Karyotype evolution in Curimatidae (Teleostei, Characiformes) from the Amazon region. II. Centric fissions in the genus *Potamorhina*

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Using cytogenetic analysis following Giemsa staining, nucleolar organizer region (NOR) staining, and C-banding, three distinct karyotypes in three species of curimatids belonging to the fish genus *Potamorhina* were identified: $2n$ = 54/44 M + 10 SM (*P. pristigaster*), $2n$ = 56/52 M + 2 SM + 2 ST (*P. latior*), and $2n$ = 102/2 M + 2 SM + 98 A (*P. altamazonica*). A $2n$ = 54 was considered to be the ancestral diploid number and the different karyotypes were probably the result of centric fissions. Both the NOR pattern and constitutive heterochromatin pattern are species specific.

Key words: centric fissions, karyotypic evolution, Amazon fish, Curimatidae.

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Des analyses cytogénétiques postérieures à l'emploi des méthodes de Giemsa pour la coloration des bandes C et des régions organisatrices de nucléoles (RON) ont permis d'identifier trois caryotypes distincts chez trois espèces de curimatides appartenant au genre *Potamorhina*, savoir :: $2n$ = 54/44 M + 10 SM (*P. pristigaster*), $2n$ = 56/52 M + 2 SM + 2 ST (*P. latior*) et $2n$ = 102/2 M + 2 SM + 98 A (*P. altamazonica*). Le nombre diploïde ancestral a été $2n$ = 54 et les différents caryotypes sont probablement la résultante de fissions centrales. Les profils des RON et de l'hétérochromatine constitutive sont spécifiques aux espèces.

Mots clés : fissions centrales, évolution caryotypique, poissons de l'Amazone, Curimatidae.

[Traduit par la rédaction]

Introduction

Taken as a whole, fish form a karyotypically diversified group in which diploid number, chromosome formula, and DNA content are extremely variable. The major alterations involved in the chromosomal evolution of fish are Robertsonian rearrangements (centric fusion and fission) and non-Robertsonian rearrangements (inversions, deletions, duplications, and translocations). However, all of these rearrangements are often inferred by conventional staining (Giemsa) given the difficulty in obtaining appropriate chromosome banding patterns among fish (Kirpichnikov 1981).

Approximately 20 species belonging to the family Curimatidae have been studied cytogenetically and have been characterized as a group having basically $2n$ = 54 metacentric–submetacentric (M–SM) chromosomes, with a few exceptions (i.e., *Curimatopsis* spp., Scheel 1973; *Curimata ocellata*, Feldberg *et al.* 1992; *Steindachnerina* sp., Rocha and Giuliano-Caetano 1990; and *Cyphocharax* sp., Venere 1991). As a contribution to the karyotypic study of this family, in the present study we investigated three additional species belonging to the genus *Potamorhina*. According to Vari (1984), this genus is considered to be a monophyletic subunit of the family Curimatidae and comprises five species, of which *P. latior* and *P. pristigaster* are endemic to the Amazon basin and *P. altamazonica* occurs in the Amazon and Orinoco basins.

Material and methods

Thirteen specimens (5 males and 8 females) of *Potamorhina pristigaster*, 22 (12 males and 10 females) of *P. altamazonica*, and 18 (8 males and 10 females) of *P. latior* were analyzed. The specimens were caught in the Amazon basin (60°W, 3°S), in the region of confluence of the Negro and Solimões rivers (Catalão Lake), in the Solimões river (Janauacá Lake and Camaleão Lake), and in the Amazonas river (Rei Lake).

Somatic chromosome preparations were obtained from renal cell suspensions by the air-drying technique of Bertollo *et al.* (1978), which was slightly modified in concentration (0.025–0.05%) and time exposure of colchicine (40–60 min). Meiotic preparations were obtained from testes by the method of Bertollo *et al.* (1978). The nucleolar organizer regions (NORs) were identified by silver staining according to the technique of Howell and Black (1980) and constitutive heterochromatin was detected by the technique of Sumner (1972).

