-A	70		
M	60	Tetraodontiformes	Tetraodontidae	*Sphoeroides greeleyi*	48	10M+8SM+12ST+16A	76		

Tabla 3. Análisis de varianza no paramétrico de Kruskal-Wallis para el rango medio del número diploide de cromosomas entre peces Dulceacuícola y Marinos.

Grupo	N	Rango medio	H	Grados de Libertad	P
Dulceacuícolas	26	70.0200	26.6341	1	$0.0005x10^{-4}$
Marino	67	37.7239			

Tabla 4. Análisis de varianza no paramétrico de Kruskal-Wallis para el rango medio del número de brazos cromosómicos entre peces Dulceacuícolas y Marinos.

Grupo	N	Mean Rank	H	DF	P
Dulceacuícolas	25	71.4800	30.0434	1	$0.0002x10^{-4}$
Marinas	67	37.1791			

Tabla 5. Frecuencia de especies con 48 cromosomas exclusivamente acrocéntricos y aquellos que poseen cariotipos distintos entre especies marinas y dulecacuícolas, y Test de independencia Chi-Cuadrado (χ^2).

Grupo	2n=48A	2n≠48A	Total
Dulceacuícolas	1	24	25
Marinas	25	42	67
Total	26	66	92

Tamaño Muestral: 92
Estadístico de contraste Chi-Cuadrado (χ^2): 9.9661
G.L.: 1
P-valor: 0.0016
Nº de celdas con frecuencias absolutas esperadas < 5: 0 de 4, un 0.0000%
Nº de celdas con frecuencias absolutas esperadas < 1: 0 de 4, un 0.0000%

Tabla 6. Proporción de especies con complementos 2n=48A entre Perciformes y no Perciformes y Test de independencia Chi-Cuadrado (χ^2)

Grupo	2n=48A	2n≠48A	Total
Perciformes	30	6	36
No Perciformes	12	44	56
Total	42	50	92

Tamaño Muestral: 92
Estadístico de contraste Chi-Cuadrado (χ^2): 33.8460
G.L.: 1
P-valor: <0.0001
Nº de celdas con frecuencias absolutas esperadas < 5: 0 de 4, un 0.0000%
Nº de celdas con frecuencias absolutas esperadas < 1: 0 de 4, un 0.0000

Tabla 7. Correlación por Rangos de Spearman entre la posición filogenética de los Ordenes (Ancestralidad), número diploide (2n) y Número de brazos cromosómicos (NF) con base en 92 especies de Actinopterygii

Ancestralidad	**2n**	**NF**
Coeficiente de Correlación	-0,46146442	-0,69146478
N	92	92
Probabilidad	$1,83049x10^{-06}$	$1,17044x10^{-14}$

FIGURAS

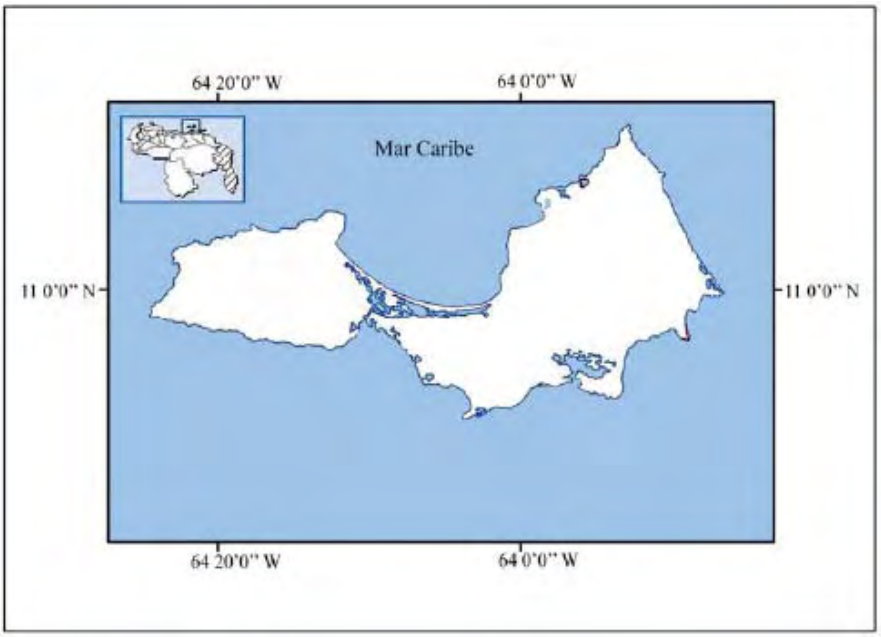

Figura 1. Localización de la Isla de Margarita en Venezuela.

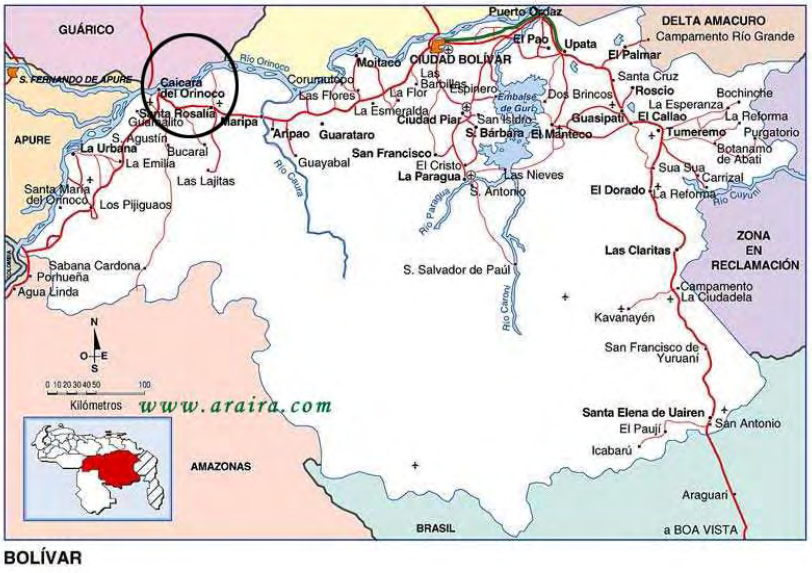

Figura 2. Ubicación de la localidad de Caicara del Orinoco**.** En el círculo destaca la ubicación

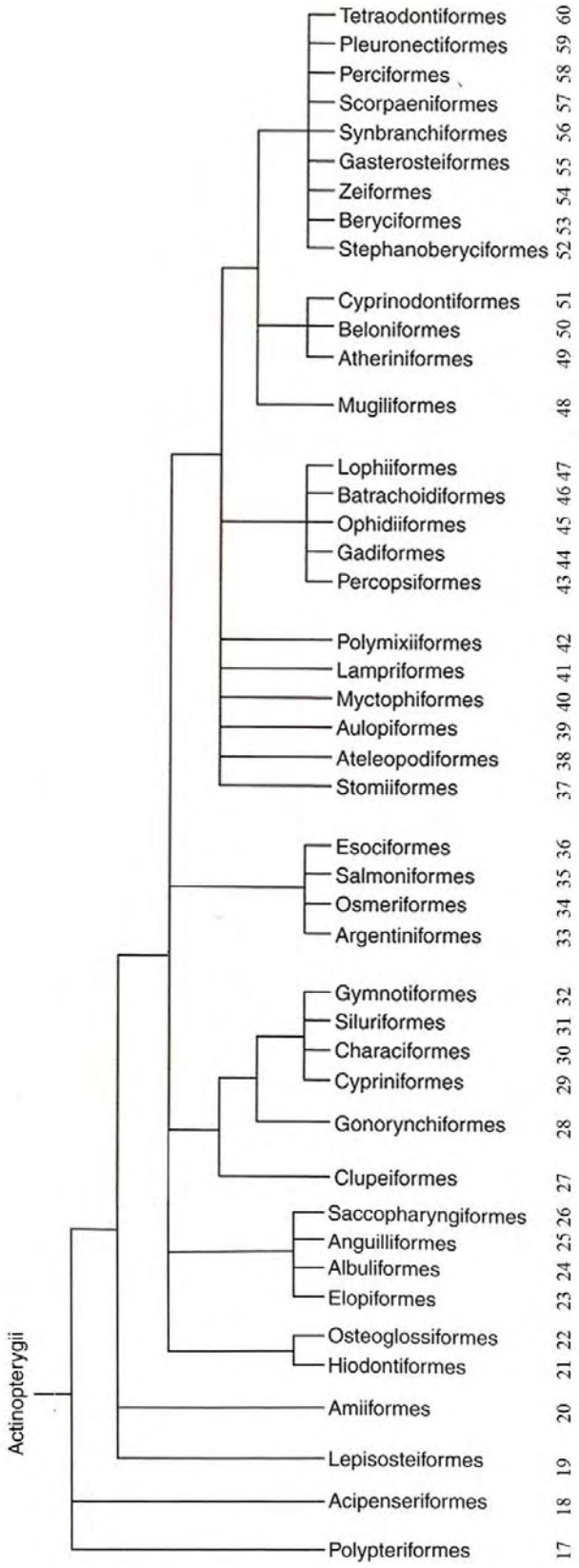

Figura 3. Filogenia de los Actinopterygii. El número al lado de cada Taxón representa la posición de cada Orden en el arbol filogenético, según Nelsón, 2006.

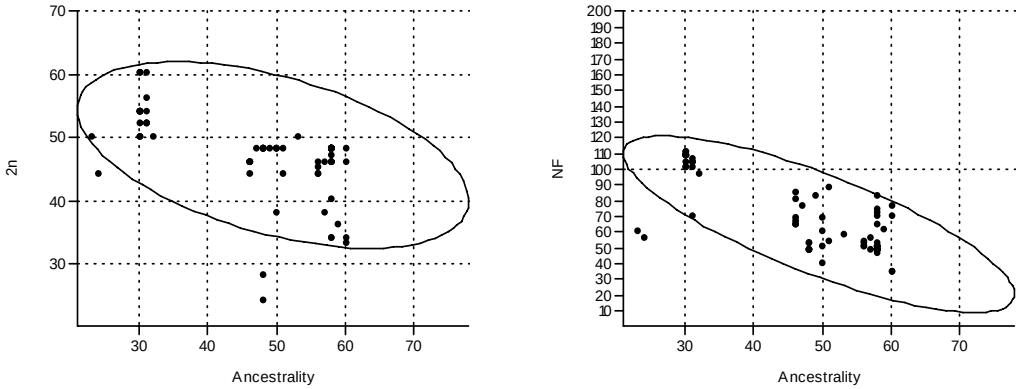

Figura 4. Ploteo del número diploide de cromosomas (2n) y el Número fundamental (NF) para peces de Venezuela contra la ordenación filogenética propuesta por NELSON (2006). El elipsoide define los límites que agrupan el 95% de los datos.

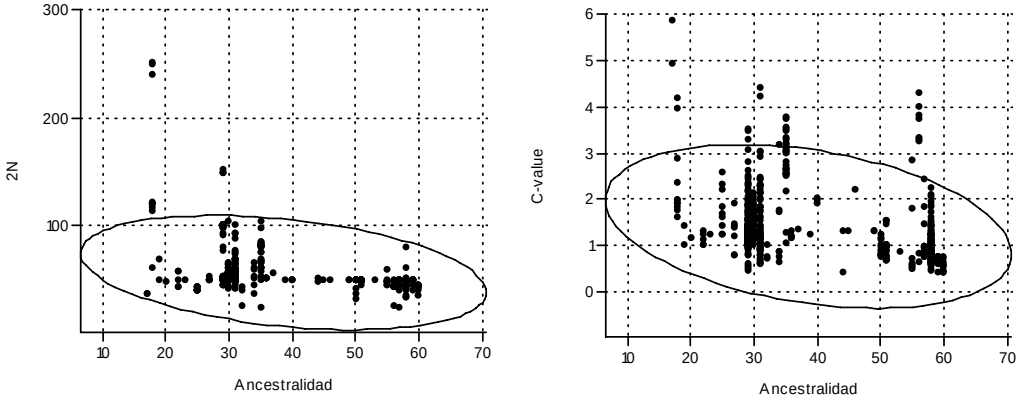

Figura 5. Ploteo del número diploide de cromosomas (2n) y el Número fundamental (NF) para 638 especies de peces obtenidos de la base de datos *Animal Genome Size Database* (Gregory, 2009, disponible en www.genomesize.com), contra la ordenación filogenética propuesta por NELSON (2006). El elipsoide define los límites que agrupan el 95% de los datos.

