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Research Article

Karyological characterization of four Neotropical fish species of the genus *Hisonotus* (Teleostei, Loricariidae, Hypoptopomatinae) from distinct Brazilian river basins

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Abstract

The karyotypes of four *Hisonotus* species (two provisionally-named species A and D, *H. nigricauda*, and *H. leucofrenatus*) were found to have the same diploid number of 2n = 54 and interstitial silver-staining nucleolus organizer regions (Ag-NORs) located on the long arm of the largest metacentric pair. The C-banding pattern appeared to be species-specific, with one group (*H. nigricauda* and the unnamed species A and D) being characterized by small amounts of positive C-banded segments and containing a sub-group (species A and D) identified by a large positive C-banded segment on a small metacentric chromosome pair. The second group contained different samples of *H. leucofrenatus*, characterized by a larger amount of C-band positive segments spread over several chromosome arms. Heterochromatin appears to play an important evolutionary role in chromosome differentiation in *Hisonotus* species, especially in *H. leucofrenatus*. The geographic isolation of several *H. leucofrenatus* populations seems to have favored chromosome evolution of each sample analyzed.

Key words: fish cytogenetics, karyotypes, Ag-NORs, C-band, chromosome evolution.

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Introduction

The subfamily Hypoptopomatinae is a monophyletic group of loricariids composed of 79 species distributed in 15 genera (Schaefer, 2003). Recent taxonomic studies have revealed the existence of new genera and species (Reis and Schaefer, 1998; Isbrücker *et al.,* 2001) and provided information for better taxa delimitation. The two Hypoptopomatinae genera *Microlepidogaster* and *Hisonotus* have, until recently, been considered as synonymous, although they are now recognized as separate and valid taxa (Schaefer, 1998). The genus *Microlepidogaster* is monotypic containing only *M. perforatus*, while all of the remaining species previously referred to as *Microlepidogaster* (Andreata *et al.*, 1993; Andreata *et al.,* 1994) have now been allocated to the genus *Hisonotus* (Schaefer, 1998).

Differences either in the amount or distribution of chromosomal heterochromatin as identified by the C-banding technique has been reported to be an important evolutionary component in some fish groups, with some differences in the distribution of positive C-band segments having lead to the cytogenetic characterization of genera, species and even populations (Mantovani *et al*., 2000). In some species of Neotropical fish, changes in the amount or distribution of positive C-band segments have also been associated with sex chromosome differentiation (Almeida-Toledo *et al*., 2001) and B chromosomes (Jesus *et al*., 2003).

Cytogenetic information about *Hisonotus* is restricted to four species for which the heterochromatin apparently played a fundamental role in karyotypic diversification, at least at a local level (Andreata *et al.*, 1993; Andreata *et al.,* 1994). In this paper we present the karyotypes of four *Hisonotus* species from distinct Brazilian river basins and compare our results with the available data for this genus.

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Materials and Methods

Cytogenetic studies were carried out on four *Hisonotus* species from southeastern and southern

Brazil, voucher specimens of which were deposited in the collection of the Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Universidade Estadual Paulista (UNESP), Botucatu, São Paulo, Brazil. The specimens studied were: eight females *H. nigricauda* (LBP 579) from the Guaíba river near the town of Eldorado do Sul in Rio Grande do Sul state; four females *H. leucofrenatus* (LBP 735) from the Cavalo stream near the town of Jaraguá do Sul in Santa Catarina state; one female and three males *Hisonotus* sp., provisionally named as species A, (LBP 869) from the Paraitinga river near the town of Salesópolis in São Paulo state; and one female and two males *Hisonotus* sp., provisionally named as species D, (LBP 791) from Grande stream near the town of Pindamonhangaba in São Paulo state. The *Hisonotus* species provisionally named A and D are new species which will be described elsewhere (H. A. Britski, Museu de Zoologia, Universidade de São Paulo, personal communication). The collection sites are shown in Figure 1.

Mitotic chromosome preparations were obtained from kidney and gill tissues using the air-drying technique (Foresti *et al*., 1993). Chromosome morphology was determined on the basis of arm ratio (Levan *et al*., 1964) and chromosomes were classified as metacentric, submetacentric, subtelocentric or acrocentric. Nucleolar organizer regions (Ag-NORs) were silver-stained following Howell and Black (1980) and C-banding was performed according to Sumner (1972).

