

## COMPARATIVE CYTOGENETIC STUDY OF THE ARMORED CATFISHES OF THE GENUS *Hoplosternum* (SILURIFORMES, CALLICHTHYIDAE)

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### ABSTRACT

Karyotypes of two *Hoplosternum* species revealed dissimilar chromosome complements consisting of  $2n=60/FN=68$  in *H. littorale* and  $2n=64/FN=84$  in *H. thoracatum*. The nucleolar organizer regions (NORs) were characteristic for each species. We suggest that these karyotypic differences are due to pericentric inversions and Robertsonian rearrangements. The results are compared with those of other callichthyid karyotypes in order to evaluate the possible derivations of the chromosomal patterns of *Hoplosternum*.

### INTRODUCTION

The Callichthyidae family includes eight genera: *Aspidoras*, *Brochis*, *Callichthys*, *Cascadura*, *Cataphractops*, *Corydoras*, *Dianema* and *Hoplosternum*. This family is quite distinct from other catfish groups. Although its ingroup phylogenetic status is poorly known (Gosline, 1940), it has been tentatively included in the superfamily Loricarioidea (Schaefer and Lauder, 1986).

The available numerical and morphological data indicate that the Callichthyidae is cytogenetically diverse. The diploid and fundamental number vary from  $2n=40/FN=80$  in *Corydoras nattereri*, to  $2n=132/FN=222$  in *Corydoras aeneus*. There is a general agreement that gene duplications and/or polyploid events occurred during the evolutionary history of the callichthyid catfishes (Hinegardner and Rosen, 1972; Scheel *et al.*, 1972; Dunham *et al.*, 1980; Oliveira *et al.*, 1988a).

Burgess (1989) reported that the genus *Hoplosternum* apparently is composed of five species: *H. littorale*, *H. thoracatum*, *H. magdalenae*, *H. pectorale* and *H. shirui*. The first two species have been reported from the Amazon basin, and the last three have uncertain taxonomic validity.

*Hoplosternum* species live in streams, rivers, swamp pools and/or floodplain areas, including Paraguayan chaco, Guiana savanna, Venezuelan llanos and Amazonian flood forest (várzea or igapó). Sometimes, the water in these environments is stagnant and poor in oxygen. As a result *Hoplosternum* spp. have evolved morphological (Carter and Beadle, 1931) and molecular adaptations (Garlick *et al.*, 1979; Perez, 1980; Affonso, 1990).

Additional cytogenetic investigations are necessary for this group in order to determine whether chromosome changes occurred during the speciation processes. Here we describe the karyotype (Giemsa and NORs) of the two congeneric species from the central Amazon basin and discuss their cytotaxonomic implications.

## MATERIALS AND METHODS

Ten specimens of *H. littorale* (two males and eight females) and five specimens of *H. thoracatum* (one male, one female and three juveniles) were collected in Camaleão lake on Marchantaria island (3°14'S, 60°00'W) located in the Amazon river, approximately 15 Km above its confluence with the Negro river. The specimens remain preserved as part of a permanent fish collection at the Instituto Nacional de Pesquisas da Amazônia. Cytogenetic analyses were performed using the following procedures: the animals were injected subcutaneously in the dorsal or caudal regions with a suspension of Fleischmann's yeast (0.5 ml/100 g of body weight) to stimulate kidney cell mitosis (Lee and Elder, 1980). 24 hours after inoculation, each fish received an intraperitoneal injection of colchicine 0.05% (1 ml/100 g of body weight) and was killed 50 minutes later. Chromosome preparations were obtained from kidney cells by the air-drying technique described by Bertollo *et al.* (1978), and the nucleolar organizer regions (NORs) were detected by the method of Howell and Black (1980). Chromosome pairs were classified according to centromere position as metacentric, submetacentric, subtelocentric and acrocentric. Metacentric (M) and submetacentric (SM) refer to biarmed chromosomes, while subtelocentric (ST) and acrocentric (A) refer to monoarmed chromosomes.

## RESULTS

The diploid number, fundamental number and karyotypic formula of *H. thoracatum* were determined from 145 cells ( $2n=64$ , FN=84, 8M + 12SM + 44ST-A,

respectively). Their karyotype is shown in Figure 1. We attempted the characterization of the monoarmed chromosomes; however, they were difficult to assign correctly into single categories because of different degrees of condensation in different metaphase plates. The NORs of this species are located on a small-sized acrocentric pair (27<sup>th</sup> pair) and coincide with the secondary constrictions. The secondary constrictions were visible in few metaphases. Due to the scarcity of secondary constrictions and similarity within the small-sized acrocentric group, the NORs were not considered good chromosome markers.

The diploid number, fundamental number and karyotypic formula of *H. littorale* were determined from 278 cells ( $2n=60$ ,  $FN=68$ ,  $4M + 4SM + 52ST-A$ , respectively). Their karyotype is shown in Figure 2. The NORs of this species were located on a large-sized acrocentric group (7<sup>th</sup> pair), coinciding, also, with the secondary constrictions. In *H. littorale* the secondary constrictions on the NOR carriers were always visible and they can be considered good chromosome markers.

