

Chromosome Relationships between Anostomidae and Chilodontidae Fish (Characiformes)

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Summary Although many chromosome studies have been conducted in Anostomidae, the data are still concentrated in some genera. Other Characiformes families, such as Chilodontidae, are still cytogenetically unknown. In order to contribute to the cytogenetical knowledge on Anostomidae and Chilodontidae, the mitotic chromosomes of 3 anostomid species, *Abramites solarii*, *Anostomus ternetzi* and *Pseudanus trimaculatus*, and 2 chilodontids, *Caenotropus labyrinthicus* and *Chilodus punctatus*, were analyzed using Giemsa staining, C-banding and nucleolus organizer region distribution detected by silver nitrate (Ag-NOR) and mithramycin (MM) staining. Although 54 banded chromosomes and 1 chromosome pair bearing NOR sites represent a general trait, heterochromatin distribution and NOR chromosome pair position are variable among Anostomidae and Chilodontidae species. This trend suggests that cryptic changes along the chromosome complement of these fishes have occurred, even though the gross karyotype structure has been strongly conserved during their chromosome evolution. Furthermore, it appears to have a parallelism between morphological/ecological diversity and chromosomal evolution rate among anostomids and chilodontids.

Key words Anostomidae, Chilodontidae, Chromosome evolution, Fish, Heterochromatin, Nucleolus organizer region.

The freshwater fish of the Anostomidae and Chilodontidae families (Characiformes) are typical from neotropical region. While anostomids show wide geographic distribution from Central to South America, chilodontids are restricted to the Amazon and Orinoco basins and rivers in the northeast coast of Brazil (Vari *et al.* 1995).

The family Anostomidae comprises 12 genera thus far recognized: *Abramites*, *Anostomoides*, *Anostomus*, *Gnathodolus*, *Laemolyta*, *Leporellus*, *Leporinus*, *Pseudanus*, *Rhytidodus*, *Sartor*, *Schizodon* and *Synaptaemus* (Greenwood *et al.* 1966, Géry 1977, Winterbottom 1980). *Gnathodolus* and *Synaptaemus* are monotypic and together with *Sartor* are quite specialized (Santos and Jégui 1987). These 3 late genera plus *Anostomus* and *Pseudanus* have been considered a monophyletic unit (Anostominae, sensu Winterbottom 1980), assembling small sized fishes with a geographic distribution restricted to peripheral areas of the Amazon basin. The other hand, Chilodontidae is represented by only 2 genera, *Chilodus* and *Caenotropus* (Vari *et al.* 1995). Anostomidae and Chilodontidae have been joined to Curimatidae and Prochilodontidae in a monophyletic unit (Vari 1983).

Despite of their morphological diversity, the anostomids have been characterized by a common karyotype pattern showing $2n=54$ meta- and submetacentric chromosomes (Galetti Jr. and Foresti 1986, 1987, Galetti Jr. *et al.* 1981a, b, 1984, 1991a, b, Mestriner *et al.* 1995). Similar karyotype pat-

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tern has been reported for other Characiformes, such as Curimatidae (Venere and Galetti Jr. 1989, Feldberg *et al.* 1992, 1993), Prochilodontidae (Pauls and Bertollo 1983, 1990, Feldberg *et al.* 1987), Hemiodontidae (Porto *et al.* 1993) and Parodontidae (Moreira-Filho *et al.* 1984, 1985, Jesus *et al.* 1999). No cytogenetic data are available for Chilodontidae. Even in anostomids, the chromosomes were more extensively studied in few genera such as *Leporinus*, *Schizodon* and *Leporellus* (Galetti Jr. *et al.* 1981a, b, 1991a, b, 1995a, b, Galetti Jr. and Foresti 1986, 1987, Martins and Galetti Jr. 1997, 1998, Mestriner *et al.* 1995). The remaining genera are still cytogenetically unknown.