Cariotipos y fotografías ilustrativas de cada especie analizada

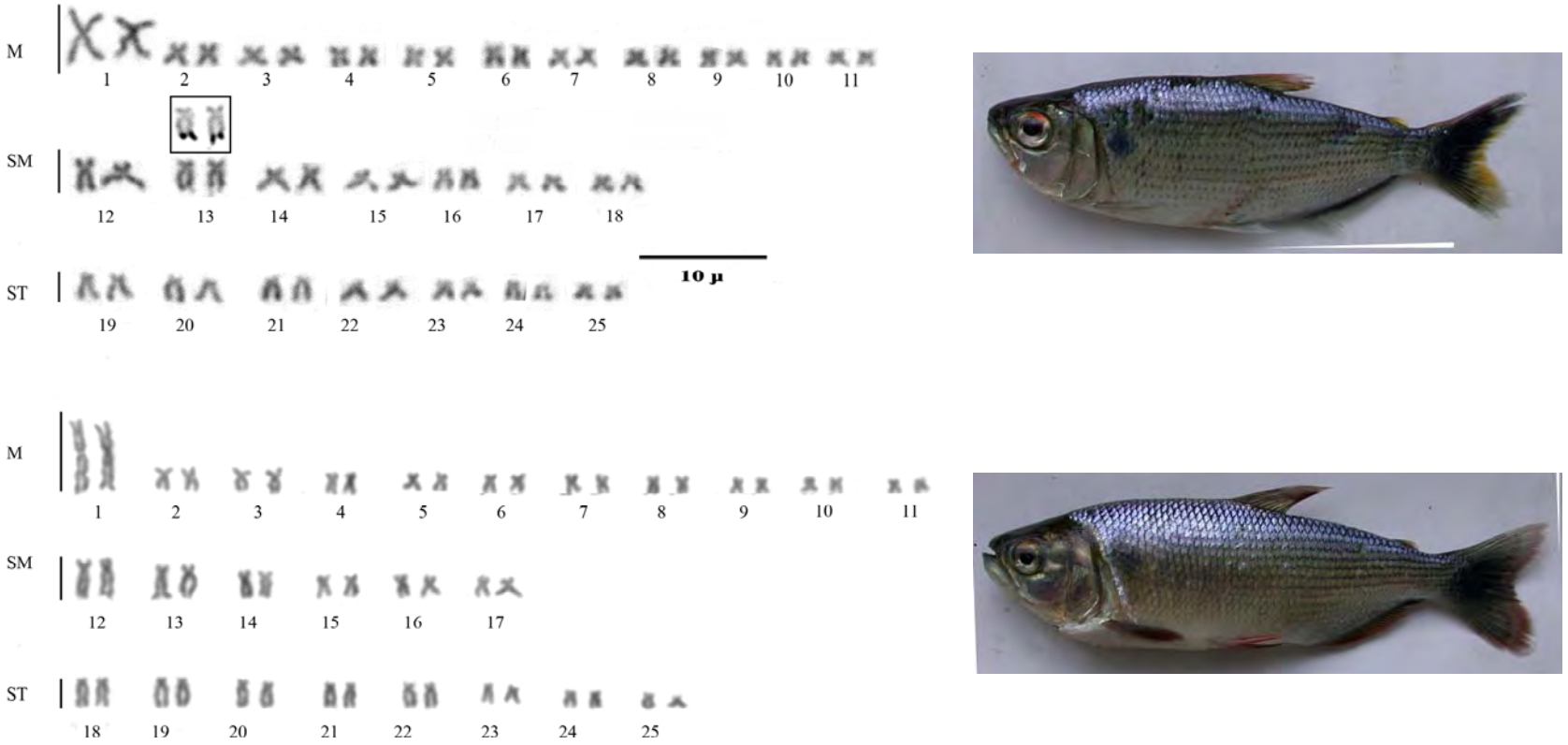

Figura 6. Cariotipos de *Brycon amazonicus* (arriba) y *B. Falcatus* (abajo). En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

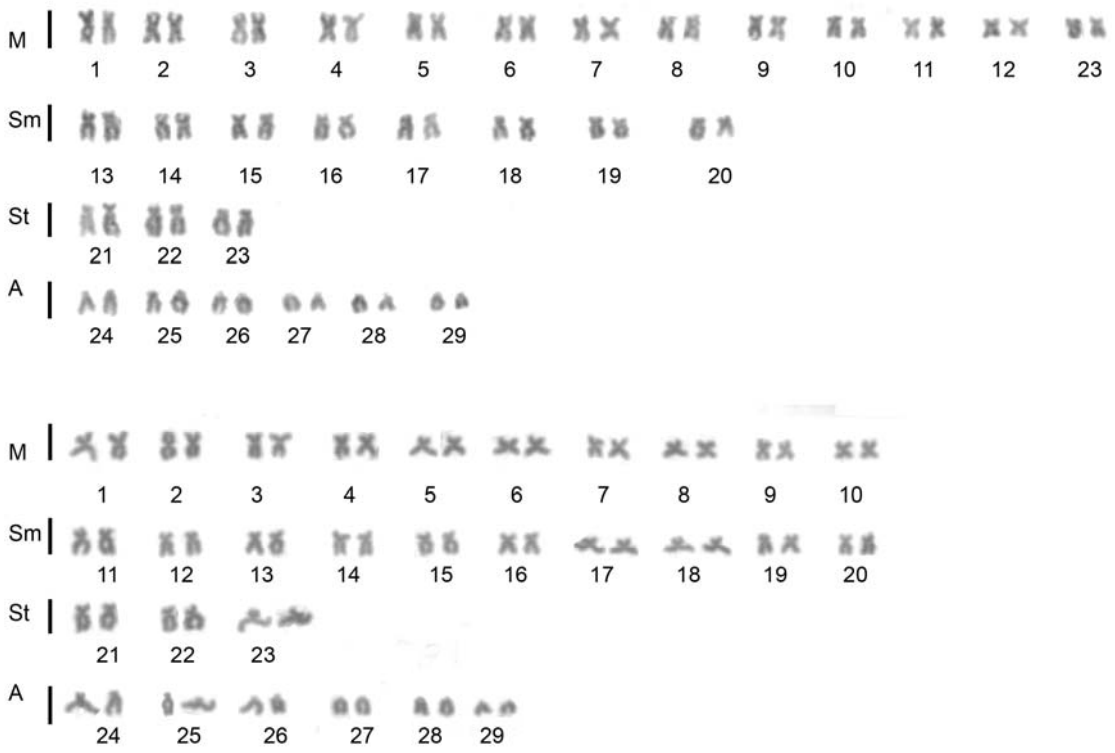

Figura 7. Cariotipos de *Serrasalmus altuvei* (arriba) y *S. Irritans* (abajo). En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

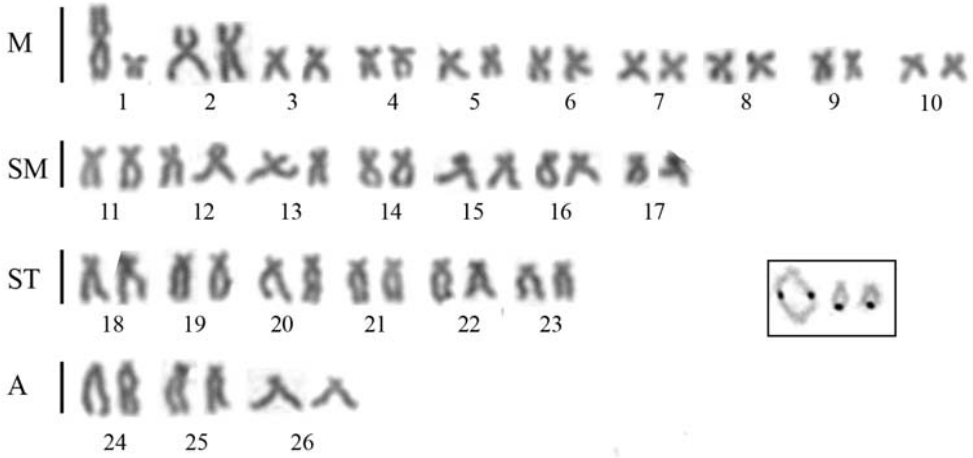

Figura 8. Cariotipo de *Triportheus orinoscensis* hembra. En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

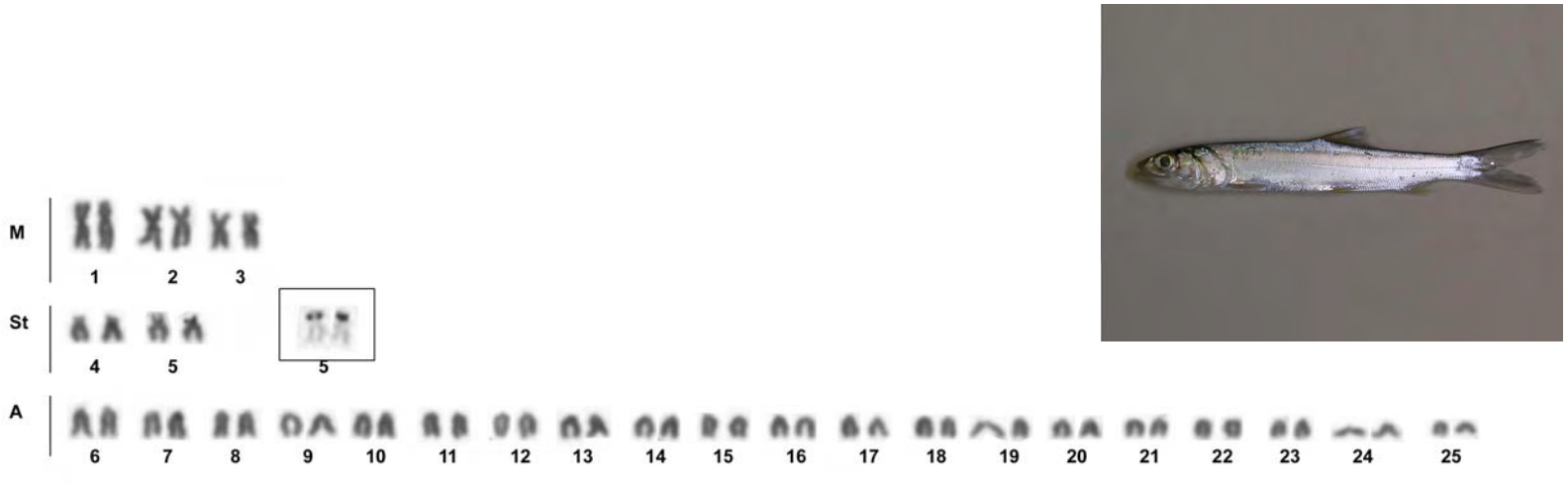

Figura 9. Cariotipo de *Elops saurus* Linnaeus, 1766. En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

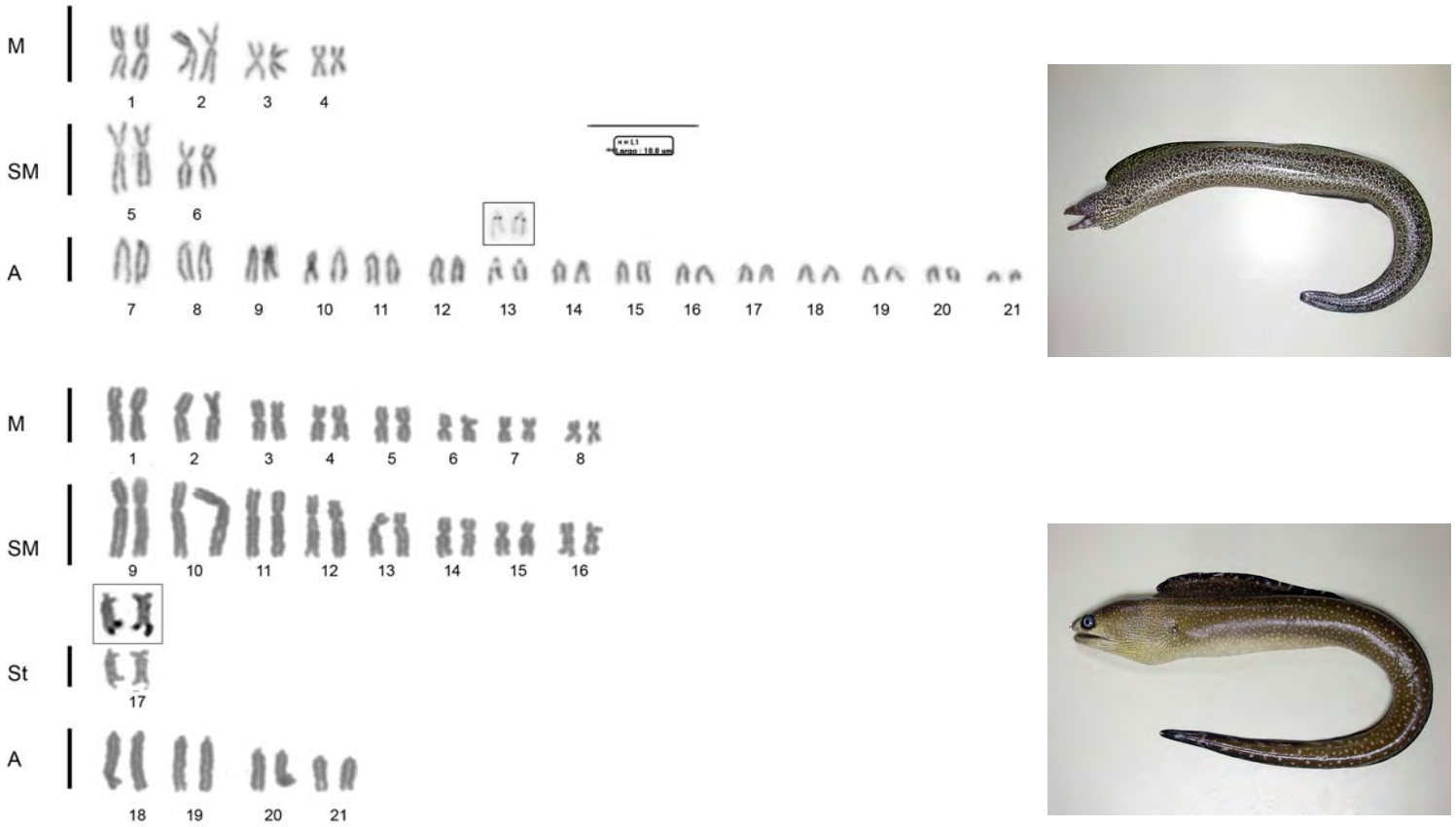

Figura 10. Cariotipo de *Gymnothorax moringa* (Cuvier, 1829) *y Gymnothorax ocellatus* Agassiz, 1828 *(abajo)*. En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

