

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

CARIOTIPO CONVENCIONAL, HETEROCROMATINA CONSTITUTIVA Y REGIONES ORGANIZADORAS 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, constitutive heterochromatin, *Hoplosternum littorale*.

RESUMEN

Se reporta el estudio citogenético de *Hoplosternum littorale* mediante tinción convencional con Giemsa, bandeo C e impregnación con nitrato de plata. La especie posee un número diploide $2n=60$, compuesto por 6M, 2SM, 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 pericentromérica de casi todos los cromosomas. Regiones banda C positiva teloméricas e intersticiales fueron también observadas en algunos cromosomas. La comparación de estos resultados con los 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, heterocromatina constitutiva, *Hoplosternum littorale*.

INTRODUCTION

The family Callichthyidae is a group of Neotropical fishes that comprises eight 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, environments sometimes 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).

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. littorale* 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), which could be evidenced by silver staining, since this is a procedure that reveals the residues of the Ag-stainable rRNA-protein 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).

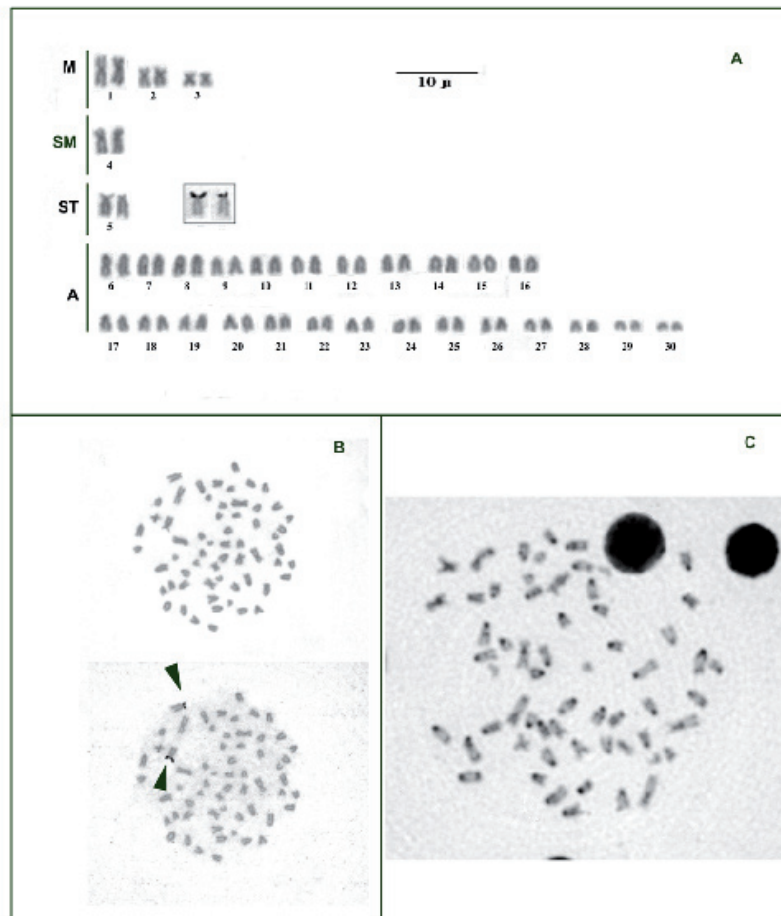


Figure 1. Karyotype of *Hoplosternum littorale*. NOR-bearing chromosomes appear in the square (A). Sequential staining of chromosomes for identifying correspondence among NOR bearing chromosomes when stained with AgNO_3 with the same chromosomes previously stained with Giemsa (B). Metaphase plates after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