To visualize the gradient in chromosome size, morphometric analyses were performed to determine the relative length (RL%) of each chromosome pair in relation to the total haploid length. The chromosomes were then grouped into relative size classes and chromosome frequency was determined for each class in each species. Chromosomes were classified into metacentric

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TABLE 1. Karyotypic characteristics of the *Potamorhina* species studied

Species	No. of specimens		No. of cells				Chromosome types				NOR location		
	Males	Females	Males	Females	2*n*	FN	M	SM	ST	A	Pair	Position	Arm
P. pristigaster	05	08	155	231	54	108	42	12	—	—	25th SM	Terminal	SA
P. altamazonica	12	10	180	167	102	106	02	02	—	98	5th A	Terminal	LA
P. latior	08	10	199	397	56	112	52	02	02	—	25th M	Terminal	LA

NOTE: 2*n*, diploid number; FN, fundamental number; M, metacentric; SM, submetacentric; ST, subtelocentric; A, acrocentric; SA, short arm; LA, long arm.

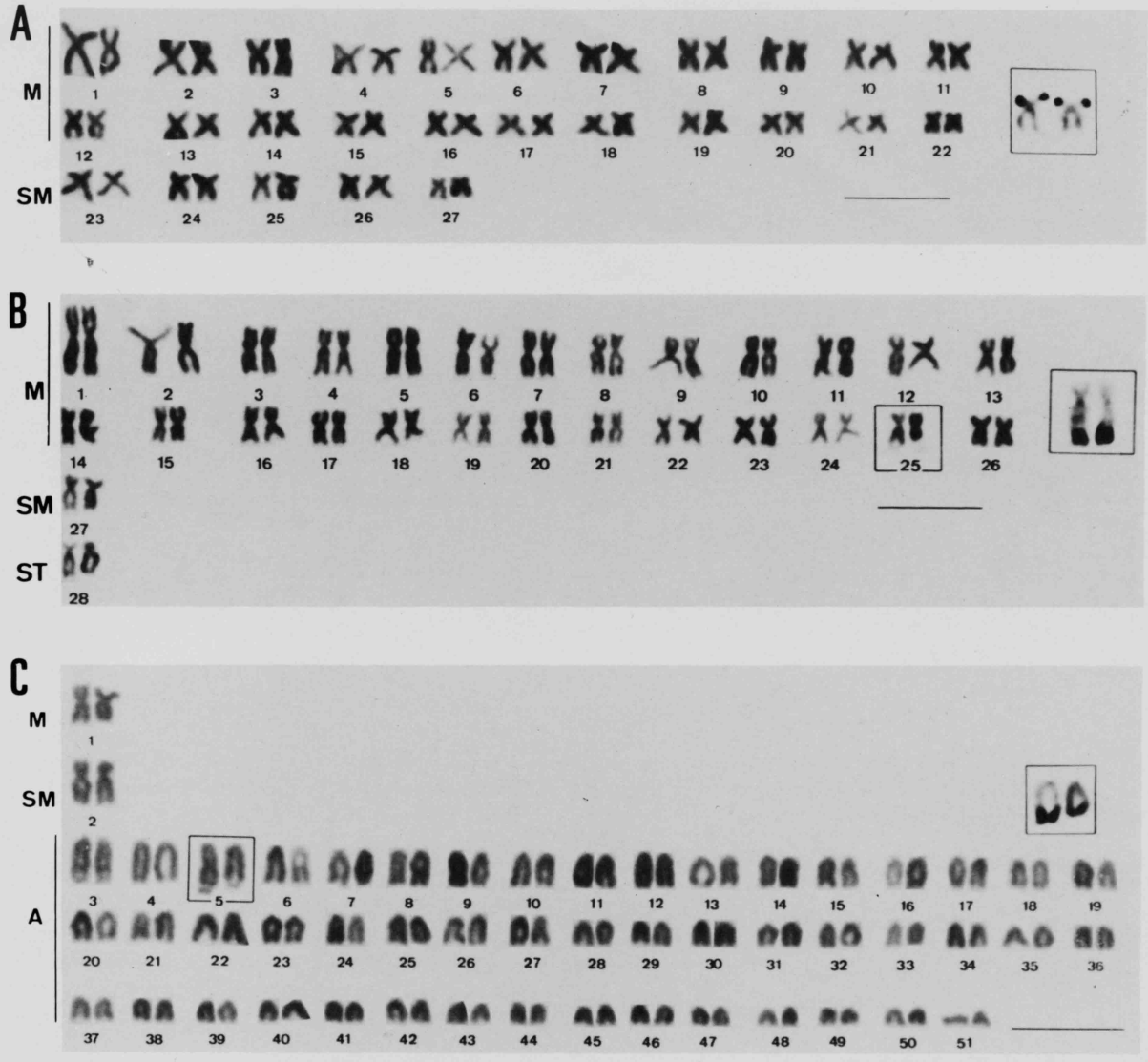

FIG. 1. Karyotypes and nucleolar chromosomes of (A) *Potamorhina pristigaster*, (B) *P. latior*, and (C) *P. altamazonica*. Scale bar = 10 μm. M, metacentric; SM, submetacentric; ST, subtelocentric; A, acrocentric.

(M), submetacentric (SM), and subtelocentric (ST) types, considered to be biarmed, and into acrocentrics (A), considered to be one armed.

Results

The three curimatid species analyzed are characterized by different karyotypic characters (Table 1; Figs. 1, 2, and 3). *Potamorhina pristigaster* has 2*n* = 54/44 M + 10 SM and NORs located on the terminal region of the short arm of a metacentric chromosome pair (probably the 25th pair of complement). *Potamorhina latior* has 2*n* = 56/52 M + 02 SM + 02 ST and NORs located on the terminal region of the long arm of a submetacentric chromosome pair (25th pair of complement). *Potamorhina altamazonica* has 2*n* = 102/02 M + 02 SM + 98 A and NORs located on the terminal region of the long arm of an acrocentric chromosome pair (5th pair of complement).

With respect to patterns of constitutive heterochromatin, *P. pristigaster* has heterochromatin blocks almost exclusively in the pericentromeric region of all chromosomes. In