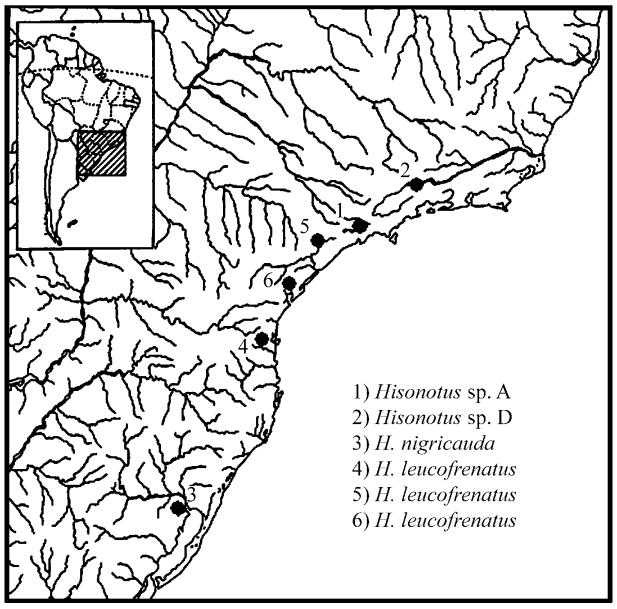

Figure 1 - Map showing the sites where the *Hisonotus* samples were collected. 1) Paraitinga river, Salesópolis, São Paulo State; 2) Grande stream, Pindamonhangaba, São Paulo State; 3) Guaíba river, Eldorado do Sul, Rio Grande do Sul State; 4) Cavalo stream, Jaraguá do Sul, Santa Catarina State; 5) Poço Grande stream, Juquiá, São Paulo State; 6) Marumbi river, Morretes, Paraná State. The numbers 5 and 6 correspond to the collecting sites of *H. leucofrenatus* studied by Andreata *et al*. (1993).

Results

We found that *Hisonotus* species A and D shared a similar karyotypic structure composed of 13 metacentric pairs, 13 submetacentric pairs and 1 subtelocentric pair (Figure 2), the only conspicuous difference between these two species being that in species A the first and second metacentric pairs were of almost the same size while in species D the first metacentric pair was larger than the second metacentric pair (Figure 2).

Regarding the other two species, *H. nigricauda* had 13 metacentric pairs, 10 submetacentric pairs and 4 subtelocentric pairs (Figure 3A), whilst *H. leucofrenatus* had 11 metacentric pairs, 12 submetacentric pairs, 3 subtelo-