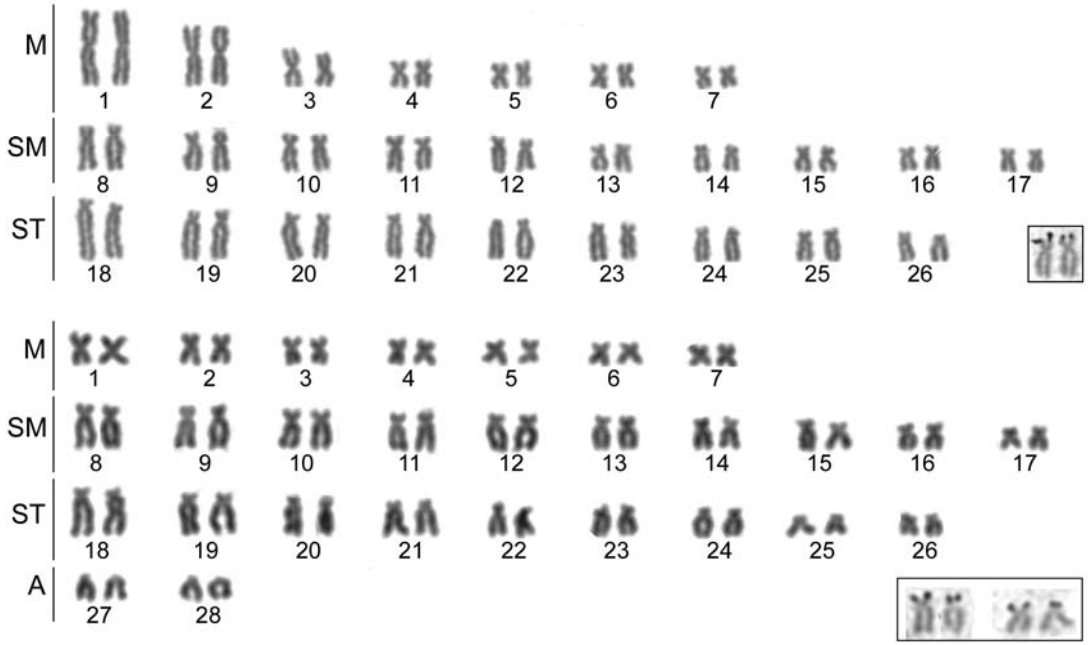

Figura 11. Cariotipo de *Cathorops spixii* (Agassiz, 1829) *(arriba) y Selenaspis herzbergii* (Bloch, 1794) *(abajo)*. En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

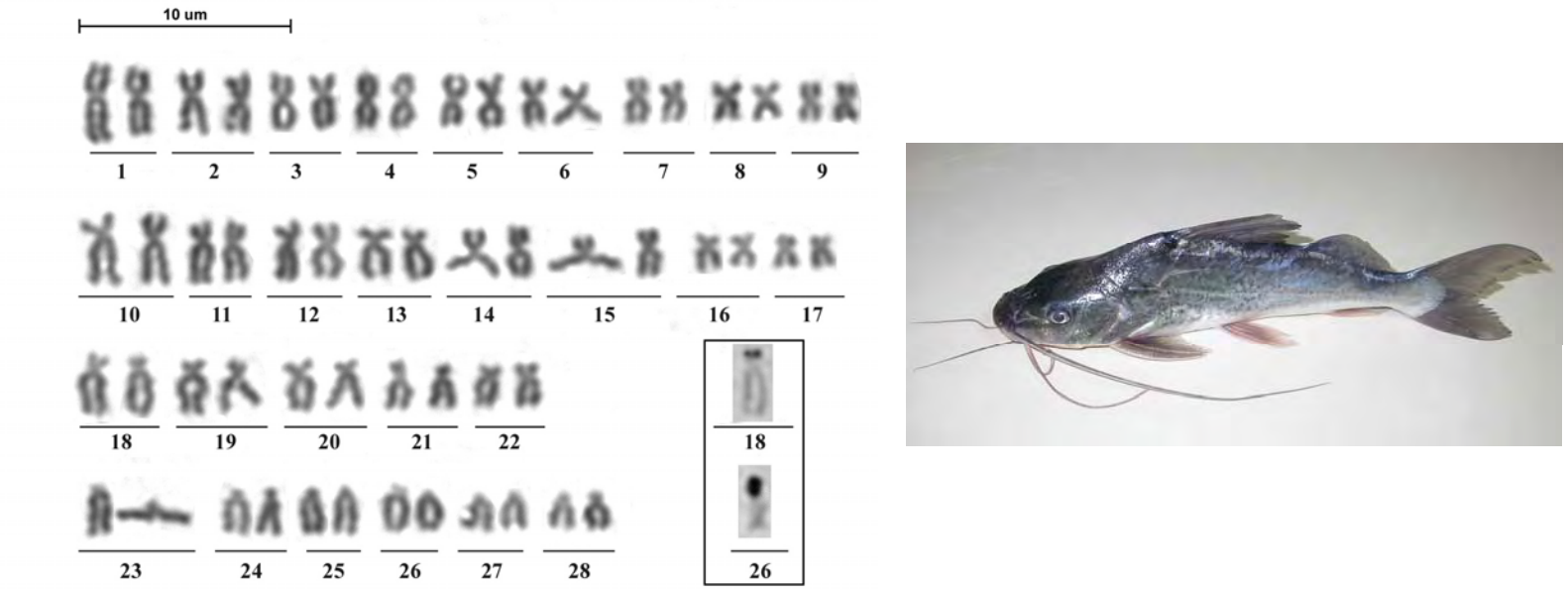

Figura 12. Cariotipo de *Pimelodus blochii* Valenciennes, 1840. En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

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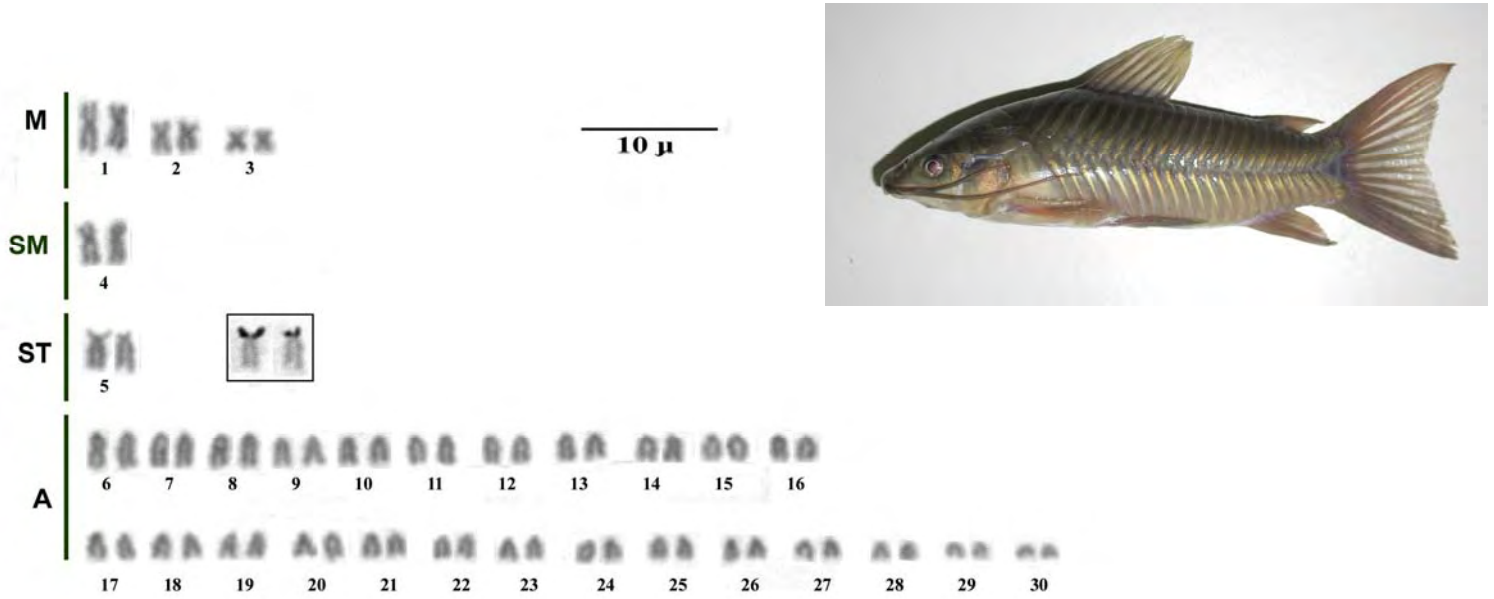

Figura 13. Cariotipo de *Hoplosternum littorale*. En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

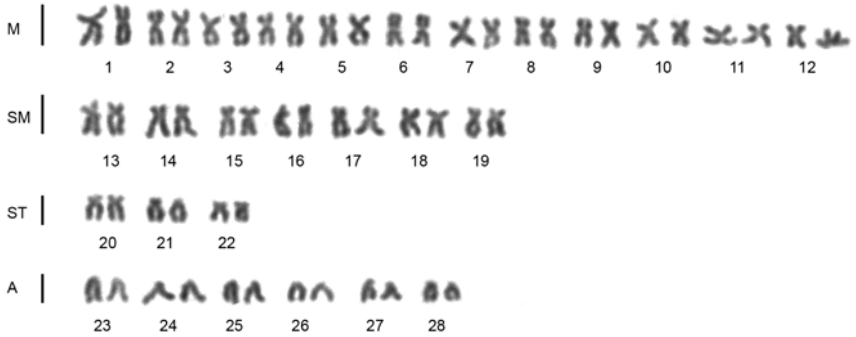

Figura 14. Cariotipo de *Pseudoplatystoma metaense (arriba) y Pseudoplatystoma orinocoense (abajo).* A la derecha del cariotipo se muestra una fotografía de la especie.

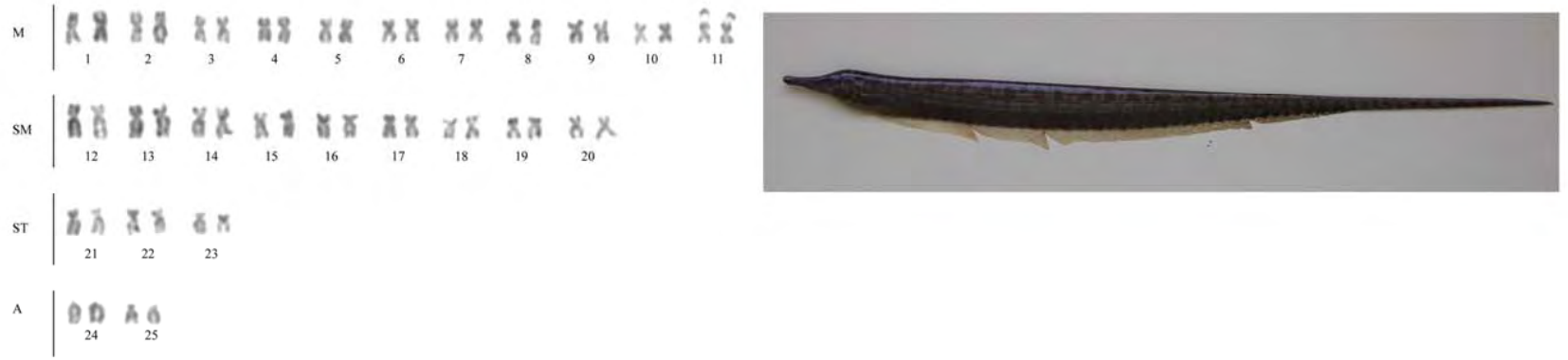

Figura 15. Cariotipo de *Ramphichthys sp.* A la derecha del cariotipo se muestra una fotografía de la especie.

Figura 15. Cariotipo de *Synodus foetens (Linnaeus, 1758).* A la derecha del cariotipo se muestra una fotografía de la especie.

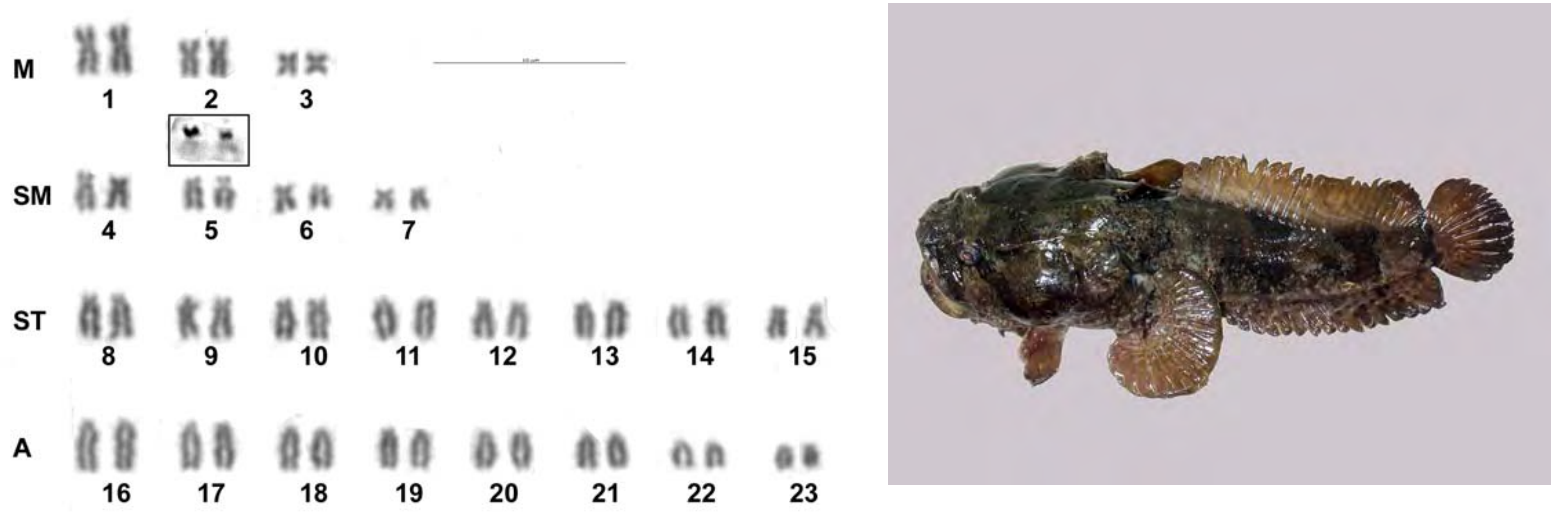

Figura 16. Cariotipo de *Batrachoides manglae* Cervigón, 1964. En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

Figura 17. Cariotipo de *Antennarius multiocellatus* (Valenciennes, 1837). A la derecha del cariotipo se muestra una fotografía de la especie.