No chromosomal differences were observed between sexes in either species.

## DISCUSSION

The high incidence of closely related species with different karyotypes suggests that chromosome changes can play an important role in evolution and speciation (White, 1968, 1978). We observed that the karyotypes of *H. littorale* and *H. thoracatum* are quite different, reinforcing what several workers have stated concerning extensive chromosomal evolution in the Callichthyidae. However, although included in the same genus, the phylogenetic relationships between the two species and among the callichthyids have not been determined and a close relationship remains to be demonstrated.

Beside the different karyotypes and NOR locations, the two *Hoplosternum* species present different morpho-anatomical characteristics (Hoedeman, 1952; Alexander, 1965) and different haemoglobin patterns (Galdames-Portus *et al.*, 1982; Affonso, unpublished data). The distribution patterns and population size of these two species in the Amazon basin also apparently differ considerably (personal observations). *H. thoracatum* seems to form small populations and to be distributed in both "blackwater" (Negro river, Uatumã river) and "clearwater" (Tocantins river, Jari river) rivers, whose origins are the archaic shields of central Brazil and the Guianas, while *H. littorale* forms large populations and is nearly restricted to the "whitewater" rivers (Amazon river, Madeira river), originated from Andean and Pre-Andean regions.

In spite of the different karyotypic features which we have pointed out between these species, the chromosome changes that have probably taken place in *Hoplosternum*

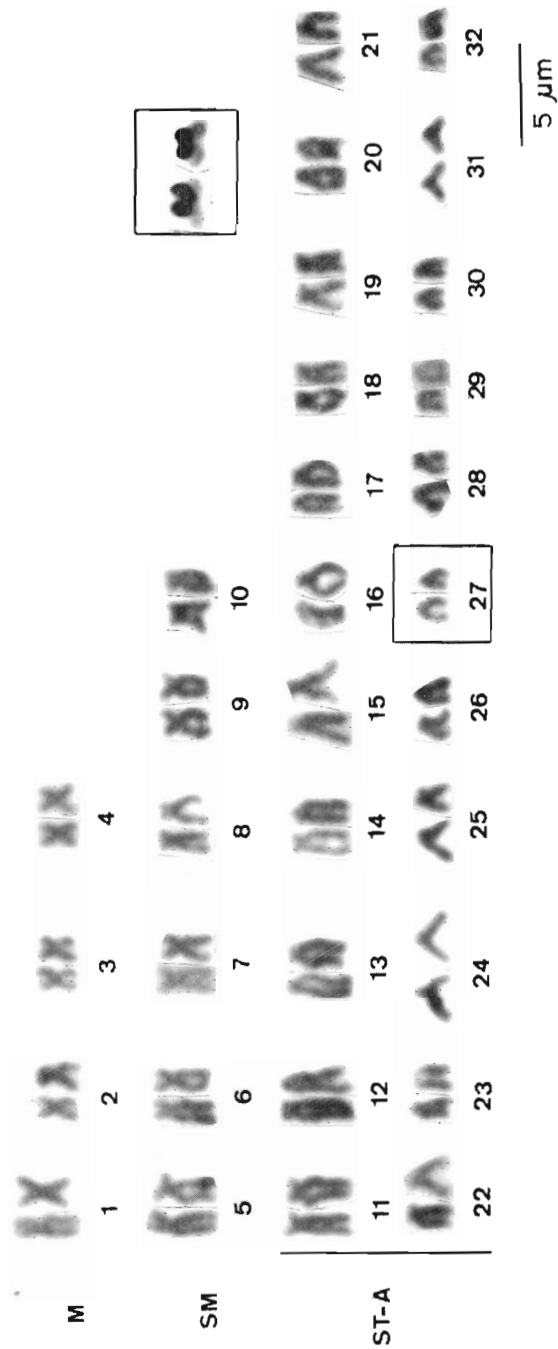


Figure 1 - Karyotype and nucleolar chromosomes of *Hoplosternum thoracatum*. The NOR bearing chromosomes appear in the square inset.