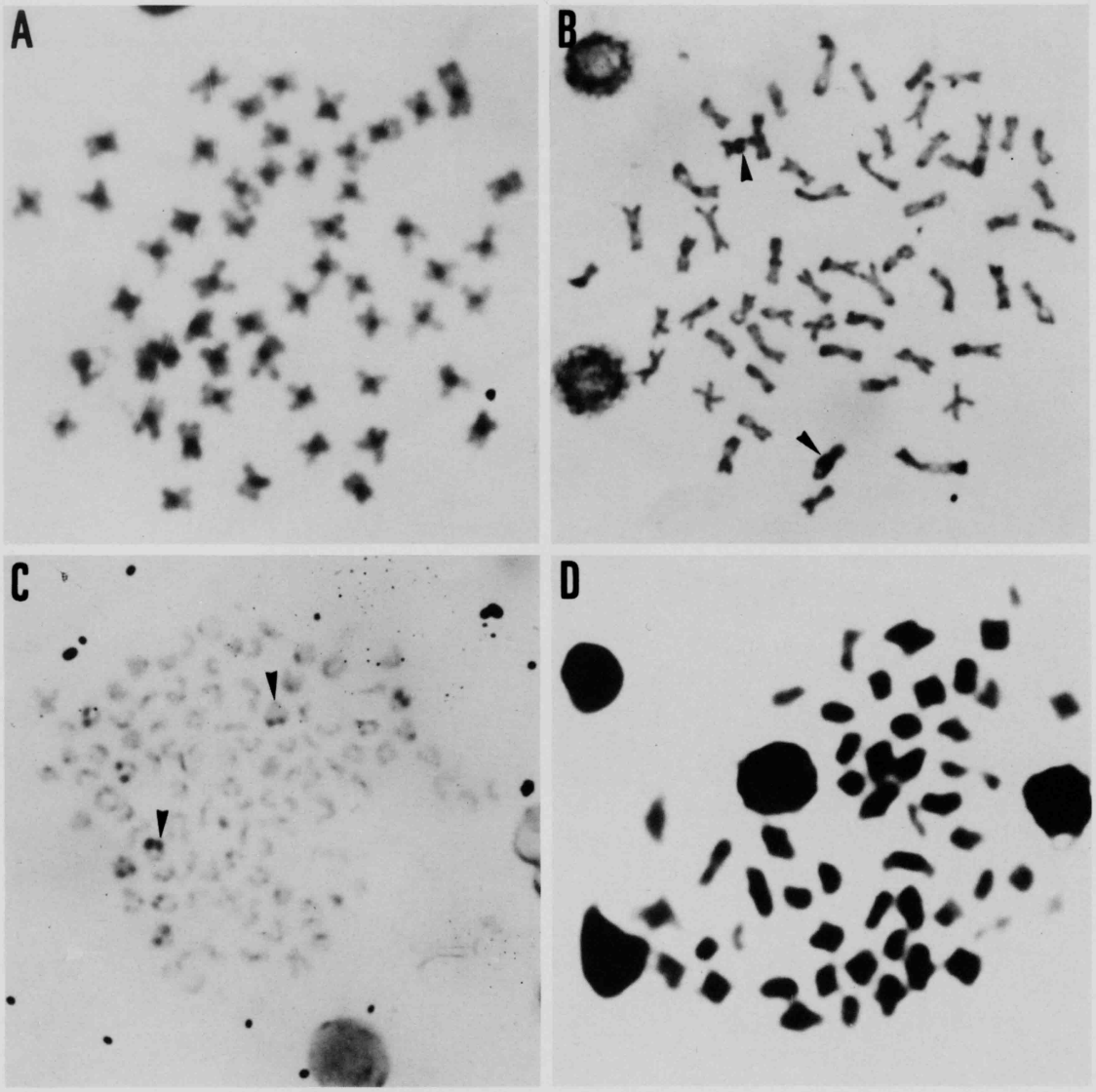

FIG. 2. (A–C) Stained somatic metaphases showing the location of constitutive heterochromatin (A, *Potamorhina pristigaster*; B, *P. latior*; and C, *P. altamazonica*). (D) Meiotic metaphase (metaphase I) showing 51 bivalents in *P. altamazonica*. The arrows point to the NOR-bearing chromosomes.

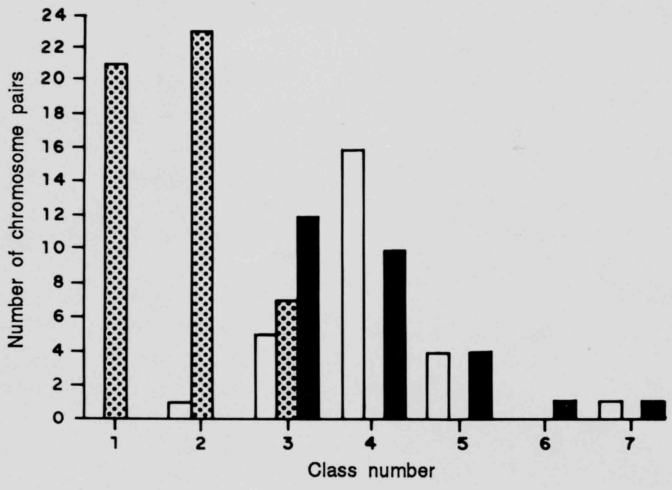

FIG. 3. Chromosome number frequency in the *Potamorhina* species analyzed (stippled bar, *P. altamazonica*; open bar, *P. pristigaster*; and solid bar, *P. latior*), distributed by relative size (% of the haploid lot) (1, 1.05–1.80; 2, 1.81–2.56; 3, 2.57–3.31; 4, 3.32–4.06; 5, 4.07–4.82; 6, 4.83–5.57; 7, 5.58–6.32).

