

## Comparative cytogenetic studies of Curimatidae (Pisces, Characiformes) from the middle Paraná River (Argentina)

M.S. Brassesco<sup>1</sup>, M.C. Pastori<sup>2</sup>, H.A. Roncati<sup>2</sup> and A.S. Fenocchio<sup>2,3</sup>

<sup>1</sup>Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

<sup>2</sup>Laboratorio de Citogenética General, Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, Misiones, Argentina

<sup>3</sup>Departamento de Biologia Geral, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Londrina, PR, Brasil

Corresponding author: M.S. Brassesco

E-mail: marsol@rge.fmrp.usp.br

Genet. Mol. Res. 3 (2): 293-301 (2004)

Received December 17, 2003

Accepted May 12, 2004

Published June 30, 2004

**ABSTRACT.** Almost all species of the Curimatidae family have a stable karyotype, with a diploid number of 54 metacentric (M) and submetacentric (SM) chromosomes, and one sole nucleolus organizer pair. This family has considerable specific diversity in Argentinean fluvial basins; however, no cytogenetic data are available. Eight species from the Paraná River (Argentina): *Cyphocharax voga*, *C. spilotos*, *C. platanus*, *Steindachnerina brevipinna*, *S. conspersa*, *Curimatella dorsalis*, *Psectrogaster curviventris*, and *Potamorhina squamoralevis* were analyzed cytogenetically. Chromosome preparations were obtained from direct samples and through cell culture, and they were processed for conventional, C- and nucleolar organizer region-banding. Six of the species exhibited the standard family karyotype, with  $2n = 54$  M-SM and fundamental number of chromosomes (FN) = 108, as well as variations in the chromosome formula, and in heterochromatic and nucleolar organizer regions. Though nucleolar organizer regions were located on only one chromosome pair, they varied in both carrier chromosomes and pairs

involved. On the other hand, *C. platanus* showed a complement of  $2n = 58$  M-SM and subtelocentric with  $FN = 116$ , and *P. squamoralevis* presented  $2n = 102$ , with some M-SM and a large number of acrocentric chromosomes. Even though the karyotype macrostructure appears to be conserved, the speciation process within the family has been accompanied by micro-structural rearrangements, as evidenced by pattern diversity in the heterochromatin and nucleolar organizer regions. Some changes in chromosome macrostructure have also occurred in this group, primarily in *C. platanus* and *P. squamoralevis*, in which there have been centric dissociations and inversions.

**Key words:** Paraná River, Curimatidae, Nucleolar organizer regions, Cytogenetic studies

## INTRODUCTION

The Curimatidae family comprises 8 genera, and it includes almost 140 species that are widely distributed throughout the Neotropics (Vari, 1988), encompassing the Atlantic drainage basins of South America, from northern Colombia to the Río Negro in Argentina, as well as across the trans-Andean basins in the Punta Arenas (Chile) province to Peru. These species are popularly called “sabalitos” and they inhabit various types of freshwater ecosystems, including lakes, rivers and small streams. In some regions, Curimatidae, and its sister group Prochilodontidae (Vari, 1991), are the main types of detritivorous fishes and they can account for more than 50% of the ichthyofauna (Venere, 1991).

Cytogenetic studies of Neotropical fishes have revealed wide chromosome diversity. Two strong trends are apparent among Characiformes: karyologically homogeneous groups versus taxa with high cytogenetic variability (Bertollo et al., 1986). Curimatidae fall into the first group and they rarely deviate from a determined karyotype. The macro-structural stability of this family allows one to infer the evolutionary path taken by this family (Galetti Jr. et al., 1994). Despite the high specific diversity of this group, its evolutionary divergence in morphological, metric and continuous characteristics (Azpelicueta and Braga, 1991) has not been accompanied by macro-structural karyotype changes. More than 70% of the species that have been studied presented a diploid number of 54, with metacentric (M) and submetacentric (SM) chromosomes and fundamental number of chromosomes ( $FN = 108$ ) (Navarrete and Julio Jr., 1997).

Curimatidae, Prochilodontidae, Anostomidae, Chilodontidae, and Parodontidae are considered a monophyletic group, due to shared, derived osteological and anatomical characters (Vari, 1983). Conservation of a basic karyotype with 54 banded chromosomes in these four families suggests that this chromosome number comes from an ancestor of these families (Brum and Galetti, 1997; Venere, 1998).