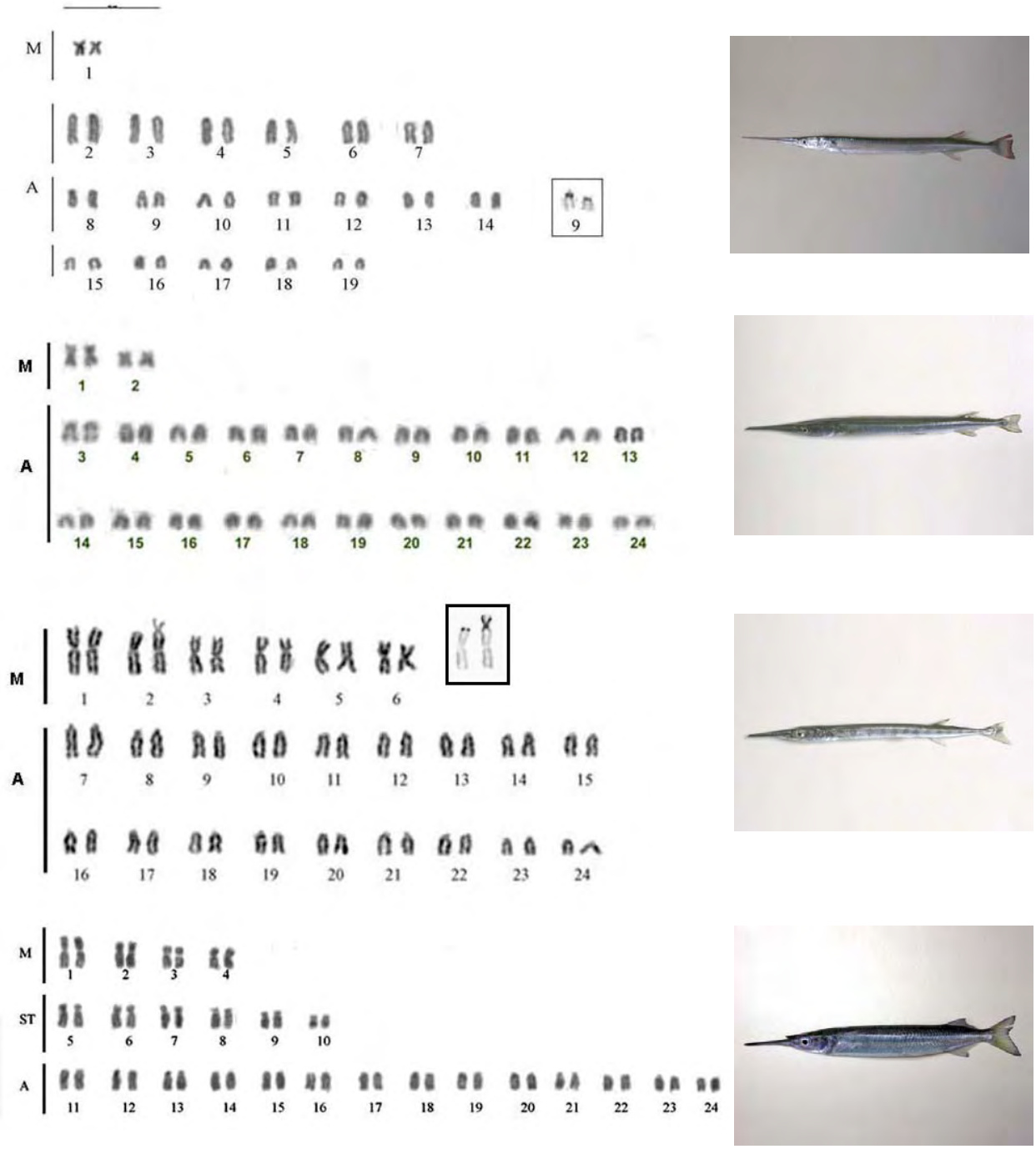

Figura 18. Cariotipo de (a) *Strongylura marina* (Walbaum, 1792), (b) *Strongylura timucu* (Walbaum, 1792), (c) *Tylosurus crocodilus crocodilus* (Péron and Lesueur, 1821), (d) *Hyporhamphus unifasciatus* (Ranzani, 1841). En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

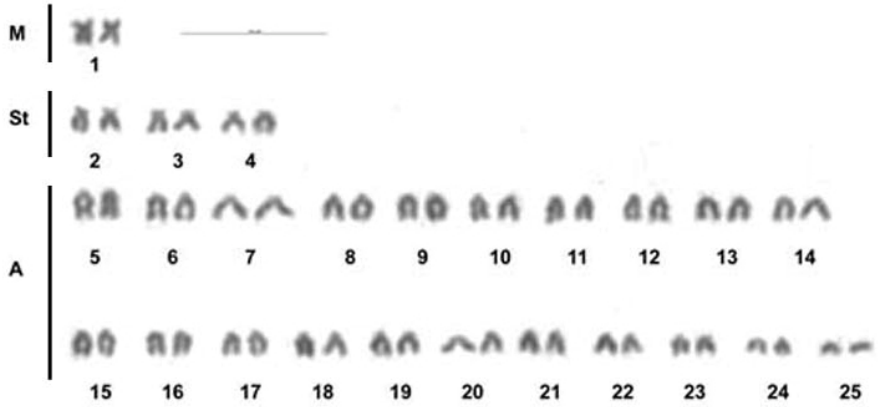

Figura 19. Cariotipo de *Holocentrus adscensionis* (Osbeck, 1765). A la derecha del cariotipo se muestra una fotografía de la especie.

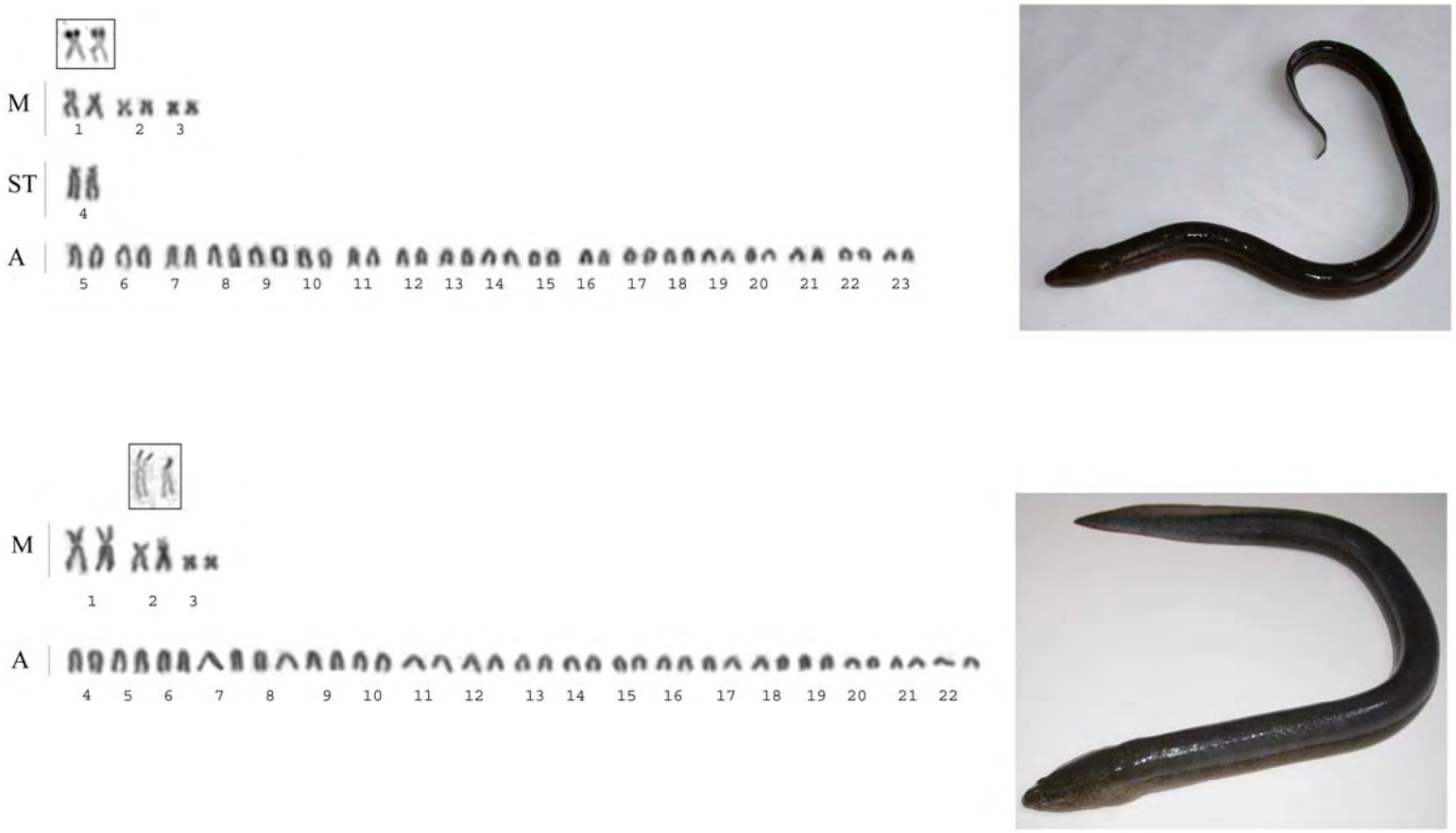

Figura 20. Cariotipos de *Ophisternom aenigmaticum* (a, a, c) y *Symbranchus marmoratus* (d). En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

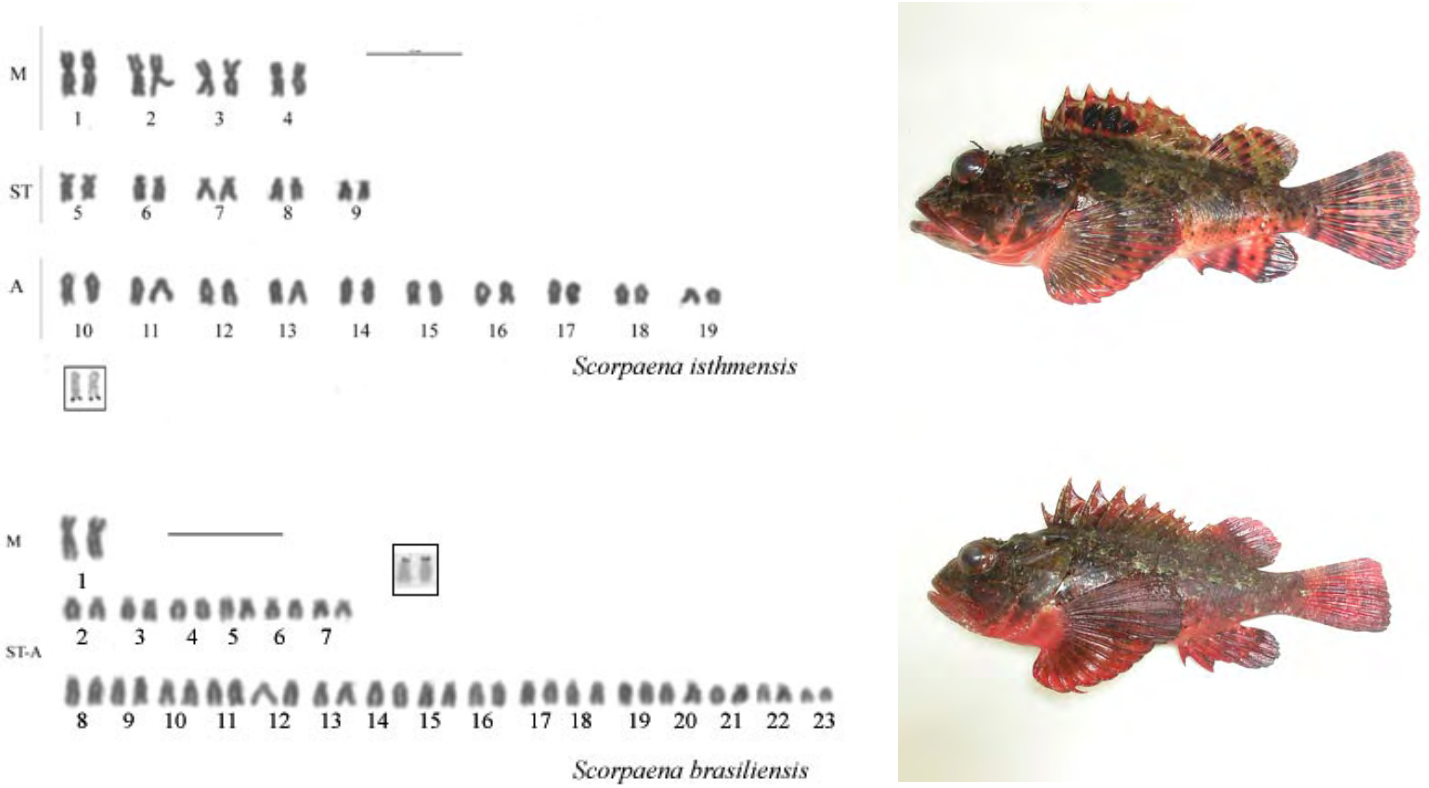

Figura 21. Cariotipos de *Scorpaena isthmensis* Meek and Hildebrand, 1928 (arriba) *y S. brasiliensis* Cuvier, 1829 (abajo) En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

