

**KARYOTYPE AND NUCLEOLAR ORGANIZER REGIONS
IN *Serrasalmus rhombeus* (SERRASALMINAE) FROM CAICARA
DEL ORINOCO, VENEZUELA**

Mauro Nirchio, Alberto Granado, Ernesto Ron and Julio E. Pérez

SUMMARY

Cytogenetic studies were conducted on *Serrasalmus rhombeus* from Caicara del Orinoco, Venezuela. The diploid chromosome number $2n=60$ was composed of 30 metacentric, 16 submetacentric, 4 subtelocentric and 10 acrocentric chromosomes ($NF=110$). Four pairs of NOR-bearing chromosomes were detected. NOR are located terminally on the short arms of

one pair of medium sized subtelocentric chromosomes, and on three small sized acrocentric chromosome pairs. The karyotype diversity in this species suggests that karyotype formulae and NOR number could be useful for characterization of intraspecific stocks or populations.

Introduction

"Piranhas" or "caribes", as they are commonly known, are a group of Neotropical fishes of high diversity and predatory habits, and an important part of South American freshwater communities. This group comprises 5 genera: *Pygopristis*, *Pygocentrus*, *Pristobrycon*, *Serrasalmus* and *Catoprion*, thoroughly distributed in South America (Machado-Allison and Fink, 1996).

In Venezuela four genera and 16 species have been identified: *Pygopristis* (*P. denticulatus*); *Pygocentrus* (*P. cariba*); *Pristobrycon* (*P. calmoni*, *P. careospinus*, *P. maculipinnis* and *P. striolatus*); and *Serrasalmus* (*S. altuvei*, *S. eigenmanni*, *S. elongatus*, *S. gouldingi*, *S. irritans*, *S. manueli*, *S. medinai*, *S. nalseni*, *S. neveriensis*, and *S. rhombeus*) (Machado-Allison and Fink, 1996). Several species have been karyotyped to date

(Muramoto *et al.*, 1968; Prado and Galetti, 1986; Cestari and Galetti, 1992; Nakayama, 1997; Nakayama *et al.*, 2000, 2001, 2002; Centofante *et al.*, 2002) but, as far as we know, there are no cytogenetic studies reported on these species in Venezuela.

This paper describes the diploid number, chromosome formula and NOR locations in *S. rhombeus* from Caicara del Orinoco, Venezuela.

Materials and Methods

Sixteen individuals (9 males and 7 females) of white piranha *Serrasalmus rhombeus* (Linnaeus, 1766; Characidae, Serrasalminae) were captured with seine nets in the lowland flood plains near Caicara del Orinoco, Bolivar State, Venezuela, and transported to the *Instituto Limnológico* of the *Universidad de Oriente*. Voucher specimens were deposited at

KEYWORDS / Karyotype / NOR / *Serrasalmus rhombeus* /

Received: 07/29/2002. Modified: 09/12/2002. Accepted: 10/01/2002

Mauro Nirchio. M.Sc. in Marine Sciences, Universidad de Oriente (UDO), Venezuela. Professor, UDO. Address: Escuela de Ciencias Aplicadas del Mar, Núcleo de Nueva Esparta, Universidad de Oriente. Apartado 174, Porlamar, Margarita, Venezuela. e-mail: nirchio@cantv.net

Alberto Granado. M.Sc. in Marine Sciences. Professor, UDO. Address: Instituto Limnológico, Universidad de Oriente. Caicara del Orinoco, Estado Bolívar, Venezuela. e-mail: limnológico@cantv.net

Ernesto Ron. B.S. in Marine Biology, UDO. Instructor, Escuela de Ciencias Aplicadas del Mar, Núcleo de Nueva Esparta, UDO. e-mail: ronernesto@hotmail.com

Julio E. Pérez. M.A., University of Kansas. Ph.D., Southampton University U.K. Professor, UDO. Address: Instituto Oceanográfico de Venezuela, Universidad de Oriente. Apartado 243, Cumaná, Venezuela. e-mail: jperez@telcel.net.ve

RESUMEN

Se estudió el cariotipo de *Serrasalmus rhombeus* de Caicara del Orinoco, Venezuela. El número diploide de cromosomas $2n=60$ estuvo compuesto de 30 metacéntricos, 16 submetacéntricos, 4 subtelocéntricos y 10 acrocéntricos (NF=110). Fueron detectados cuatro pares de cromosomas portadores de NOR. Las NOR están localizadas en la región terminal de los brazos

cortos de un par de cromosomas subtelocéntricos de mediano tamaño y en la región terminal de tres pares acrocéntricos pequeños. La diversidad del cariotipo en esta especie sugiere que la fórmula del cariotipo y el número de NOR podrían ser de utilidad para la caracterización de stocks o poblaciones intraespecíficas.