In order to contribute to the cytogenetical knowledge on Anostomidae and Chilodontidae, the mitotic chromosomes of 3 anostomid species, *Abramites solarii*, *Anostomus ternetzi* and *Pseudanos trimaculatus*, and 2 chilodontids, *Caenotropus labyrinthicus* and *Chilodus punctatus*, were studied. The chromosome relationships within and between Anostomidae and Chilodontidae are discussed.

Materials and methods

Three anostomid species, *Abramites solarii* (3 females, 1 male), *Anostomus ternetzi* (3 females, 3 males) and *Pseudanos trimaculatus* (1 female, 1 male), and 2 chilodontid species, *Chilodus punctatus* (1 female, 4 males) and *Caenotropus labyrinthicus* (1 female, 3 males), were analyzed. Exception to the latter one which was wild-caught in the Araguaia river (Tocantins-Araguaia basin), township of Barra do Garças, MT, all the remaining species are originally from the Amazon region, but the studied individuals were bought in pet stores.

Mitotic chromosomes were obtained from anterior kidney cell suspensions described elsewhere (Bertollo *et al.* 1978). The heterochromatin was identified by C-banding (Sumner 1972) and nucleolus organizer region distribution was analyzed by silver nitrate (Ag-NOR) (Howell and Black 1980) and mithramycin (MM) staining (Schmid 1980).

Results

The anostomid and chilodontid species showed a common diploid karyotype with 54 banded chromosomes (Figs. 1, 2, 3). Two Ag-NOR sites were detected in the chromosome complement of all studied species. In anostomids, Ag-NORs were found at the end of the long arm of a medium-sized metacentric (pair 5) in *A. solarii* (Fig. 2a), proximal to the centromere in the long arm of a large metacentric (pair 2) in *A. ternetzi* (Fig. 2b), and at the end of the long arm of a medium-sized submetacentric (pair 7) in *P. trimaculatus* (Fig. 2c). In both chilodontids, the Ag-NORs were observed at the end of the long arm of a medium-sized submetacentric, comparable to the chromosome pair 5 (Fig. 3a, b). C-bands were mostly weaker in *A. solarii* and *A. ternetzi*. Darker heterochromatin blocks were restricted to few chromosomes in both species, including the NOR-bearing chromosomes (Fig. 2a, b). Of note, a well-defined intercalary heterochromatin was observed in the long arm of a large metacentric (pair 3) of *A. ternetzi*. The heterochromatin was more abundant in *P. trimaculatus*, mainly in the centromere areas (Fig. 2c). Large amount of proximal heterochromatin was detected in the long arm of several chromosomes in *C. punctatus* (Fig. 3a), while few C-bands were observed in centromeres and telomeres of *C. labyrinthicus* (Fig. 3b). In the former species, the Ag-NOR sites also appear C⁺. Of note, one out of the four studied individuals of *C. labyrinthicus* showed a third Ag-NOR bearing chromosome and MM fluorescent staining revealed at least 5 chromosomes bearing MM bands (Fig. 4).

Discussion

As previously described in *Leporinus*, *Leporellus*, *Schizodon* (Galetti Jr. *et al.* 1981a), *Laemolyta* and *Rhytiodus* (Venere 1998, Krichanã 1999), the species of the genus *Abramites*,