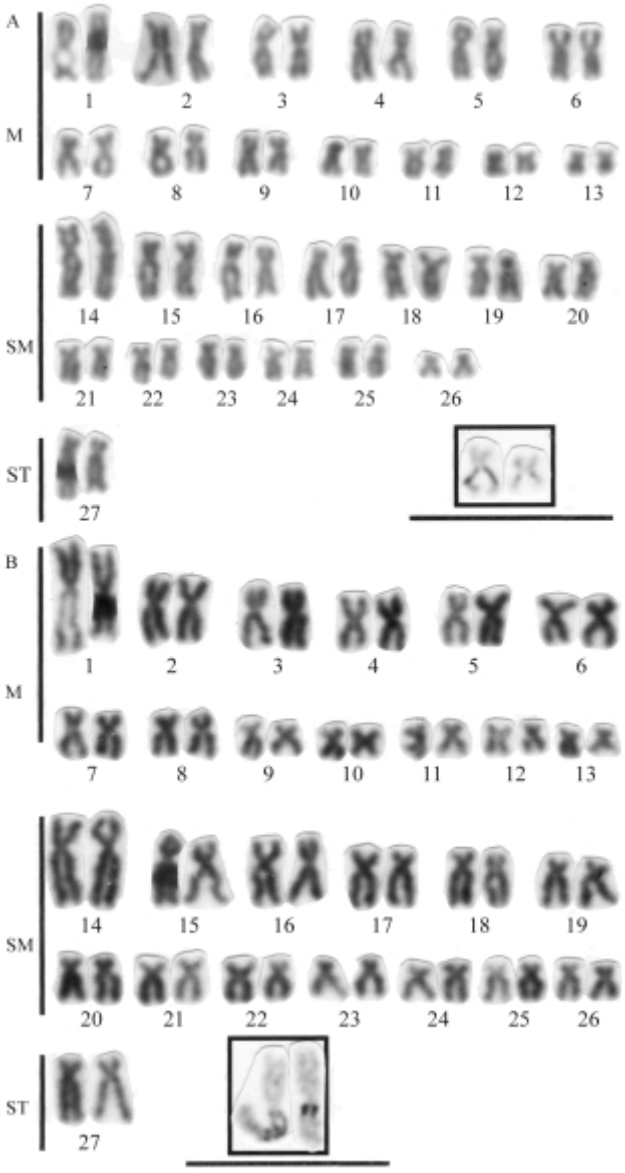

Figure 2 - Giemsa stained karyotypes of *Hisonotus* species A and D from São Paulo State. A = species A from Salesópolis; B = species D from Pindamonhangaba. Inset, the Ag-NOR-bearing chromosome pairs. Bars = 10 μm.

centric pairs and 1 pair of acrocentric chromosomes (Figure 3B).

All four species showed interstitial Ag-NORs located on the long arms of the largest metacentric pair (Figures 2 and 3). Positive C-banded segments were restricted to the pericentromeric and terminal regions of several chromosomes of *Hisonotus* species A (Figure 4A), *H. nigricauda* (Figure 4B) and *Hisonotus* species D (Figure 4C). We also found that *Hisonotus* species A and D presented a small pair of metacentric chromosomes with a large positive C-banded block on the long arm (Figures 4A and 4C). Comparing the same chromosome segments, the Ag-NOR segments were negatively C-banded in *Hisonotus* species A and positively C-banded in species D (Figure 4). In the case of *H. leucofrenatus* we found several positive C-band segments located on the long arm of several chromosomes, including the largest Ag-NOR-bearing chromosomes (Figure 4D).