RESUMO

Estudou-se o cariotipo de *Serrasalmus rhombeus* de Caicara del Orinoco, Venezuela. O número diploide de cromossomas $2n=60$ esteve composto de 30 metacéntricos, 16 submetacéntricos, 4 subtelocéntricos e 10 acrocéntricos (NF=110). Foram detectados quatro pares de cromossomas portadores de NOR. As NOR estão localizadas na região terminal dos

brazos curtos de um par de cromossomas subtelocéntricos de tamanho médio e na região terminal de três pares acrocéntricos pequenos. A diversidade do cariotipo nesta espécie sugere que a fórmula do cariotipo e o número de NOR poderiam ser de utilidade para a caracterização de stocks ou povoações intraespecíficas.

the Ichthyology Collection of the *Escuela de Ciencias Aplicadas del Mar, Universidad de Oriente*.

Each specimen was injected with 0.1% colchicine i.p. (1ml/100g fish weight). The fish were maintained in a well aerated aquarium and after 2hr they were sacrificed. The kidneys were removed and placed in a hypotonic solution of 0.4% KCl. Each kidney was minced with fine forceps and a fine cellular suspension was obtained then by repeated aspiration and forced release with a glass syringe. After 30min in the hypotonic solution, the cellular suspension was centrifuged at 1,000rpm for 3min. The hypotonic solution was discarded and the cellular pellet was suspended and washed 3 times in a methanol-acetic acid mixture 3:1 (V:V).

One droplet of the cellular suspension was dropped on a clean microscope slide, previously chilled in a freezer, from a height of 45cm. The slides were briefly passed over a flame and then allowed to air-dry.

For conventional karyotype the preparations were stained during 20min with 5% Giemsa in phosphate buffer pH 6.88. Detection of Nucleolar Organizer Regions (NOR) followed the silver staining method (Howell and Black, 1980). Staining with $AgNO_3$ was performed sequentially to identify the correspondence among

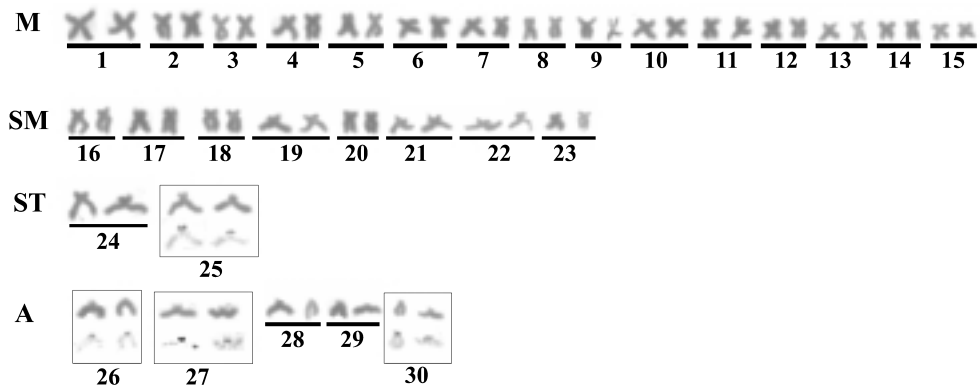


Figure 1. Karyotype of *Serrasalmus rhombeus*. NOR-bearing chromosomes revealed after sequential staining are in squares.

NOR bearing chromosomes after staining them with Giemsa.

The mitotic figures were photographed using a green filter and ASA 50 film. The resulting photographs were scanned (1200dpi) and stored as tif images. Long arm (L), short arm (S) and total chromosome length were measured from each chromosome to the nearest 0.01mm using the measuring tool of Adobe Photoshop Software v.5.0. The relative length (RL%) of each chromosome pair in relation to the total length of the haploid chromosome length was obtained from these measurements. Chromosomes were identified by the arm ratio criteria (Levan *et al.*, 1964). Metacentric (M), submetacentric (SM) and subtelocentric (ST) chromosomes were considered to be biarmed, and acrocentric (A) chromosomes were considered to be uniarmed.

Results and Discussion

All the specimens of *S. rhombeus* analyzed were characterized by a modal karyotype $2n=60$, obtained in 79% of all cells examined (182 cells). The hypomodal and hypermodal counts, on the whole, hardly reach 21% of all the cells recorded, and probably result from preparation-caused defects such as chromosome loss, overlap, miscounting and additional chromosomes from another spread. The karyotype was composed of 30 M, 16 SM, 4 ST and 10 A chromosomes with an arm number (*Nombre Fundamentale*) of NF=110 (Figure 1). No heteromorphic sex chromosomes were observed. The difference in relative length (RL%) between the two first chromosome pairs was 0.4%. Ag-stained metaphases (Figure 1) showed four pairs of NOR-bearing

chromosomes, located terminally on the short arms of one pair of medium sized ST chromosomes (N° 25), and on three small sized A chromosome pairs (N° 26, 27, 30).