P. latior, the heterochromatin blocks are more visible than in the other two species and are present in the pericentromeric and telomeric regions of most chromosomes and in the interstitial regions of at least three M–SM chromosome pairs. In this species, well-differentiated blocks adjacent to the NOR are also visible (Fig. 2). In *P. altamazonica* heterochromatin blocks are also distributed in the pericentromeric region of all chromosomes, although lightly staining for the most part, and there are also conspicuous bands in the telomeric and interstitial regions of some acrocentric chromosomes.

Figure 3 illustrates the relative size classes distribution of the chromosome. Specific distributions for the three species are clearly distinguishable, particularly *P. altamazonica* whose chromosomes belong only to the smallest size classes 1, 2, and 3.

Discussion

On the basis of the karyotypic data obtained previously for Curimatidae species, related families (Prochilodontidae, Anostomidae, and Chilodontidae), and for two out-groups

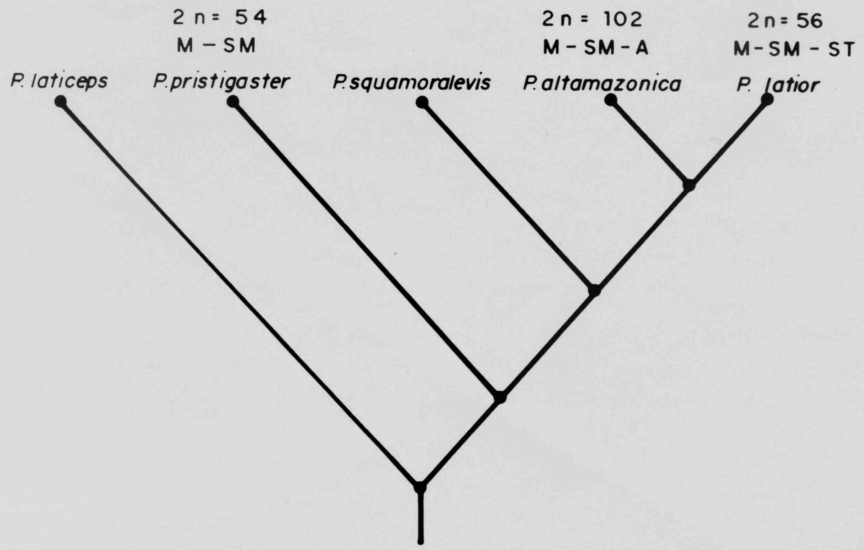

FIG. 4. Diploid numbers and karyotypic formulas of species studied superimposed to the phylogenetic cladogram proposed by Vari (1984).

(Hemiodidae and Parodontidae), almost all species (about 70) have a diploid number of $2n = 54$ chromosomes and an arm number (FN) of approximately 108 (Oliveira *et al.* 1988; Venere and Galetti Jr. 1989; Feldberg *et al.* 1992).

According to Venere and Galetti Jr. (1989) and Feldberg *et al.* (1992), the chromosome rearrangements that have occurred in the family Curimatidae appear to have been mainly due to inversions, leading to some modifications in chromosome structure but not to significant changes in chromosome number. Feldberg *et al.* (1992) have, therefore, suggested that the ancestral karyotype of the family Curimatidae probably consists of $2n = 54$ metacentric and submetacentric chromosomes.

However, the two different diploid numbers ($2n = 56$ and $2n = 102$) observed in the genus *Potamorhina* investigated here contrast with the evolutionary trend of the Curimatidae and related families.