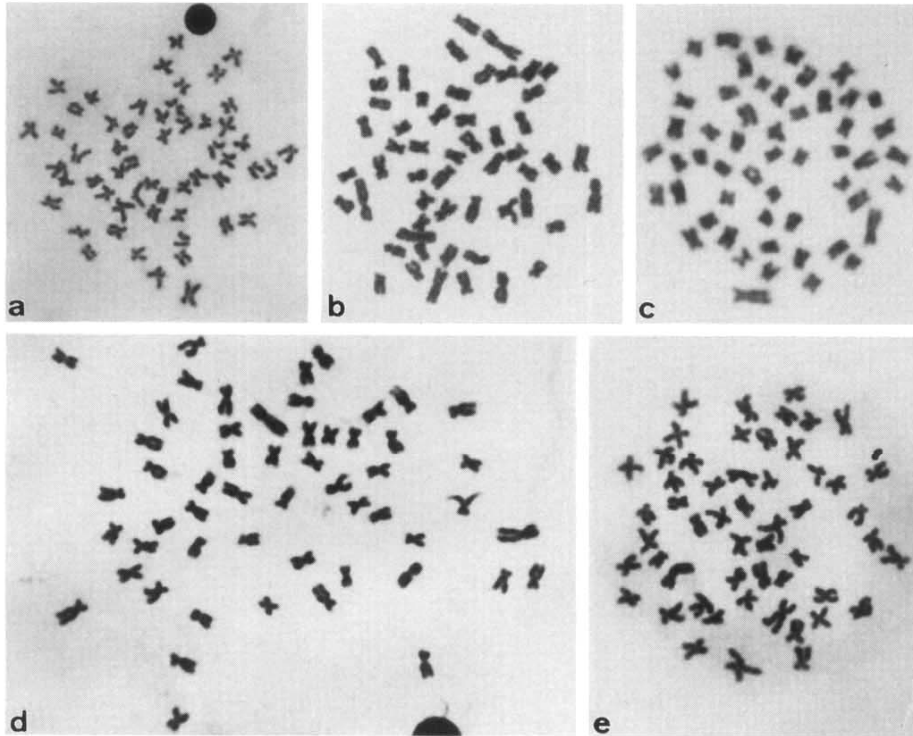


Fig. 1. Giemsa-stained metaphases of a) *Abramites solarii*, b) *Anostomus ternetzi*, c) *Pseudanos trimaculatus*, d) *Caenotropus labyrinthicus* and e) *Chilodus punctatus*.

Anostomus and *Pseudanos* studied here showed a common karyotype pattern with $2n=54$ meta- and submetacentric chromosomes. These results confirm the ideas that this karyotype should be ancestral in the family Anostomidae (Galetti Jr. *et al.* 1981a). In fact, it has been shown that this karyotype pattern is more ancient among Characiformes (Venere and Galetti Jr. 1989, Galetti Jr. *et al.* 1994). In an elegant phylogenetic interpretation based on morphological traits, Vari (1983) has shown that Curimatidae, Prochilodontidae, Anostomidae and Chilodontidae constitute a major monophyletic unit comprised by two sister clades, one joining the two former groups and another one with Anostomidae and Chilodontidae. Since most Curimatidae and Prochilodontidae cytogenetically studied have also shown $2n=54$ biarmed chromosomes, it has been believed that this karyotype pattern is a synapomorphy among all these groups (Venere and Galetti Jr. 1989, Galetti Jr. *et al.* 1994). However, the lack of chromosome data in Chilodontidae did not allow to test such hypothesis before the present work. The occurrence of $2n=54$ biarmed chromosomes in *C. labyrinthicus* and *C. punctatus*, further the similar incidence of this karyotype pattern also in Parodontidae (Moreira-Filho *et al.* 1984, Jesus *et al.* 1999) and Hemiodontidae (Porto *et al.* 1993), strongly suggest that $2n=54$ biarmed chromosomes have arisen early in the evolutionary process of Characiformes.

Variations of this karyotypic pattern were only related to a peculiar ZZ/ZW sex chromosome system of some anostomids and parodontids (Galetti Jr. *et al.* 1981b, Galetti Jr. and Foresti 1986, 1987, Moreira-Filho *et al.* 1985, 1993), supernumerary chromosomes in anostomids, curimatids and prochilodontids (Martins *et al.* 1996, Venere *et al.* 1999) and few cases of reduction or increase of chromosome number reported in curimatids (Venere 1991, Feldberg *et al.* 1993, Navarrete and