Published data on cytogenetic features in the piranhas group show that the variation in the diploid chromosome number ranges from $2n=58$ to $2n=64$ (Porto *et al.*, 1991). In the genus *Serrasalmus*, some species have been karyotyped, and all have shown multiple NOR localized on the short arms of ST-A chromosomes, varying in number and staining intensity (Galetti *et al.*, 1985; Cestari and Galetti, 1992; Nakayama, 1997).

In the particular case of *S. rhombeus*, karyological studies performed in nominal forms of the species from Central Amazon near Manaus, Brazil, revealed two cytotypes that occur sympatrically at Catalão and Camaleão lakes,

without presence of intermediates between the cytotypes, suggesting that each cytotype represents a different fish species. Cytotype 1 consists of $2n=60$, $20M + 24SM + 6ST + 10A$ and had a difference of less than 0.5% in relative length (RL%) between the first two chromosome pairs. Cytotype 2 consists of $2n=58$, $30M + 16SM + 2ST+10A$ and had 2.1% of difference in RL% between the first and second chromosome pairs, thus the largest pair is about twice the size of chromosome pair 2. Multiple NORs in short arms of 5 to 12 ST-A chromosomes were detected in both cytotypes, and number, size and intensity of NORs were variable intraindividually (Nakayama *et al.*, 2001).

The karyotype of *S. rhombeus* from Caicara del Orinoco, falls within the chromosomal pattern of the piranhas, in number and NOR multiplicity. While these agree with those of Cytotypes 1

from Catalão and Camaleão lakes in Brasil (Nakayama *et al.*, 2001) both in chromosome number as in relative length between the first two chromosome pairs, it differs in the number of each type of chromosomes in $10M$, $8SM$ and $2ST$, revealing that the sample of the population analyzed herein possesses an additional Cytotype for the species. The presence of karyotype diversity in *S. rhombeus* suggests that karyotype formulae and NOR number could allow the characterization of intraspecific stocks or populations.

REFERENCES

Centofante L, Porto JIR, Feldberg E (2002) Chromosomal polymorphism in *Serrasalmus spilopleura* Kner, 1858 (Characidae, Serrasalminae) from Central Amazon Basin. *Caryologia* 55: 37-45.

Cestari MM, Galetti PM (1992) Chromosome evolution in the genus *Serrasalmus* and cytotoxic considerations about Serrasalminae (Characidae, Pi-

sces). *Brazil. J. Genet.* 15: 555-567.

Galetti P, Silva EB, Cerminaro RT (1985) Multiple NOR system in fish *Serrasalmus spilopleura* (Serrasalminae, Characidae). *Brazil J. Genet.* 8: 479-484.

Howell WM, Black DA (1980) Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* 3: 1014-1015.

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.

Machado-Allison A, Fink W (1996) *Los peces caribes de Venezuela: diagnosis, claves, aspectos ecologicos y evolutivos*. Consejo de Desarrollo Científico y Humanístico. Universidad Central de Venezuela. Colección Monografías N° 52. Caracas. 52 pp.

Muramoto JI, Ohno S, Atkins NB (1968) On the diploid state of fish order Ostariophysi. *Chromosoma* 24: 59-66.

Nakayama CM (1997) *Caracterização cariotípica de peixes da subfamília Serrasalminae (Characiformes) da bacia amazônica*. Dissertação. Instituto Nacional de Pesquisas da Amazônia (INPA). Manaus-AM. 90 pp.

Nakayama C, Porto JR, Feldberg E (2000) Ocorrência de dois citótipos em *Serrasalmus spilopleura* Kner, 1858 (Characiformes, Serrasalminidae) da região de confluência dos rios Negro e Solimões, Amazonas, Brasil. *Acta Amazonica* 1: 149-154.

Nakayama C, Jégu M, Porto JIR, Feldberg E (2001) Karyological Evidence for a Cryptic Species of Piranha within *Serrasalmus rhombeus* (Characidae, Serrasalminae) in the Amazon. *Copeia* 101: 866-869.

Nakayama C, Porto JIR, Feldberg E (2002) A comparative cytogenetic study of five piranha species (*Serrasalmus*, Serrasalminae) from the Amazon basin. *Genetica* 114: 231-236.

Porto JIR, Feldberg E, Nakayama CM, Maia RO, Jégu M (1991) Cytotaxonomic analysis in the Serrasalminae (Ostariophysi, Characiformes). *Bull. Zool. Mus. Amsterdam (Abstracts)*: 66.

Prado CHBA, Galetti PM (1986) Considerações iniciais sobre a estrutura cromossômica no gênero *Pigocentrus* (Serrasalminae-Characiformes). *Ier Simp. Citogenet. Evol. e Aplic. de Peixes Neotropicais*. São Carlos, SP. Brasil. p. 56.