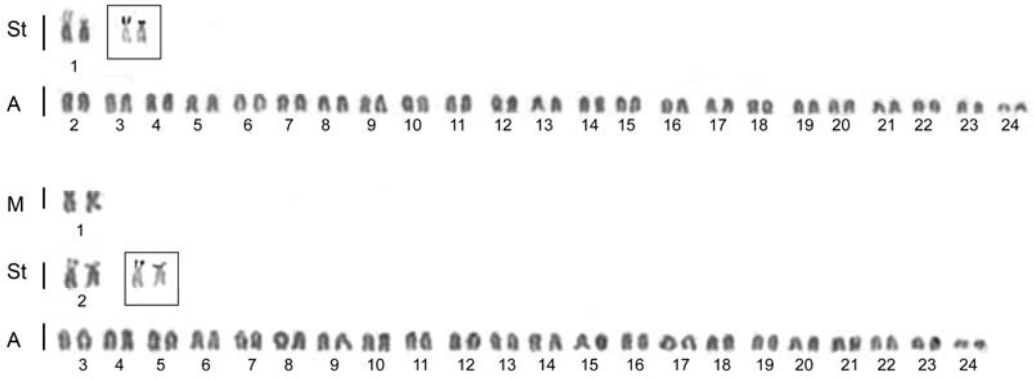

Figura 22. *Cariotipo de Selene vomer* (Linnaeus, 1758) (arriba) y *Trachinotus falcatus* (Linnaeus, 1758) (abajo). En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

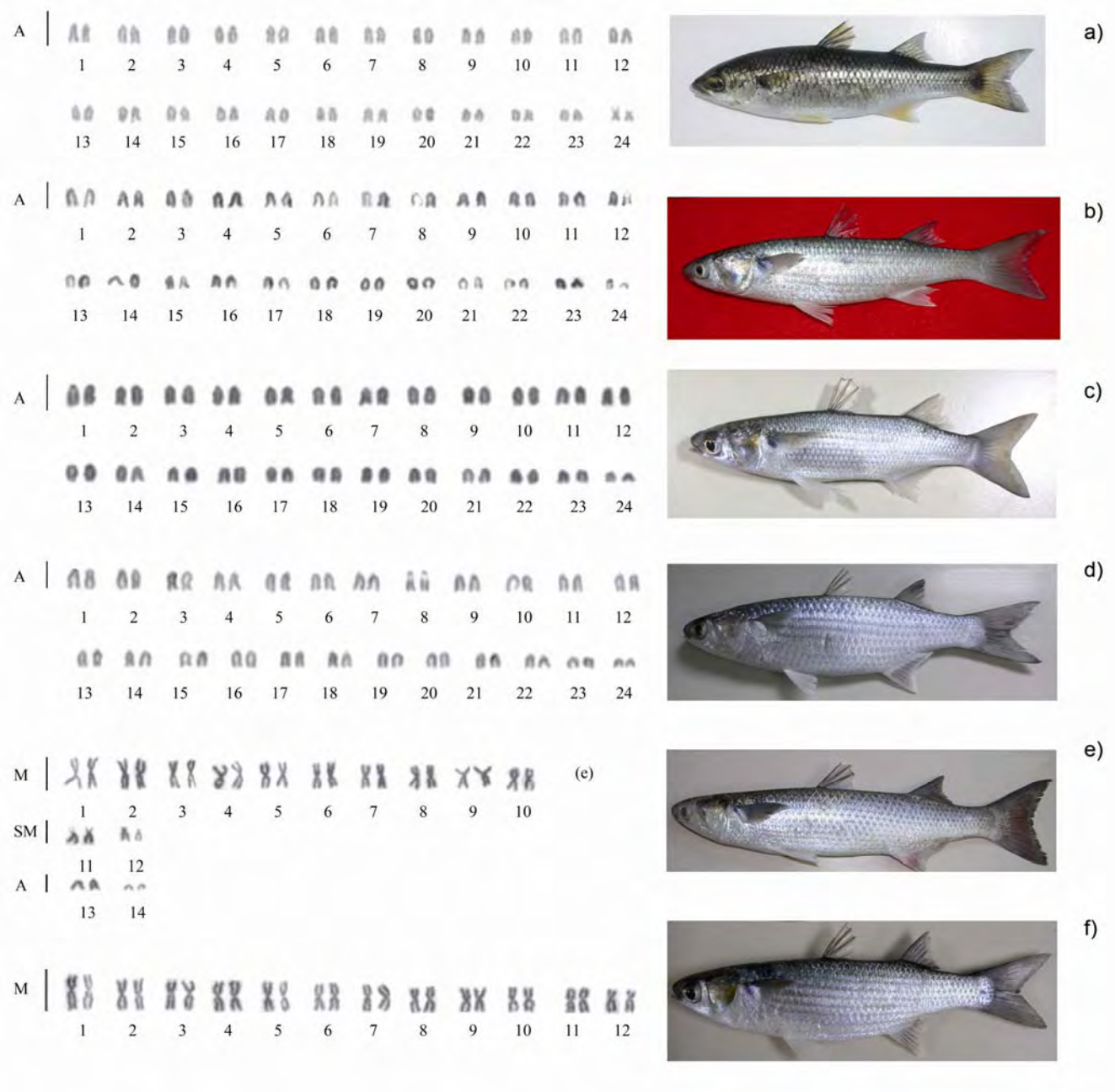

Figura 23. *Cariotipo de* (a) *Agonostomus monticola,* (b) *Mugil liza,* (c) *M. incilis*, (d) *M. ruberoculus,* (e) *M.* sp. y (f) *M. mugil curema.* A la derecha del cariotipo se muestra una fotografía de la especie.

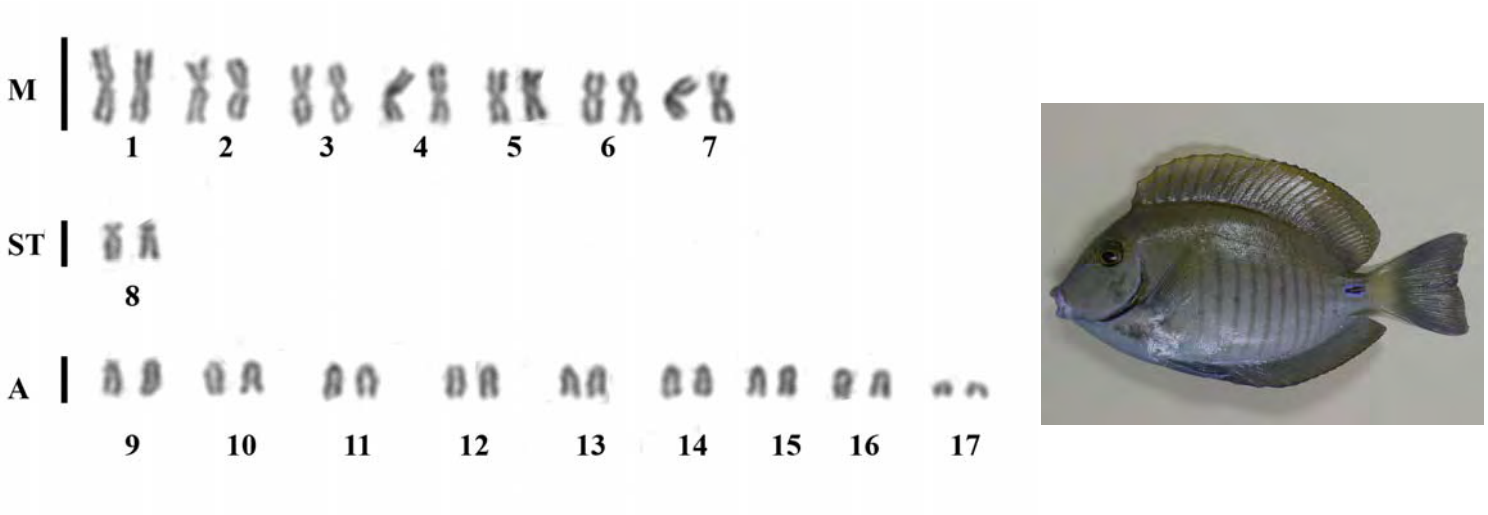

Figura 24. Cariotipo de *Achanturus chirurgus.* A la derecha del cariotipo se muestra una fotografía de la especie.

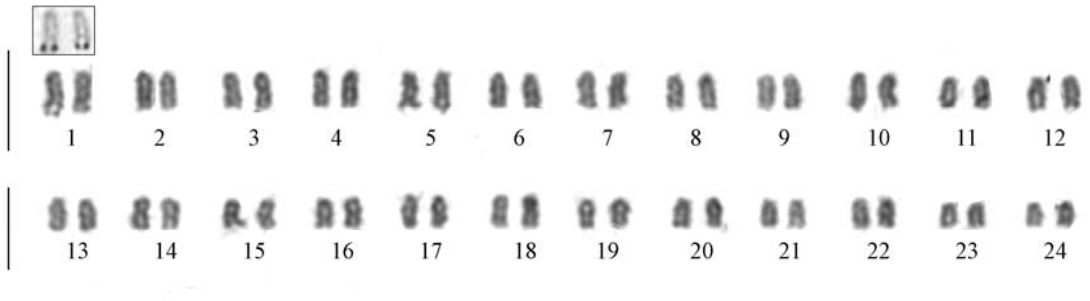

Figura 25. Cariotipo de *Centropomus undecimalis.* En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

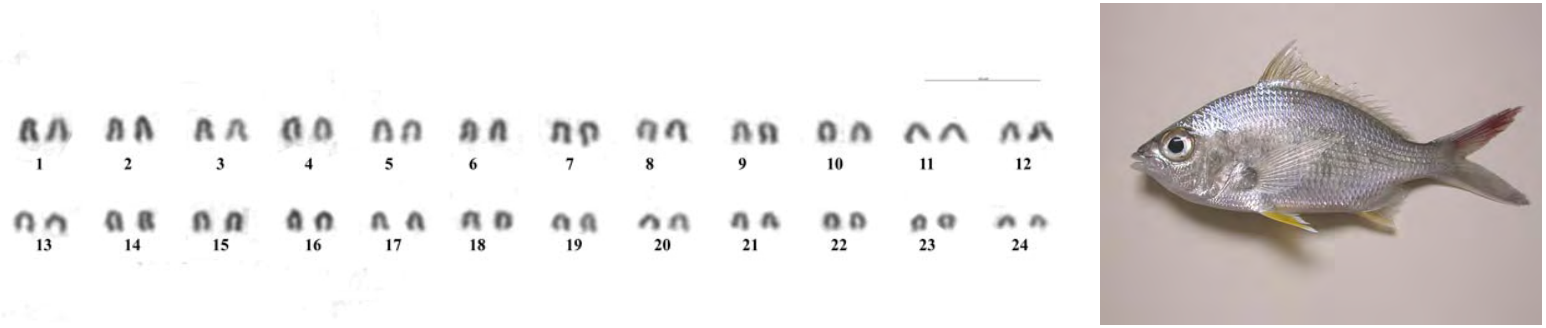

Figura 26. Cariotipo de *Euscinostomus argenteus.* A la derecha del cariotipo se muestra una fotografía de la especie.