On the other hand, some karyotypic divergences related to chromosome rearrangements ( $2n$ ;  $FN$ ) can be observed in a few Curimatidae species (Venere and Galetti, 1989; Feldberg et al., 1992 a; Navarrete and Julio Jr., 1997). Chromosome evolution in this family (i.e., *Steindachnerina* sp., *Psectrogaster* sp., *Curimatella* sp.) has also been accompanied by mi-

cro-structural changes in chromosome formula, patterns of heterochromatin distribution and nucleolar organizer regions (NORs), which are important cytotaxonomic markers (Venere and Galetti, 1989).

Curimatids show high specific diversity in Argentine fluvial basins; however, no karyotype data are available. We cytogenetically analyzed several species of this family from the Paraná River (Argentina) for basic chromosomal data, taking into account some cytotaxonomic and evolutionary considerations.

## MATERIAL AND METHODS

Eight Curimatidae species from the Paraná River (Argentina) were cytogenetically studied: *Steindachnerina brevipinna* (7 males, 15 females and 5 immatures), *Steindachnerina conspersa* (4 males and 5 females), *Curimatella dorsalis* (2 males, 3 females and one juvenile), *Cyphocharax spilodus* (12 males, 24 females and one immature), *Cyphocharax platanus* (one male and 3 females), *Cyphocharax voga* (2 males and one female), *Psectrogaster curviventris* (4 males and 2 females), and *Potamorhina squamoralevis* (1 male and 2 females). Chromosome preparations were obtained from kidney cells, by direct (Bertollo et al., 1978) and short-term culture methods (Fenocchio et al., 1991), and they were analyzed by conventional and differential staining. NORs were identified by the technique of Howell and Black (1980), and constitutive heterochromatin was revealed according to Sumner (1972).

## RESULTS

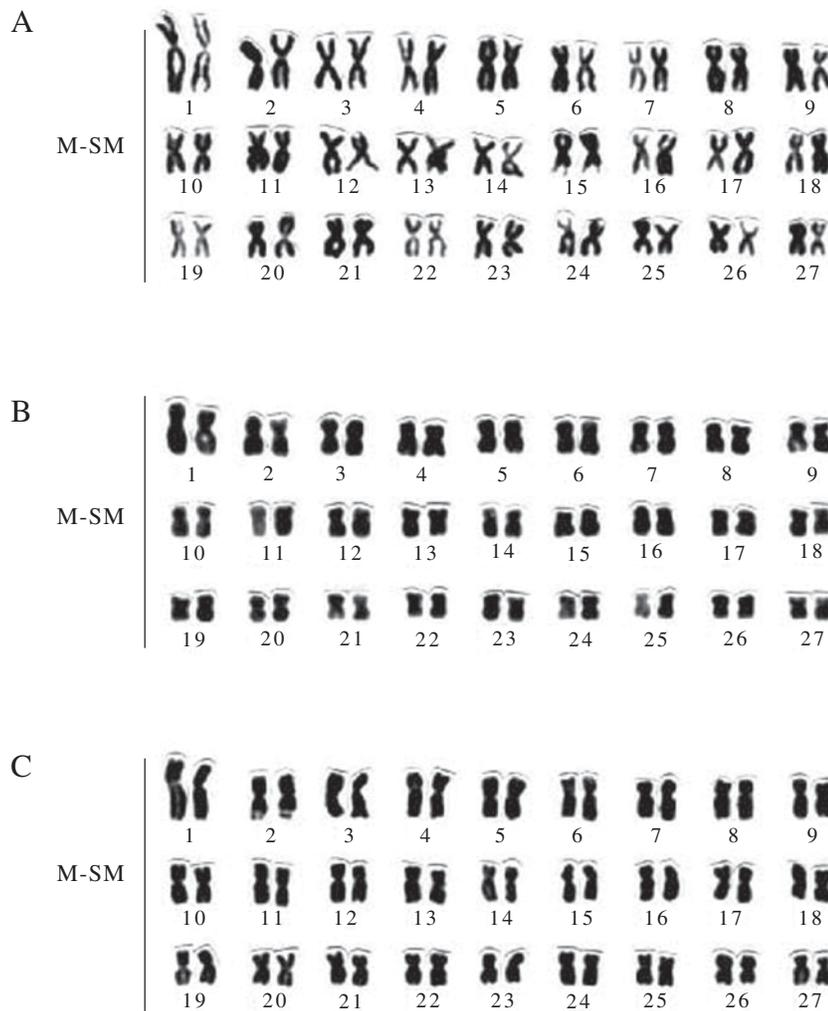
Six of the species studied, *S. brevipinna*, *S. conspersa*, *C. dorsalis*, *C. spilodus*, *C. voga*, and *P. curviventris*, presented the standard family karyotype with  $2n = 54$  bivalents chromosomes (FN = 108) (Figure 1A-C, Figure 2A-C). Complements varied in chromosome formula as well as in C- and NOR-band distributions. One *Cyphocharax spilodus* individual showed  $2n = 55$ , due to a small supernumerary chromosome (data not shown).