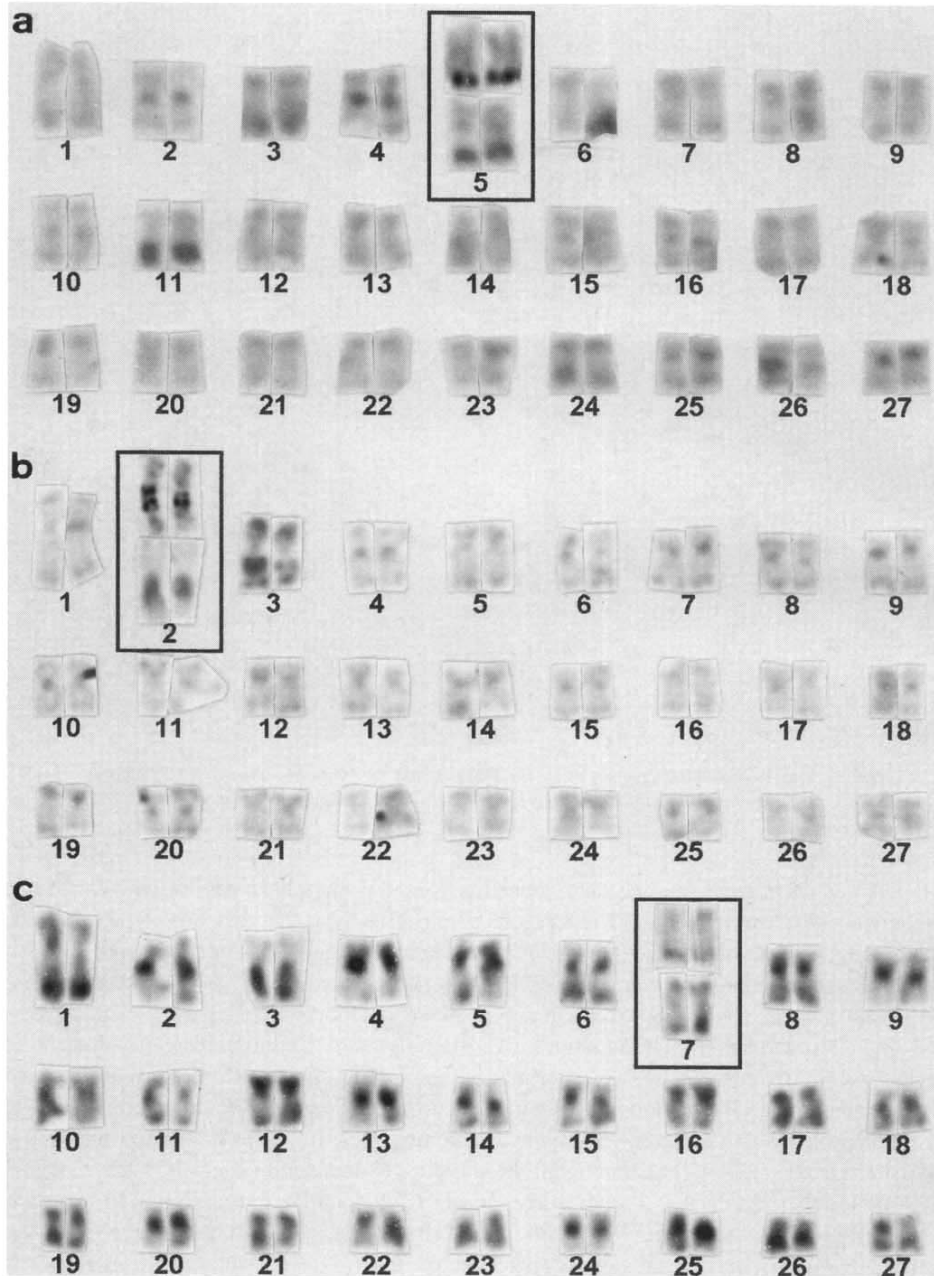


Fig. 2. C-banded karyotypes of a) *Abramites solarii*, b) *Anostomus ternetzi* and c) *Pseudanos trimaculatus*. In the box, Ag-NOR bearing chromosomes (in the top).

Júlio Jr. 1997).