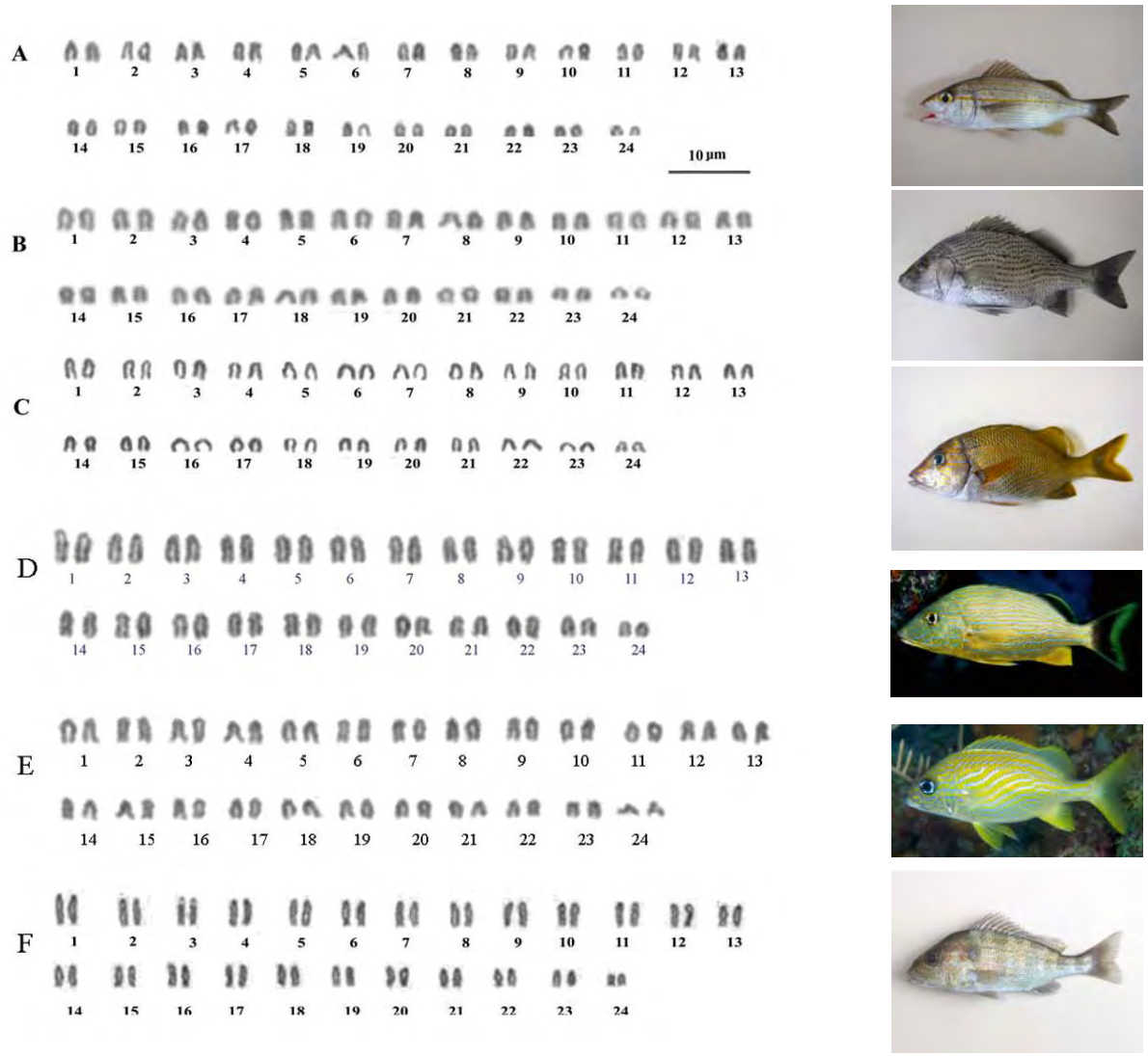

Figura 27. Cariotipos de *Haemulon aurolineatum (A), H. bonariense (B), H. plumierii (C), H. sciurus (D), H. flavolineatum(E) y Orthopristis ruber (F).* A la derecha del cariotipo se muestra una fotografía de la especie.

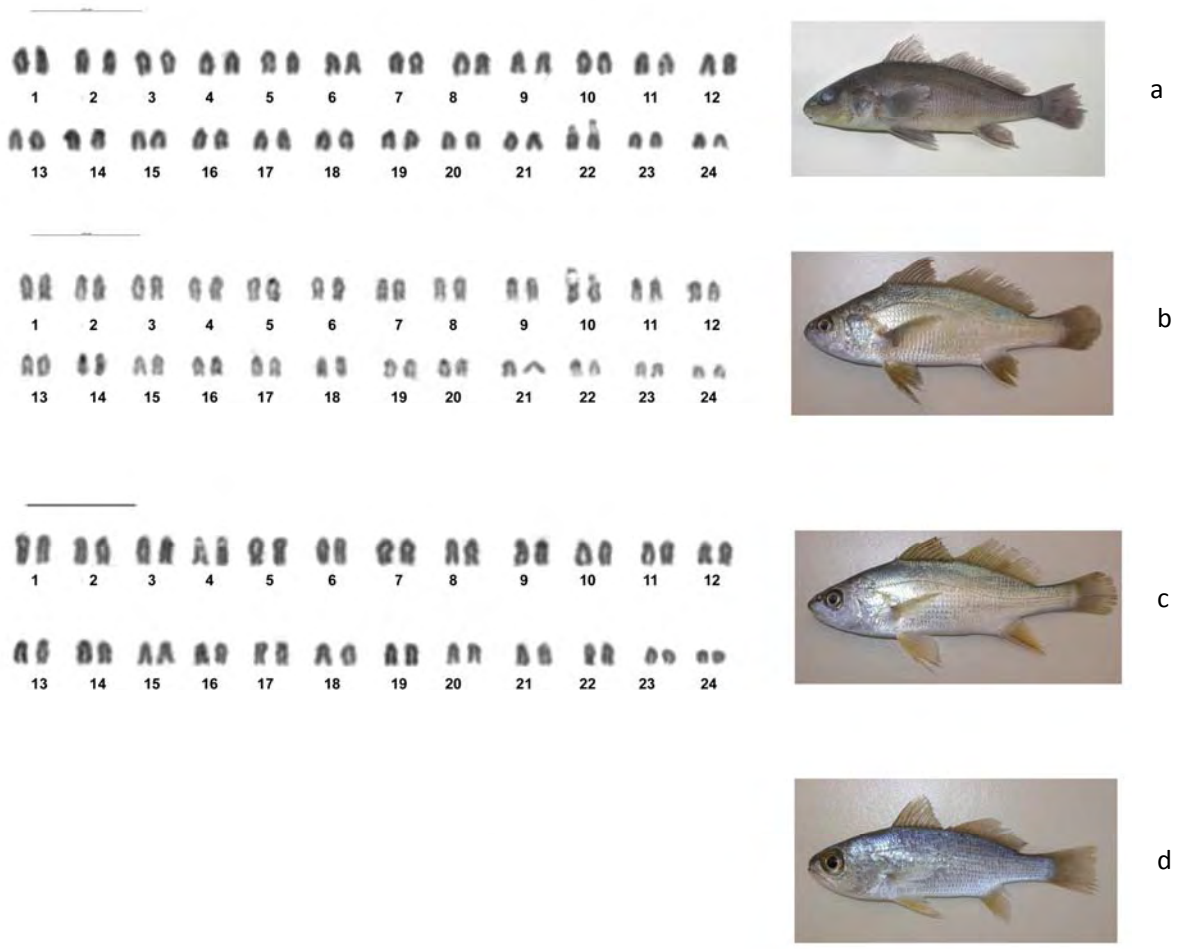

Figura 28. Cariotipo de *Ophioscion punctatissimus (a), Stellifer microps (b), Bairdiella ronchus (c) y B. sanctaluciae.* A la derecha del cariotipo se muestra una fotografía de la especie.

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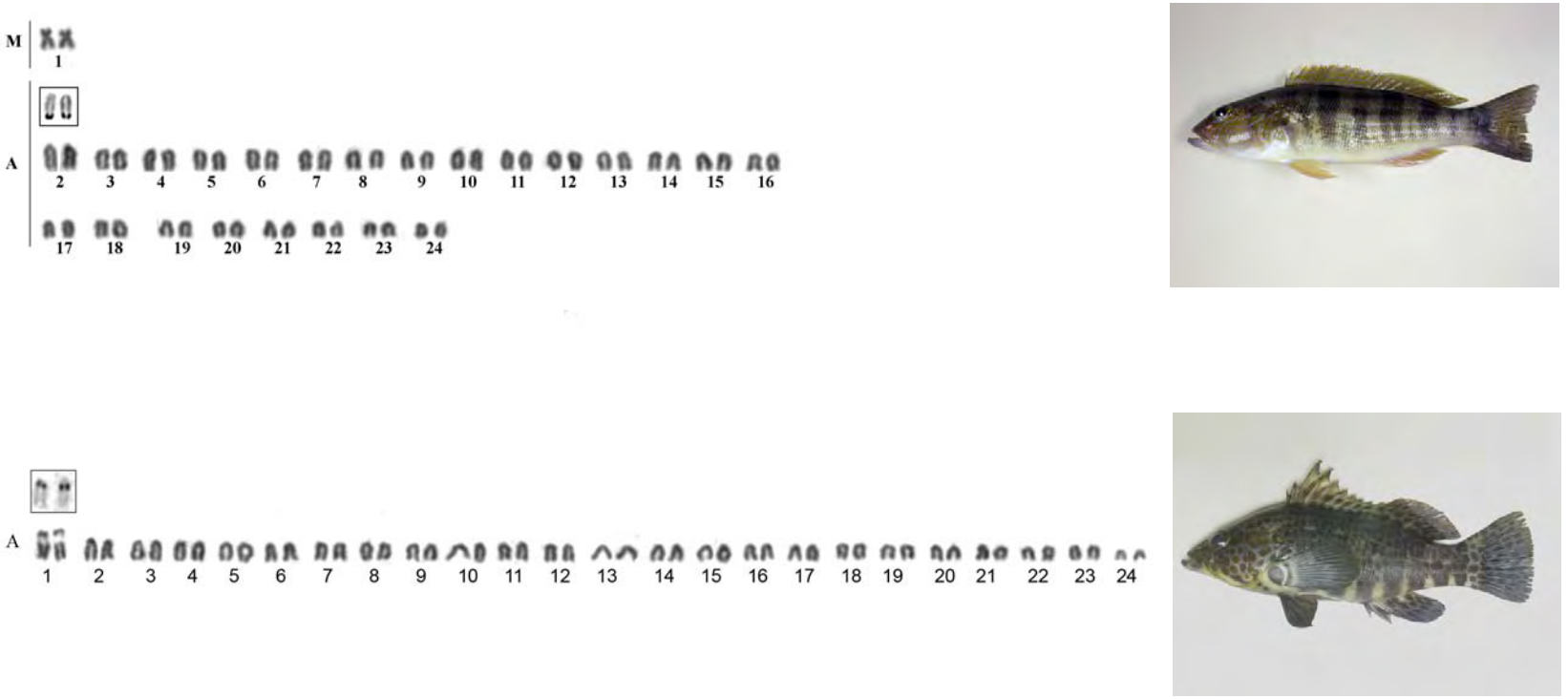

Figura 29. Cariotipo de *Diplectrum formosum (arriba) y Paralabrax degeweri (abajo)*. En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

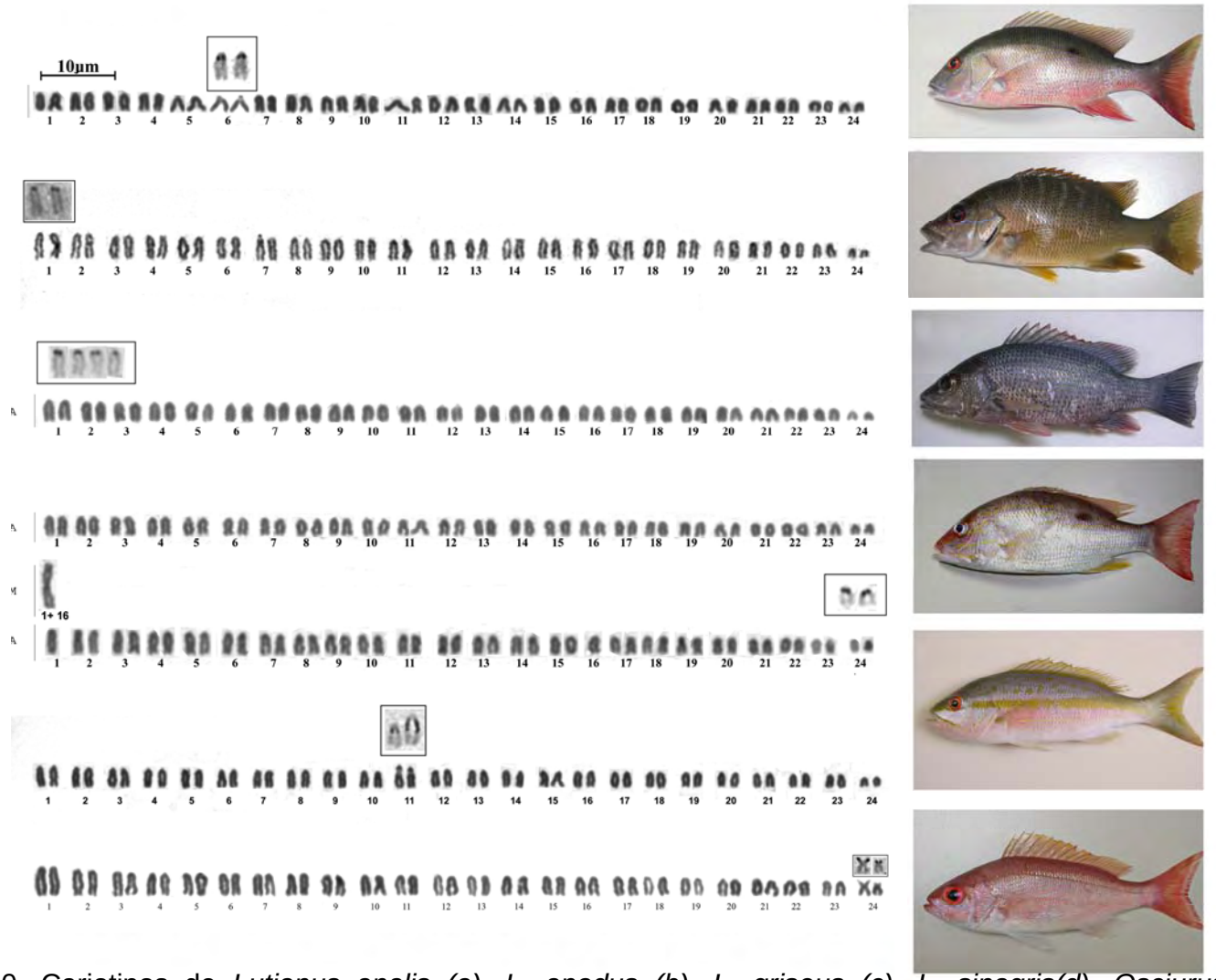

Figura 30. Cariotipos de *Lutjanus analis (a), L. apodus (b), L. griseus (c), L. sinagris(d), Osciurus chrysurus(e) y Rhomboplites aurorubens (f).* En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