On the other hand, *Cyphocharax platanus* contained a complement of 58 chromosomes M-SM-ST (FN = 116) (Figure 3A), and *Potamorhina squamoralevis* presented a diploid number of 102 chromosomes with 7 M-SM pairs and 44 A pairs (FN = 116) (Table 1, Figure 3B).

We found only one NOR-bearing chromosome pair in all 8 species (Figure 4). In addition to positional differences on the chromosome, including both telomeric and subtelomeric locations, NOR bands appeared in various positions within the complement. All the species presented mostly centromeric C-bands, but there were some clear differences in the positive segments between species with a standard karyotype. These bands were predominantly pale, with some chromosomes appearing bright. C- and NOR-bands jointly allowed identification of marker chromosomes, principally chromosomes 1 and 2. *Cyphocharax voga* had only a few, pale positive bands along the complement (Figure 5F).

## DISCUSSION

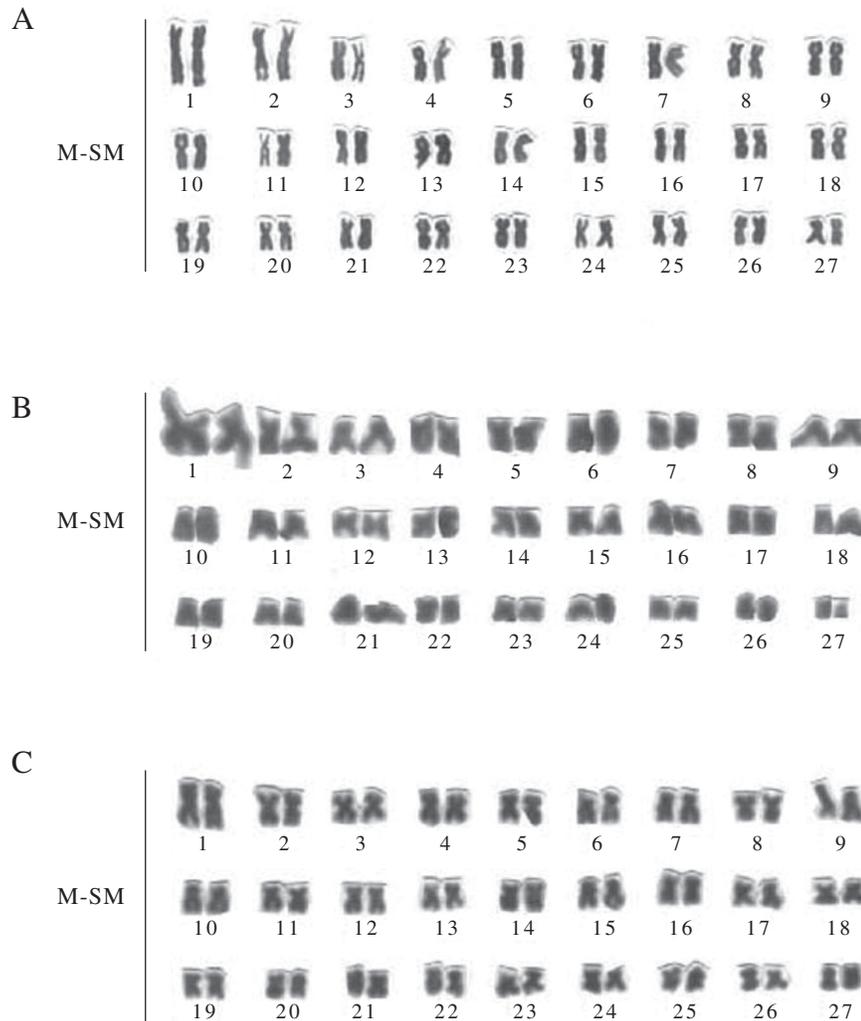
The groups Prochilodontidae, Curimatidae, Anostomidae, and Parodontidae were found to be highly stable (Feldberg et al., 1992b). In addition to being considered sister groups (Vari, 1983), the former three share common ecological characteristics. All have high mobility, consti-