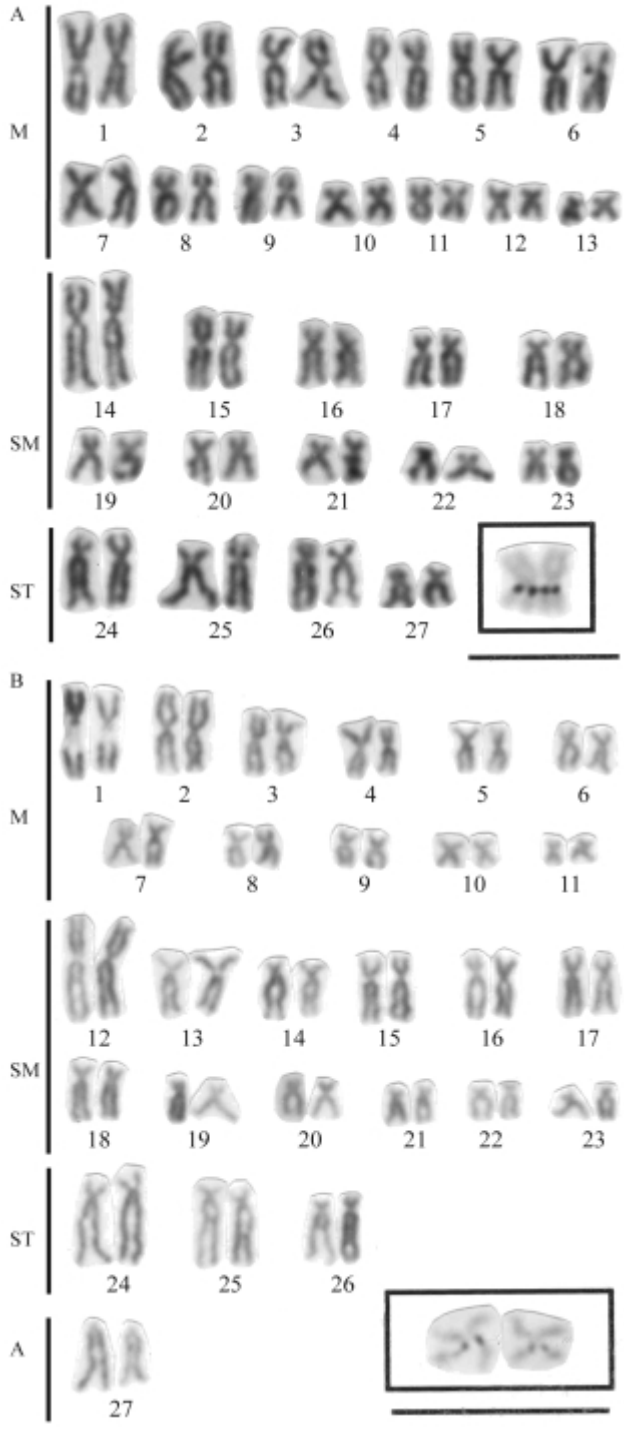

Figure 3 - Giemsa stained karyotypes of *Hisonotus* species. A = *H. nigricauda* from Eldorado do Sul (Rio Grande do Sul State); B = *H. leucofrenatus* from Jaraguá do Sul (Santa Catarina State). Inset, the Ag-NORs-carrier pairs. Bars = 10 μm.

Discussion

Our cytogenetic analyses revealed that the four *Hisonotus* species studied shared the same diploid number (2n = 54) and were also characterized by the presence of interstitial Ag-NORs located on the long arm of the largest metacentric pair. The same diploid number and interstitial Ag-NORs have being found in almost all species of Hypoptopomatinae (Andreata *et al*., 1992; Andreata *et al*., 1993; Andreata *et al*., 1994), reinforcing the hypothesis that

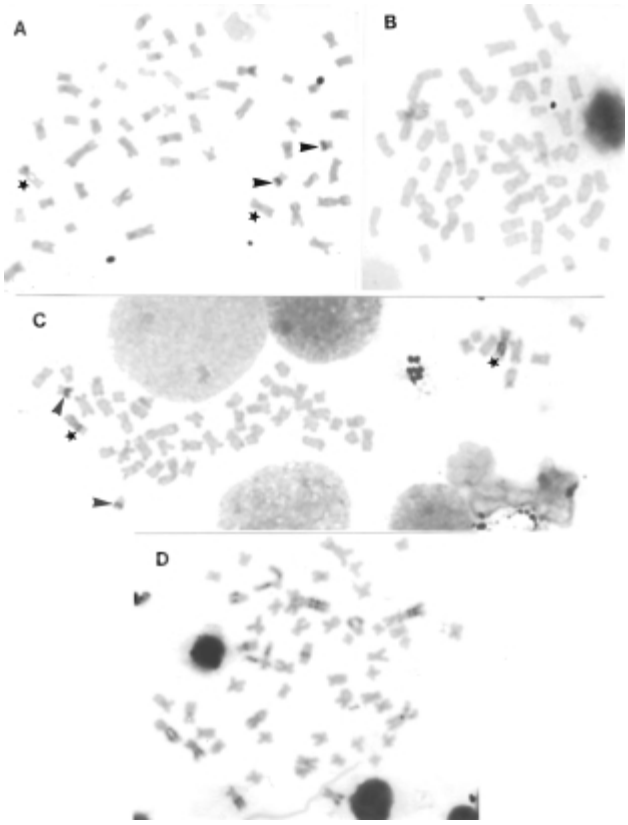

Figure 4 - C-banded mitotic metaphase of *Hisonotus* species. A = *Hisonotus* species A from Salesópolis (São Paulo State); B = *H. nigricauda* from Eldorado do Sul (Rio Grande do Sul State); C = *Hisonotus* species D from Pindamonhangaba (São Paulo State); and D = *H. leucofrenatus* from Jaraguá do Sul (Santa Catarina State). Arrows point to marker chromosomes shared by two species. Stars indicate Ag-NOR-bearing chromosomes.

this group is karyotypically very conserved (Andreata *et al*., 1994).

Although the species of the subfamily Hypoptopomatinae show a similar karyotypic macrostructure, the C-banding pattern has been useful in distinguishing species limits and determining local samples of some species (Andreata *et al*., 1993).