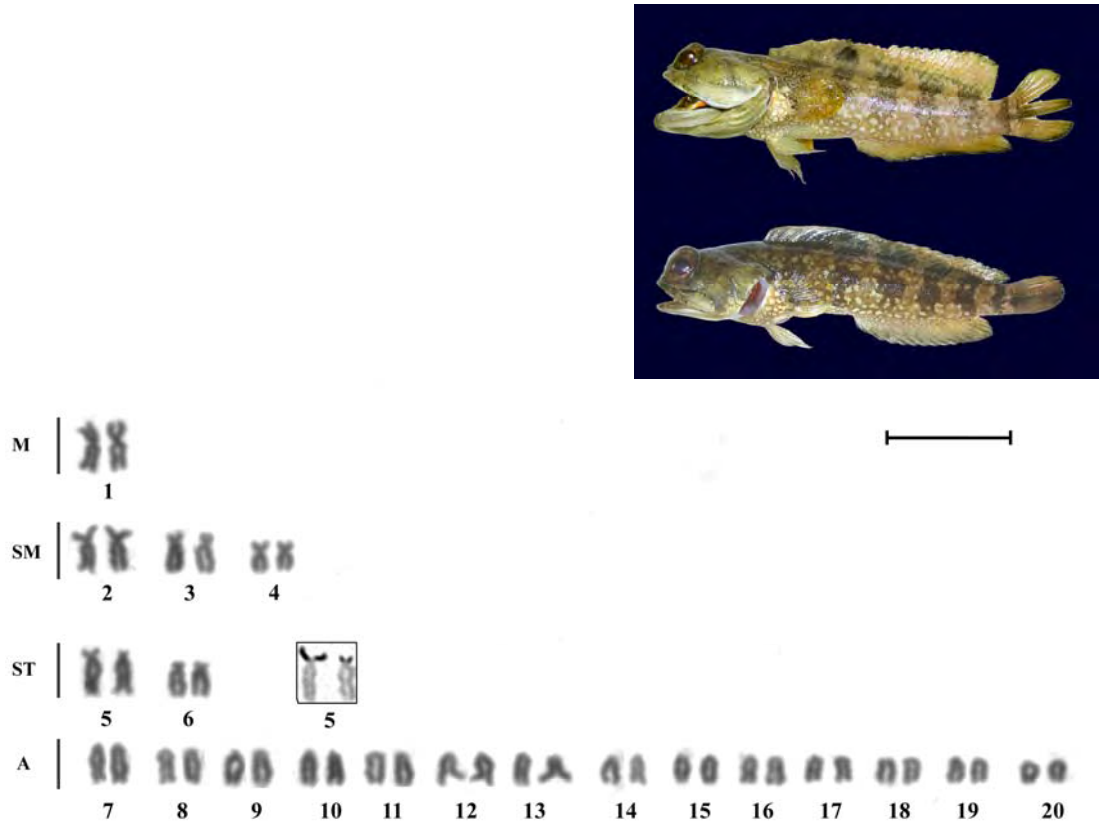

Figura 31. Cariotipo de *Opistognatus macrognatus.* En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

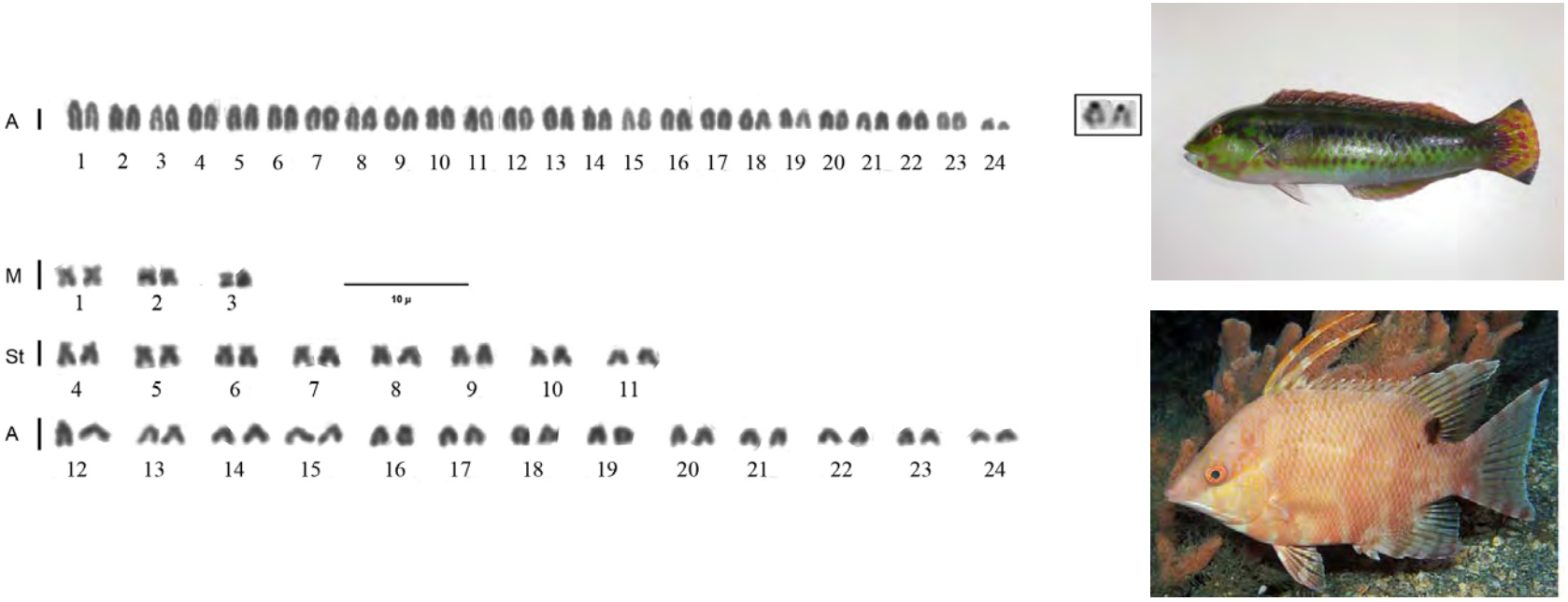

Figura 32. Cariotipo de *Halichoeres bivittatus (arriba) y Lachnolaimus maximus (abajo)*. En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

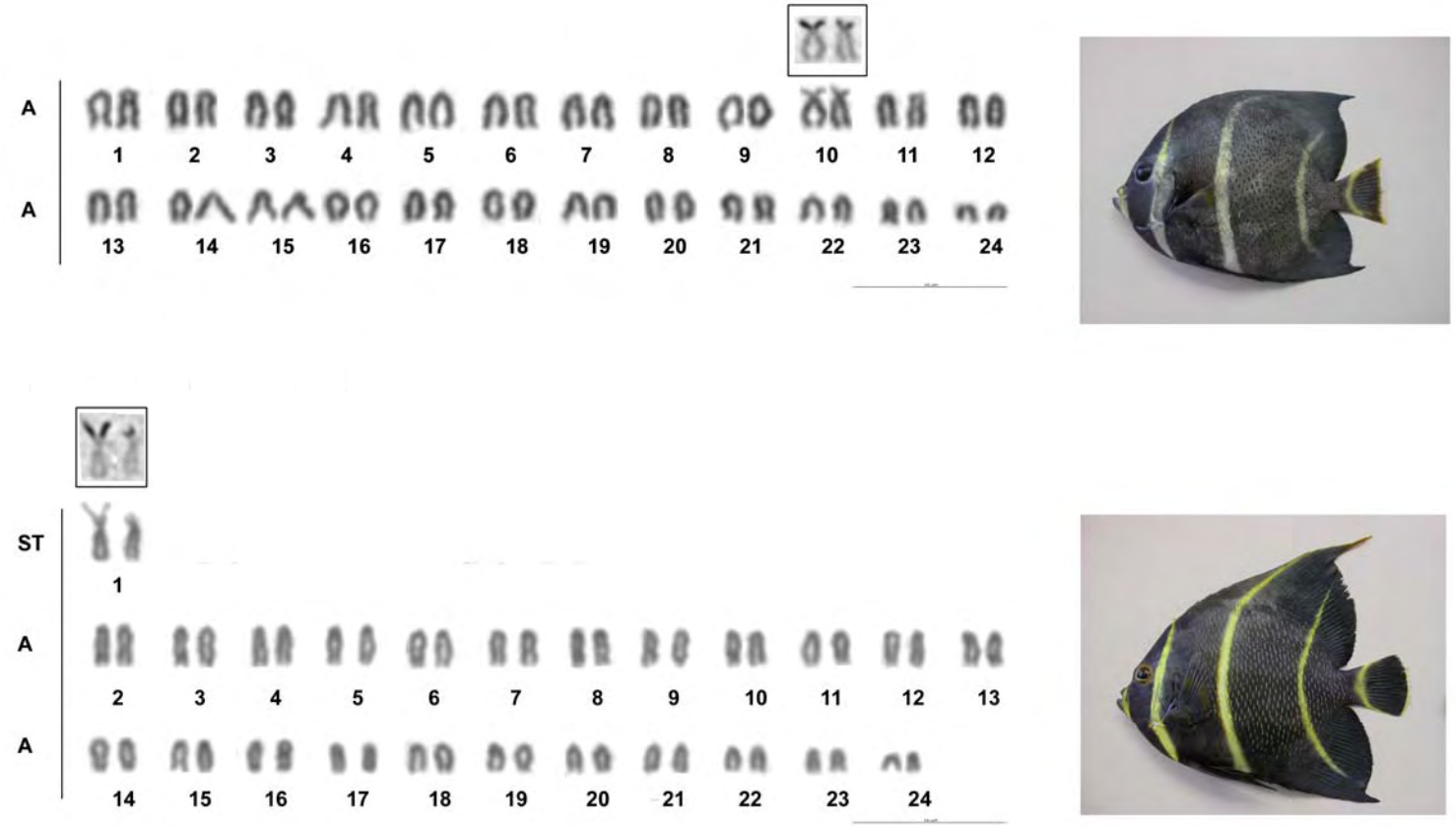

Figura 33. Cariotipo de *Pomacanthus arcuatus (arriba) y P. paru (abajo)*. En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

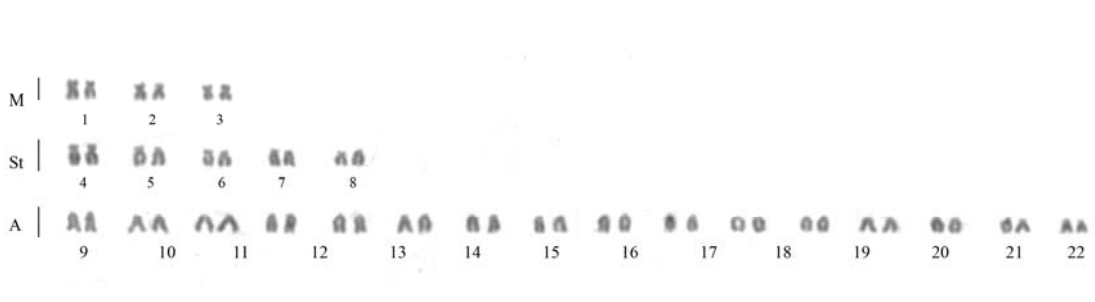

Figura 34. Cariotipo de *Archosargus rhomboidalis*. A la derecha del cariotipo se muestra una fotografía de la especie.

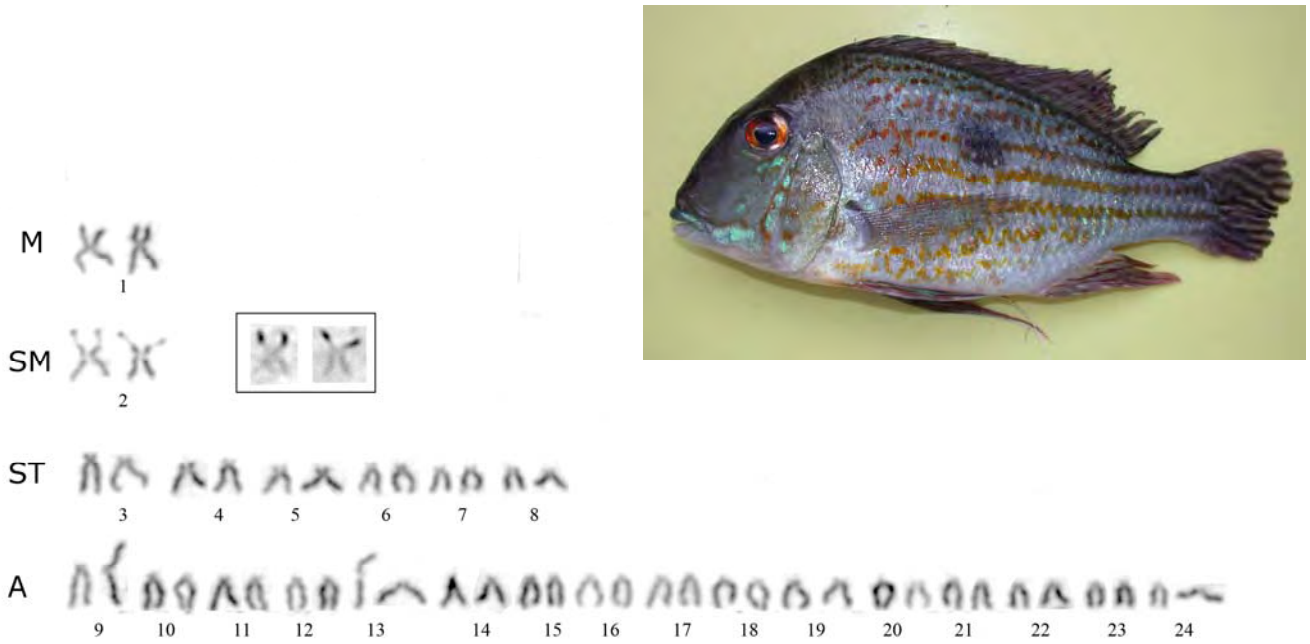

Figura 35. Cariotipo de *Geophagus surinamensis*. En el recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

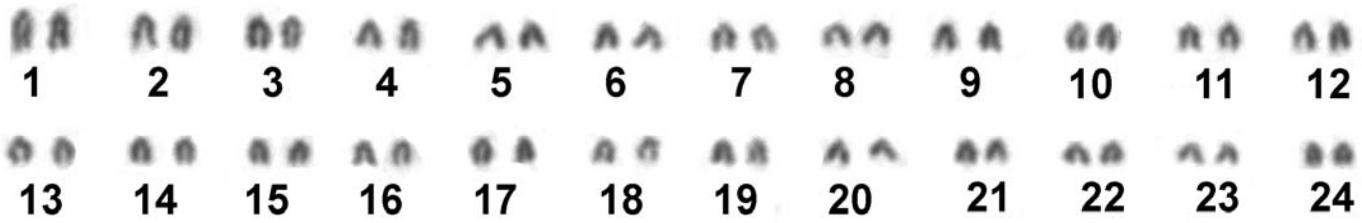

Figura 36. Cariotipo de *Ciclha sp*. A la derecha del cariotipo se muestra una fotografía de la especie.