**Figure 1.** Karyotypes with conventional Giemsa staining. **A**, *Steindachnerina brevipinna*; **B**, *S. conspersa*, and **C**, *Curimatella dorsalis*. In all cases the 1st chromosome pair is  $\sim 5 \mu$ . M = metacentric; SM = submetacentric.

tuting large populations that make seasonal migrations associated with reproductive and feeding habits (Vari, 1983). These families not only share karyotype stability, but they also present almost the same complement of  $2n = 54$  and identical chromosome morphology: metacentrics and submetacentrics (Bertollo et al., 1986; Pauls and Bertollo, 1990; Martins and Galetti, 1998).

The proposal of Vari (1983) to maintain the complement in the monophyletic group led cytogeneticists to consider that this chromosome number might have been present in a common ancestor. Consequently,  $2n = 54$  would be the plesiomorphic condition, and any other chromosome numbers would be derived characters (Venere and Galetti, 1989; Venere, 1991; Feldberg et al., 1992a). Venere and Galetti (1989) postulated that the most plausible explanation for this conservation would be cellular homeostasis, which is an equilibrium between selective forces favoring genome diversity and pressure for cell constancy. King (1993) presented an alternative hypothesis concerning maintenance of a particular chromosome complement. He believed that genome characteristics could determine the position and number of breaks, as well as possible