Thus, assuming an ancestral curimatid karyotype of the $2n = 54$ M–SM chromosome type, we suggest that in the genus *Potamorhina*, the species *P. pristigaster* may have retained the primitive structure. Among the *Potamorhina* species under study, *P. pristigaster* is considered to be the sister group of the other two (Fig. 4). *Potamorhina altamazonica* ($2n = 102$, FN = 106) and *P. latior* ($2n = 56$, FN = 112), however, show karyotype structures that may be derived and may have subsequently undergone some rearrangements, the most important probably being centric fissions.

The karyotype of *P. latior* may have arisen from a fission in the ancestral karyotype, which also had 54 biarmed chromosomes. In addition, pericentric inversions probably occurred in the chromosomes evolved from this fission leading to biarmed chromosomes.

The occurrence of a chromosome number ($2n = 102$) almost double the most frequent number for the family ($2n = 54$) in *P. altamazonica* may suggest, *a priori*, the incidence of polyploidy in this species. However, this hypothesis can be ruled out on the basis of the following considerations: (*i*) no significant differences in interphase nuclear size were detected among the three species; (*ii*) neither interphase nuclei, nor metaphase cells of *P. altamazonica*, showed an increase in nucleolus number when stained with silver nitrate; (*iii*) the size of *P. altamazonica* chromosomes is small compared with the other two species (Fig. 3), suggesting no increase in DNA content; (*iv*) during meiosis, 51 bivalents at metaphase I are observed in *P. altamazonica* (Fig. 3D); (*v*) if 54 chromosomes is the basic karyotype for the group, a derived karyotype with a predominance of M–SM chromosomes would be expected in a polyploid.

Thus, there is evidence strongly arguing in favour of the hypothesis that Robertsonian rearrangements of the centric fission type played a significant role in the karyotypic diversification of *Potamorhina*. In the case of *P. altamazonica*, these fissions probably occurred in chromosomes of the M–SM type, leading to the formation of large numbers of acrocentric chromosomes. However, complementary rearrangements certainly occurred in the karyotypic evolution of *P. altamazonica*.

Examples of centric fusion – fission among fish have been reported for species of the genus *Oncorhynchus* (Kirpichnikov 1981), *Salvelinus* (Disney and Wright Jr. 1987), *Noturus* (LeGrande 1981), *Mystus* (Sharma and Triparthi 1986), and *Corydoras* (Oliveira 1987), with centric fusions considered to be the predominant event.

With respect to the NOR pattern, three phenotypes were detected, confirming the presence of rearrangements involving NOR-bearing chromosomes in this genus (Fig. 1). Analysis of constitutive heterochromatin can be interpreted to demonstrate that NOR-bearing chromosomes went through an additional process of differentiation. Clearly visible C-banded regions were observed in the arms of the NOR chromosome pair of *P. altamazonica* and *P. latior*, which were not detected in *P. pristigaster* (Fig. 2). It is interesting to note that the NOR of *P. altamazonica* appears to be C-band positive, whereas large heterochromatin blocks are observed adjacent to the NOR in *P. latior*. Cases of C-band positive NORs, as observed in *P. altamazonica*, are relatively less frequent in fish than cases in which constitutive heterochromatin is adjacent to the NORs.

Comparisons of the C-banding data suggest that the process of heterochromatinization may have played a deversifying role in the karyotypic evolution of *Potamorhina*. *Potamorhina pristigaster* has heterochromatin blocks mainly in the pericentromeric region of the chromosomes, whereas the

other two species have heterochromatin blocks both in pericentromeric regions and in interstitial and telomeric regions of several chromosomes (Fig. 2).

Despite the trend towards conservation of chromosome macrostructure in the family Curimatidae, chromosome alterations have occurred both in chromosome number and shape in some species, as in the genus *Potamorhina* reported here. Particularly outstanding is the case of *P. altamazonica* in which centric fission represents the major mechanism of chromosome diversification.

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