The karyotype symmetrization observed in all of these groups might represent an end point of a saturated chromosome evolution (White 1978). Alternatively, the karyotype symmetrization associated to a size-chromosome homogeneity might mean an optimum organization, adjusted to the

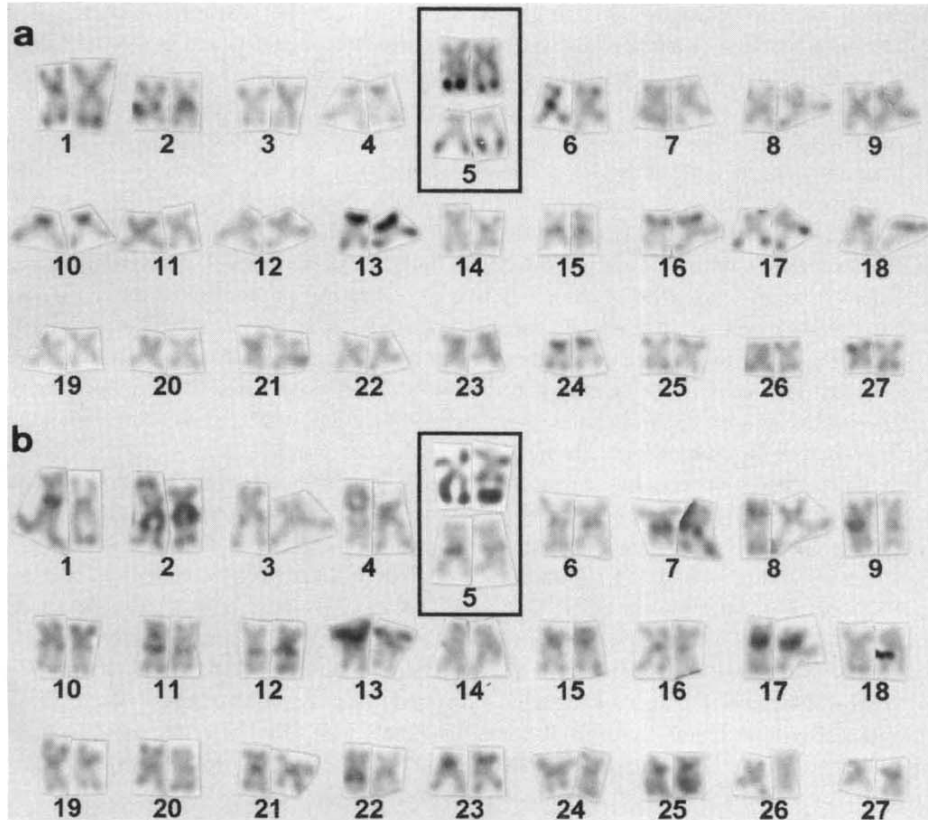


Fig. 3. C-banded karyotypes of a) *Caenotropus labyrinthicus* and b) *Chilodus punctatus*. In the box, Ag-NOR bearing chromosome (in the top).

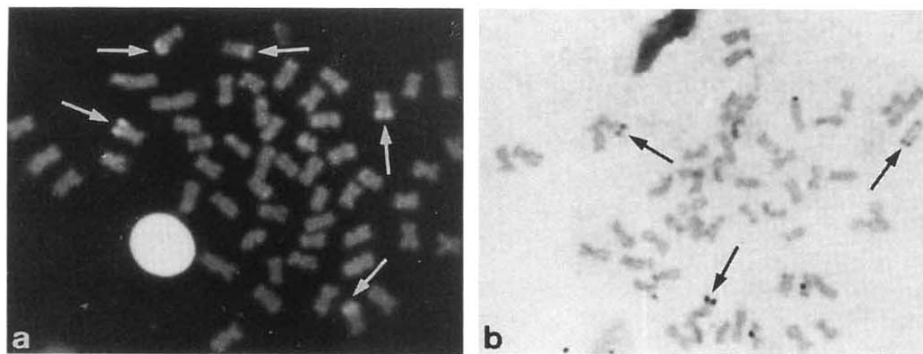


Fig. 4. a) MM and b) partial Ag-NOR metaphases of *Caenotropus labyrinthicus*. MM fluorescent signals and Ag-NOR positive sites are indicated by arrows.

cellular homeostasis during the mitotic process, and large modifications are negatively regulated (Venere and Galetti Jr. 1989, Martins and Galetti Jr. 1997). Genome sizes are intimately related to cell volume (Cavalier-Smith 1985), and the major karyotype structure itself might also be strongly related to the chromosome organization in the interphasic nuclei (Schweizer *et al.* 1987).