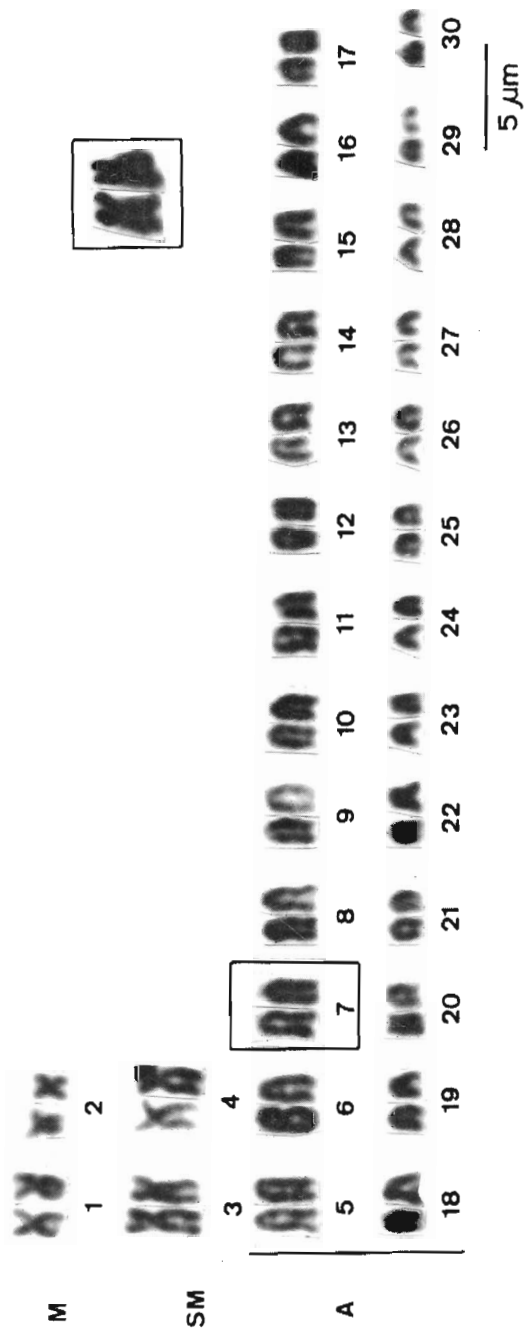


Figure 2 - Karyotype and nucleolar chromosomes of *Hoplosternum littorale*. The NOR bearing chromosomes appear in the square inset.

can mainly be attributed to pericentric inversions even though other types of rearrangements, such as Robertsonian rearrangements, certainly have occurred, as can be seen from the dissimilar karyotypes (Figures 1 and 2).

In fishes, pericentric inversions are not only present as polymorphisms (Turner *et al.*, 1985; Giuliano-Caetano and Bertollo, 1988) but can also occur as interspecific differences, as detected in several groups of fishes. However, rarely species which belong to the same genus show such karyotypic divergence as we observed in *Hoplosternum*.

The NORs of *Hoplosternum* are situated on distinct chromosomes which lead us to suppose that chromosomal evolution in *Hoplosternum* also involved the original NOR carrying chromosomes. The single NORs of *Hoplosternum* represents an opposite trend when compared with *Corydoras* species (Oliveira, 1987; Oliveira *et al.*, 1988c), *Callichthys callichthys* from Amazon basin (Porto and Feldberg, 1988) and *Brochis splendens* (Oliveira, 1990), which have multiple NORs.

Few callichthyid karyotypes among those previously described are similar to those of *Hoplosternum* (Oliveira *et al.*, 1988a). The karyotypes of *Dianema longibarbis* ( $2n=60/FN=72$ ; Hudson, 1976) and *Dianema urostriatum* ( $2n=62/FN=68$ ; Oliveira *et al.*, 1990) resemble *H. littorale*, while the karyotype of *Corydoras cf prionotus* ( $2n=68/FN=94$ ; Oliveira, 1987) resembles that of *H. thoracatum*.

Given the extreme karyotypic diversity observed in the Callichthyidae, it is possible that chromosomal changes that involved Robertsonian and non-Robertsonian translocations, inversions, in tandem growth of constitutive heterochromatin, polyploid events and/or gene duplications could be related to population size and vagility. Furthermore, the karyotypic diversity of this family seems to be a fish example of karyotypic megaevolution, *sensu* Baker and Bickham (1980).

Qumsiyeh and Baker (1988) have demonstrated that the determination of both primitive and derived karyotypes should be possible, since chromosomal banding should be adequate to infer the number and types of chromosomal rearrangements incorporated in the lineage. However, for fishes there are few works with high resolution chromosome banding (Almeida-Toledo *et al.*, 1988).

Based on karyotype sets and considering only primary karyotype data (Giemsa and NORs), we tentatively conclude that in the subfamily Callichthyinae (*sensu* Burgess, 1989) a diploid number near 60, a high incidence of monoarmed chromosomes and the presence of single NORs are probably plesiomorphic rather than apomorphic characteristics. Oliveira *et al.* (1988b) pointed out another plesiomorphic character of the Callichthyidae family, that is, low DNA value. We observed that *H. thoracatum* presents an apomorphic karyotypic structure.

Multidisciplinary research has become routine in few neotropical fish studies. Thus, cytogenetic information with detailed chromosome banding (R-, restriction enzymes), added to other types of information (ecology, systematics, biogeography,

parasitology, biochemistry), is necessary, especially in the Callichthyidae family, in order to obtain a good interpretation of the evolutionary patterns of the group.

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## RESUMO

São apresentados os cariótipos de duas espécies de *Hoplosternum*: *H. littorale* and *H. thoracatum*. Foram observados diferentes cariótipos. Na primeira espécie, encontrou-se  $2n=60/NF=68$  sendo as regiões organizadoras de nucléolos (RONs) evidenciadas num par de acrocêntricos de grande tamanho. Na segunda, encontrou-se  $2n=64/NF=84$  sendo as RONs evidenciadas num par de acrocêntricos de pequeno tamanho. Aparentemente a diversificação cariotípica das duas espécies resultou de inversões pericêntricas e rearranjos Robertsonianos. São discutidas as implicações citotaxonômicas da diversidade cariotípica do gênero *Hoplosternum* comparando-a com outros calictídeos.

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