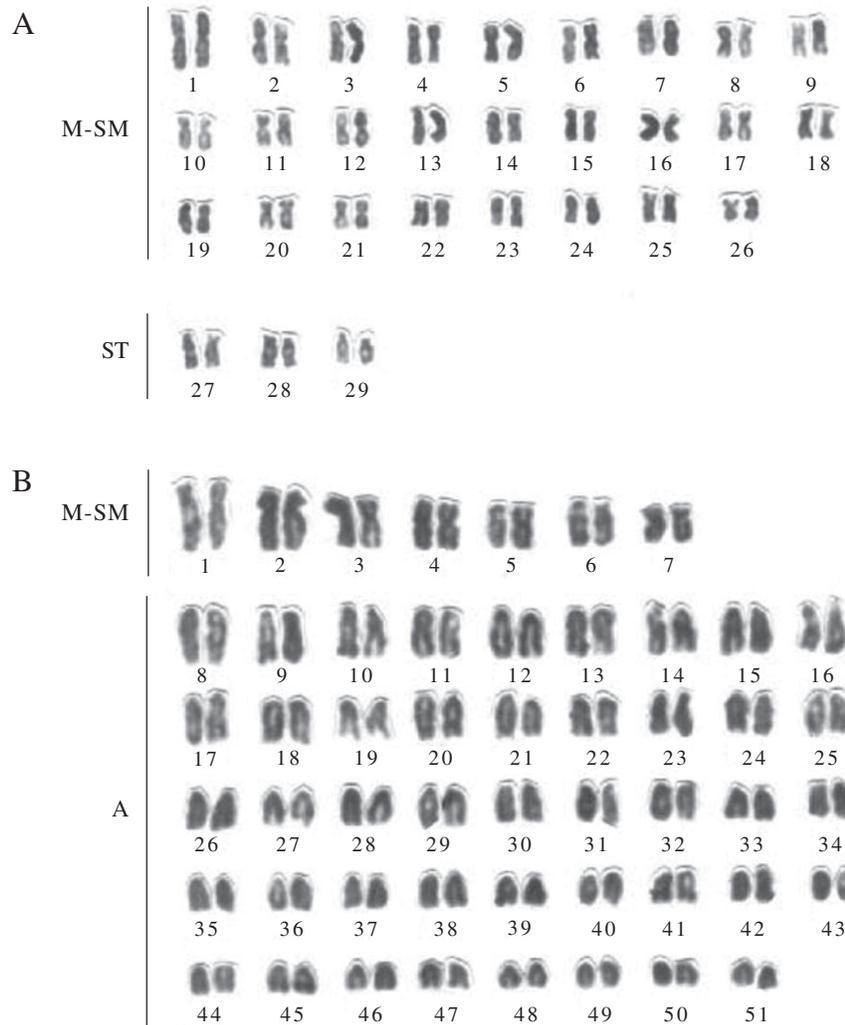


**Figure 2.** Karyotypes with conventional Giemsa staining. **A**, *Cyphocharax spilotos*, **B**, *C. voga* and **C**, *Psectrogaster curviventris*. In all cases the 1st chromosome pair is  $\sim 5 \mu$ . M = metacentric; SM = submetacentric.

types of rearrangements. In other words, if a symmetric karyotype lacks the structure to allow its reorganization, it would remain unaltered over time, as in the biarmed Curimatidae.

The *Cyphocharax platanus* karyotype ( $2n = 58$ ) could have been derived from an ancestor with  $2n = 54$ , with its origin affected by centric dissociations (Venere, 1991). However, further rearrangements involving pericentric inversions could have led to the present karyotypic structure. The same explanation could be applied to *Potamorhina squamoralevis*, where extensive fission events are indicated by the high number of acrocentric chromosomes (only 7 biarmed pairs) and FN (116), illustrating once again that the cladogenic process has been accompanied by macro-structural changes in a few of these species.

Karyotypes appeared to be homogeneous in all species presenting the standard family karyotype ( $2n = 54$  biarmed chromosomes); however, banding techniques revealed conspicuous differences (Figures 4 and 5). They showed that despite the conserved karyotype structure, the speciation process within this family has been accompanied by micro-structural rearrangements,

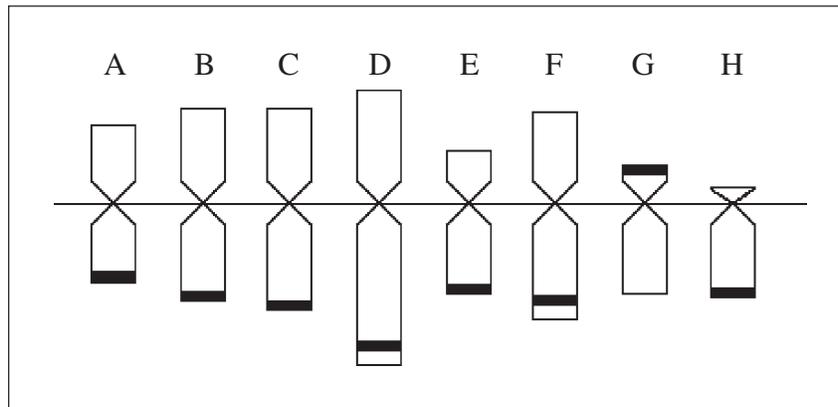


**Figure 3.** Karyotypes with conventional Giemsa staining. **A**, *Cyphocharax platanus*, and **B**, *Potamorhina squamoralevis*. In all cases the 1st chromosome pair is  $\sim 5 \mu$ . A = acrocentric; M = metacentric; SM = submetacentric; ST = subtelocentric.

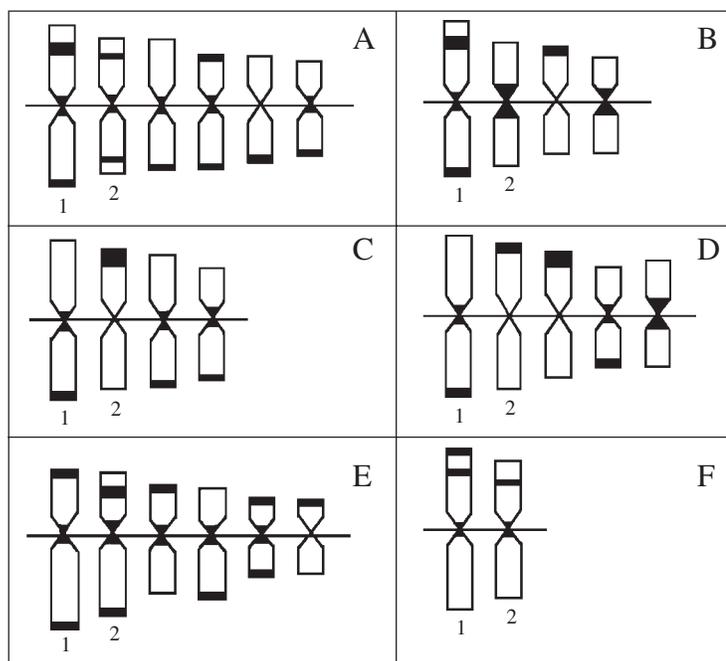
**Table 1.** Number of individuals and metaphases analyzed per species, with determination of modal diploid numbers in Curimatidae.