Our four *Hisonotus* species formed two groups based on the number and distribution of positive C-banded segments. Group 1 contained species A, D and *H. nigricauda* and was characterized by chromosomes which had a small number of positive C-banded segments restricted to the pericentromeric and terminal regions of the chromosomes. This group contained a subgroup consisting of species A and D, both of which shared the same cytotype (Figure 2) and exhibited a small metacentric chromosome pair with a large positive C-banded block in the long arms (Figures 4A and 4C). Group 2 contained the *H. leucofrenatus* specimens captured by us at Jaraguá (Figure 4D) and data from specimens of the same species captured in the Brazilian states of São Paulo and Paraná by Andreata *et al.* (1993), members of this group being characterized by a large number of positive C-band segments spread over several chromosome arms.

We found that *Hisonotus* species A and D shared similar cytotypes, Ag-NOR distribution and C-banding patterns but differed in the size of the first metacentric pair and by the fact that the C-banding was negative in species A and positive in species D. These two differences may be related in that the accumulation of positive C-band chromatin could promote an increase in the size of the first metacentric pair. These two species may have descended from a common ancestor but are now completely isolated because species A occurs in streams of the Tietê river basin and species D in streams of southeastern Brazilian coastal river basins (Figure 1). Geological studies have shown that although these two hydrological systems are now separated they were connected until the Miocene (Lundberg, 1998; Malabarba, 1998). The karyotypic differences observed between species A and D could have been fixed after their separation. Cytogenetic studies involving other groups of fish inhabiting the same region seem to support the idea that chromosome rearrangements become fixed after species separation (Oliveira *et al*., 1993; Guimarães *et al*., 1995).

The role of heterochromatin in the evolution of chromosome has long been the subject of intensive discussion. The different views on the role of heterochromatin during development and evolution have been reviewed by John (1988) who states that mechanisms such as multiple replications, unequal exchanges, amplifications, accumulations and deletions can lead to the quantitative variation of heterochromatin within and between species. Such variation could favor the formation of distinct groups of organisms and contributes to speciation, Redi *et al*. (2001) having suggested that speciation related to quantitative process of heterochromatin differentiation may have occurred in organisms as diverse as rodents and *Drosophila*.

In fish, several studies have shown that heterochromatin (identified by the C-band technique) is an important element in chromosome differentiation (Caputo *et al*., 1997; Artoni and Bertollo, 1999; Margarido and Galetti Jr, 2000). The fish *Astyanax scabripinnis* has been intensively investigated cytogenetically and has shown broad chromosome diversity, frequently related to the amount and distribution of positive C-band segments (Mantovani *et al*., 2000). Chromosomal differentiation in *A. scabripinnis* has often been related to its population structure, made up of small and isolated populations living in the headwaters of small rivers. A similar situation seems to occur with *H. leucofrenatus* in that it too exists in small isolated populations, so accumulation of heterochromatin should have occurred in the samples analyzed in our study, and other studies. Although Andreata *et al*. (1993) identified a putative sex chromosome system in two f *H. leucofrenatus* samples this was not found in the present study, suggesting that changes in positive C-banded segments had occurred and have been independently fixed in the samples.

Besides the conspicuous differences in heterochromatin distribution, the karyotype of *H. leucofrenatus* also differs from that of the other species analyzed by its different chromosome formulae. This difference may be related to the presence of several positive C-band segments since the accumulation of such segments could change chromosome morphology. However, it is not possible to discard the hypothesis that several pericentric inversions have been fixed in this species, changing its karyotypic formulae.

The karyotypic differences observed between the *H. leucofrenatus* sample studied here and those studied by Andreata *et al*. (1993) might be related to the formation of the southeastern Brazilian coastal rivers because the main rivers in this area are now separated by geographic barriers that can suppress the dispersion of individual fish. Previous studies have revealed differences between samples of several fish groups inhabiting this area, and some studies have suggested that vicariant events were the most important force acting in the speciation processes of these groups (Weitzman *et al*., 1988; Reis and Schaefer, 1998). Cytogenetic studies have also revealed the occurrence of chromosome diversification in other fish species occurring in this area (Oliveira *et al*., 1993; Guimarães *et al*., 1995). It thus appears that the chromosome differentiation observed in the *H. leucofrenatus* samples may have resulted from allopatric speciation associated with the separation of the coastal rivers in southeastern Brazil.

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