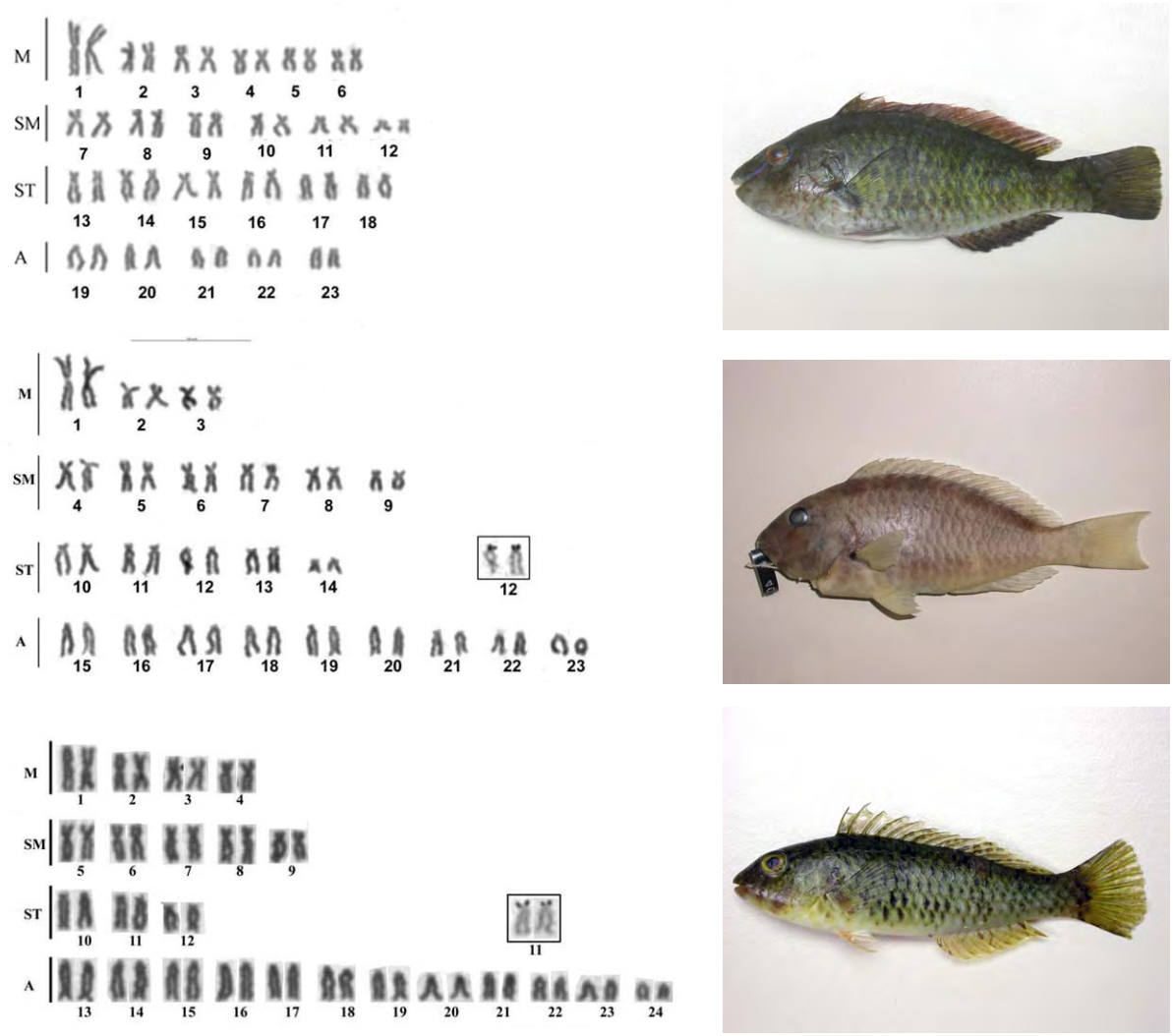

Figura 37. Cariotipo de *Sparisoma aurofrenatum, S. chrysopterum y Nicholsina usta. En el* recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

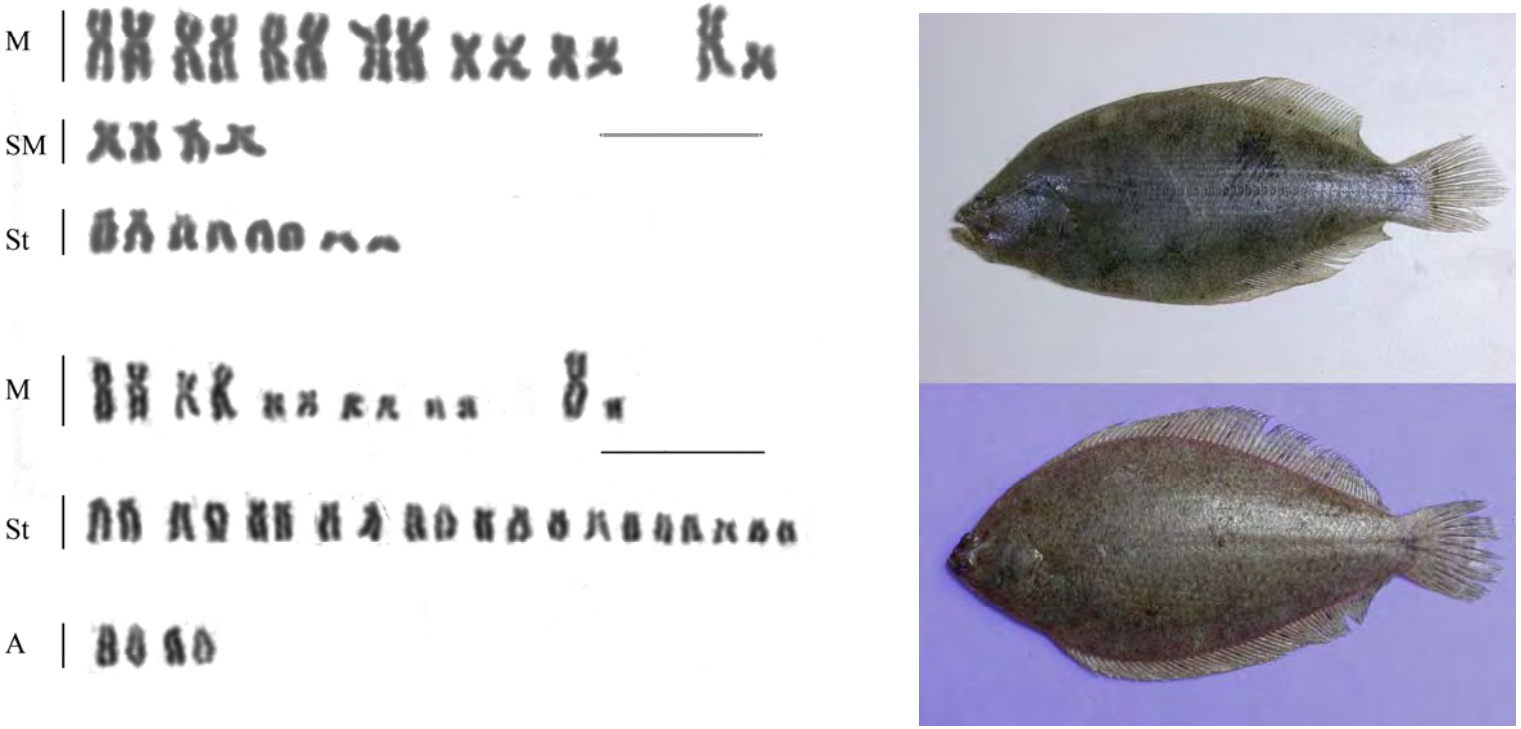

Figura 38. Cariotipo de *Citharichthys spilopterus* y *Etropus crossotus.* A la derecha del cariotipo se muestra una fotografía de la especie.

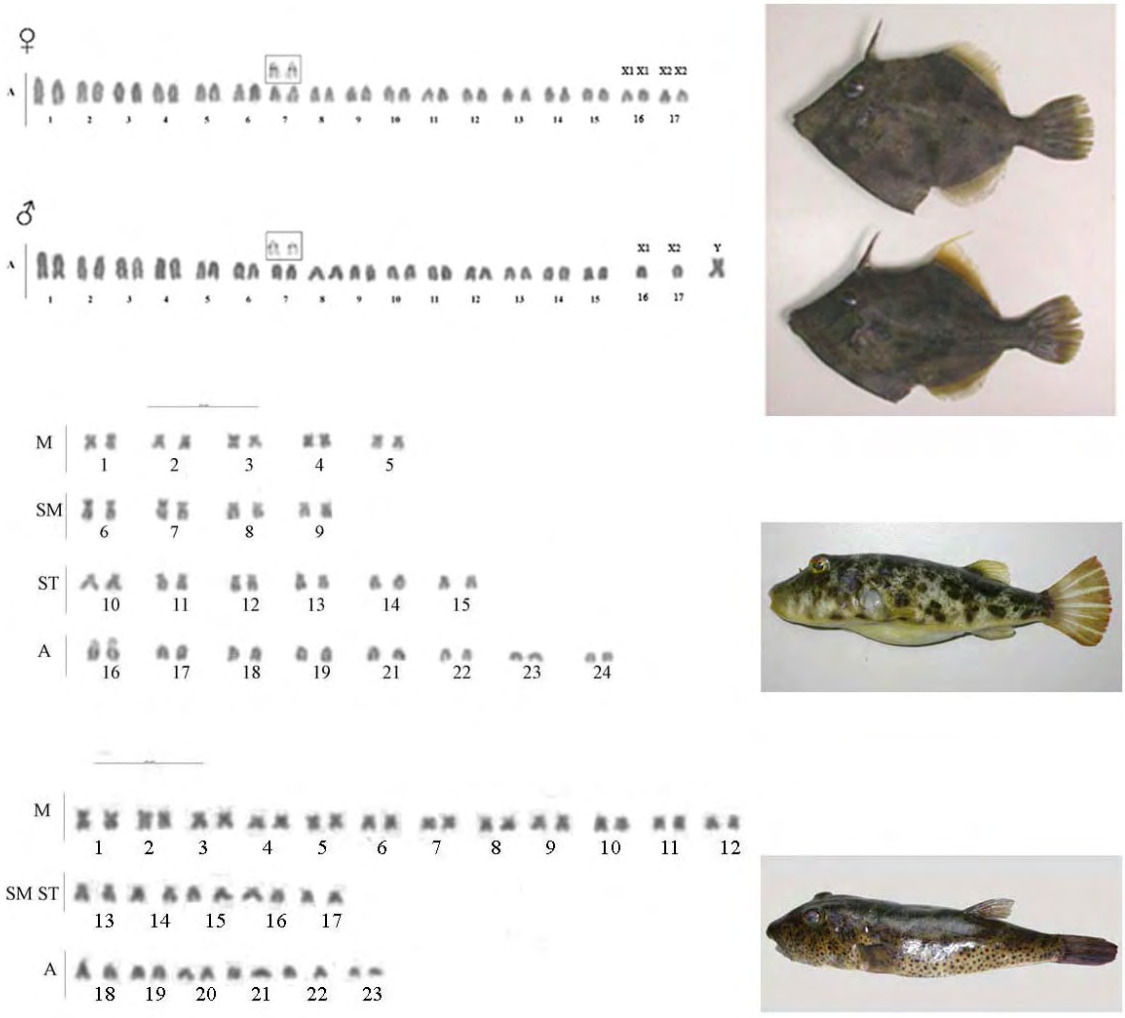

Figura 39. Cariotipo de *Stephanoepis setifer, Sphoeroides testudineus y S. greelegii. En el* recuadro se destaca el par de cromosomas portador de las Regiones Organizadoras del Nucleolo. A la derecha del cariotipo se muestra una fotografía de la especie.

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