Species	Individuals studied	Metaphases analyzed	2n	FN	Chromosome types
<i>Curimatella dorsalis</i>	5	80	54	108	M-SM
<i>Psectrogaster curviventris</i>	6	152	54	108	M-SM
<i>Steindachnerina brevipinna</i>	27	565	54	108	M-SM
<i>Steindachnerina conspersa</i>	9	199	54	108	M-SM
<i>Potamorhina squamoralevis</i>	3	57	102	116	M-SM-A
<i>Cyphocharax voga</i>	3	57	54	108	M-SM
<i>Cyphocharax platanus</i>	4	45	58	116	M-SM-ST
<i>Cyphocharax spilotos</i>	35	638	54	108	M-SM

A = acrocentric; FN = fundamental number of chromosomes; M = metacentric; SM = submetacentric; ST = subtelocentric.



**Figure 4.** Ideogram showing NOR-bands in different species. **A**, *Steindachnerina brevipinna* (pair No.15), **B**, *S. conspersa* (pair No.2), **C**, *Curimatella dorsalis* (pair No.2), **D**, *Cyphocharax spilotos* (pair No.1), **E**, *C. voga*, **F**, *Psectrogaster curviventris*, **G**, *Cyphocharax platanus* (pair No.5), and **H**, *Potamorhina squamorealevis*. In E, F and H, it was not possible to precisely identify the NOR-bearing chromosome pair.



**Figure 5.** Ideogram showing C-bands in different species with standard karyotypes. **A**, *Steindachnerina brevipinna*; **B**, *S. conspersa*; **C**, *Curimatella dorsalis*; **D**, *Psectrogaster curviventris*; **E**, *Cyphocharax spilotos*, and **F**, *Cyphocharax voga*. The general C-band pattern of the complement is represented by other chromosome schemes. *Cyphocharax voga* only shows positive bands in the first two pairs (labelled 1 + 2).

as evidenced by diverse distribution patterns of heterochromatic bands and NORs. Based on our data, we conclude that these bands, including the NORs, for example, are important cytotoxic markers. These, together with conservation of certain C-band patterns in different chromosomes (such as chromosome 1 in *Steindachnerina brevipinna* and *S. conspersa*; Figure 5) provide additional information that could be used to examine phylogenetic relationships between species.

Members of the Curimatidae family, characterized by nearly homogeneous karyotypes, are found in quite diverse ecosystems (with the exception of torrential waters) in the Neotropical region (Vari, 1988). Consequently, small isolated populations could be established under changing environmental conditions. These less morphologically and genetically differentiated populations could have been obtained by allopatric speciation, as proposed by Vari (1989) for Curimatidae. Nevertheless, the karyotypes derived from dissociations and inversions in *C. platanus* and *P. squamoralevis* could be explained by a chromosome speciation model in which negatively heterotic rearrangements could have been established by stochastic processes in isolated demes.

## ACKNOWLEDGMENTS

The authors are grateful to the Programa de Estudos Limnológicos Regionales and especially Lic. B.H. Roa and D. Aichino for facilitating fish sampling as well as the Secretaría de Políticas Universitarias, Programa Incentivos a Docentes-Investigadores.

## REFERENCES

- Azpelicueta, M. and Braga, L.** (1991). *Los Curimátidos en Argentina*. PROFADU (CONICET) Programa de fauna de agua dulce. Museo de La Plata. Vol. 40, Fascículo 1.
- Bertollo, L.A.C., Takahashi, C.S. and Moreira Filho, O.** (1978). Cytotaxonomy considerations on *Hoplias lacerdae* (Pisces: Erythrinidae). *Rev. Bras. Genet.* 1: 17-37.
- Bertollo, L.A.C., Moreira-Filho, O. and Galetti, P.M.** (1986). Cytogenetics and taxonomy: considerations based on chromosome studies of freshwater fish. *J. Fish Biol.* 28: 153-159.
- Brum, J.I. and Galetti, P.M.** (1997). Teleostei ground plan karyotype. *J. Comp. Biol.* 2: 91-102.
- Feldberg, E., Porto, J.I.R. and Nakayama, C.M.** (1992a). Karyotype evolution in Curimatidae (Teleostei: Characiformes) from the Amazon region. II. Centric fissions in the Genus *Potamorhina*. *Genome* 36: 372-376.
- Feldberg, E., Rebelo Porto, J.I. and Bertollo, L.A.C.** (1992b). Karyotype evolution in Curimatidae (Teleostei, Characiformes) of the Amazon Region. I. Studies on the Genera *Curimata*, *Psectrogaster*, *Steindachnerina* and *Curimatella*. *Rev. Bras. Genet.* 15: 369-383.
- Fenocchio, A.S., Venere, P.C., Cesar, A.C.G., Dias, A.L. and Bertollo, L.A.C.** (1991). Short term culture from solid tissues of fishes. *Caryologia* 44: 161-166.
- Galetti Jr., P.M., Bertollo, L.A.C. and Moreira Filho, O.** (1994). Trends in chromosome evolution of neotropical characiform fishes. *Caryologia* 47: 289-297.
- Howell, W.N. and Black, D.A.** (1980). Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a one step method. *Experientia* 36: 1014-1015.
- King, M.** (1993). *Species Evolution. The Role of Chromosome Change*. Cambridge University Press, Cambridge, UK.
- Martins, C. and Galetti, P.M.** (1998). Karyotype similarity between two sympatric *Schizodon* fish species (Anostomidae, Characiformes) from the Paraguay River basin. *Genet. Mol. Biol.* 21: 355-360.
- Navarrete, M.C. and Julio Jr., F.H.** (1997). Cytogenetic analysis of four Curimatidae from the Paraguay Basin, Brazil (Pisces, Characiformes, Curimatidae). *Cytologia* 62: 241-247.
- Pauls, E. and Bertollo, L.A.C.** (1990). Distribution of supernumerary chromosome system and aspects of karyotypic evolution in the genus *Prochilodus* (Pisces, Prochilodontidae). *Genetica* 81: 117-123.
- Sumner, A.T.** (1972). A simple technique for demonstrating centromeric heterochromatin. *Expl. Cell. Res.* 75: 304-306.
- Vari, R.P.** (1983). Phylogenetic relationships of the families Curimatidae, Prochilodontidae, Anostomidae and Chilodontidae (Pisces, Characiformes). *Smithson. Contrib. Zool.* 378: 1-59.
- Vari, R.P.** (1988). The Curimatidae, a lowland neotropical fish family (Pisces, Characiformes); distribution, endemism, and phylogenetic biogeography. *Proceedings of a Workshop on Neotropical Distribution Patterns*. Academia Brasileira de Ciências, Rio de Janeiro, RJ, Brazil, pp. 343-377.
- Vari, R.P.** (1989). Systematics of the neotropical Characiform Genus *Psectrogaster* Eigenmann and

- Eigenmann (Pisces: Characiformes). *Smithson. Contrib. Zool.* 481: 1-43.
- Vari, R.P.** (1991). Systematics of the neotropical Characiform Genus *Steindachnerina* Fowler (Pisces: Ostariophysi). *Smithson. Contrib. Zool.* 507: 1-118.
- Venere, P.C.** (1991). Citogenética comparativa de peixes da família Curimatidae (Characiformes). Master's thesis, Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos, São Carlos, SP, Brazil.
- Venere, P.C.** (1998). Diversificação cariotípica em peixes do médio rio Araguaia, com ênfase em Characiformes e Siluriformes (Teleostei, Ostariophysi). Doctoral thesis, Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos, São Carlos, SP, Brazil.
- Venere, P.C.** and **Galetti, P.M.** (1989). Chromosome evolution and phylogenetic relationships of some neotropical characiformes of the family Curimatidae. *Rev. Bras. Genet.* 12: 17-25.