However, the karyotype of these fish groups is not totally conserved. It has been reported the occurrence of remarkable differences along the heterochromatin segments and in the NOR distribution patterns within and between these fishes. In *Abramites* and *Anostomus* the heterochromatin was observed quite reduced, with C-positive bands present only in few chromosomes. Similar results were described in some *Leporinus* species (Galetti Jr. *et al.* 1991a, b). In contrast, the centromeric heterochromatin appears quite abundant in *P. trimaculatus*, similarly to previously described in *Leporellus*, corroborating that such heterochromatin variations have played important role in chromosome diversification of these fishes (Galetti Jr. *et al.* 1991a). Similar divergence can be observed in the NOR distribution, and in anostomids the NORs have been claimed with cytotaxonomic importance (Galetti Jr. *et al.* 1984, 1991b). Although two NOR-bearing chromosomes per cell have been detected in the karyotype of most studied anostomids, the NOR sites as observed in *A. ternetzi*, *A. solarii* and *P. trimaculatus* are located in different chromosomes in these fishes. In chilodontids, the variation in the number of NOR-bearing chromosomes detected by silver in *C. labyrinthicus* and confirmed by MM staining (Schmid and Guttenbach 1988), suggests that such NOR variations can also be important in the chromosome diversification of this fish group.

Although it can be observed a general trend in the chromosome evolution of these major fish groups, lower taxa may experience different chromosome evolutionary trends. In anostomids, the species-rich genus *Leporinus* shows wide morphological and ecological diversity, and also larger chromosome variability due to sex chromosomes (Galetti Jr. *et al.* 1981b, Galetti Jr. and Foresti 1986, 1987), NOR polymorphisms (Galetti Jr. *et al.* 1984, 1991b) and heterochromatin variations (Galetti Jr. *et al.* 1991a, b). In contrast, *Schizodon* shows fewer chromosome variations, related to heterochromatin and NOR distribution (Martins and Galetti Jr. 1997, 1998) and little morphological and ecological diversity. Similar condition has previously been described in other organisms, and chromosomal evolution seems faster in placental mammals than in other vertebrates, such as amphibians (Wilson *et al.* 1975). It appears to have a parallelism between morphological/ecological diversity and chromosomal evolution rate among anostomids (Martins and Galetti Jr. 1997). In the same way, although Chilodontidae shows some variations related to heterochromatin and NOR distribution, the conserved chromosome macrostructure observed can be explained by the lower morphological diversification among these fishes.

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References

- Bertollo, L. A. C., Takahashi, C. S. and Moreira-Filho, O. 1978. Cytotaxonomic consideration on *Hoplias Lacerdae* (Pisces, Erythrinidae). *Braz. J. Genet.* **1**: 103–120.
- Cavalier-Smith, T. 1985. *The Evolution of Genome Size*. Willey, London.
- Feldberg, E., Bertollo, L. A. C., Almeida Toledo, L. F., Foresti, F., Moreira Filho, O. and Santos, A. F. 1987. Biological aspects of amazonian fish. IX. Cytogenetic studies in two species of the genus *Semaprochilodus* (Pisces, Prochilodontidae). *Genome* **29**: 1–4.
- , Porto, J. I. and Bertollo, L. A. C. 1992. Karyotype evolution in Curimatidae (Teleostei, Characiformes) of the Amazon region. I. Studies on the genera *Curimata*, *Psectrogaster*, *Steindachnerina* and *Curimatella*. *Braz. J. Genet.* **15**: 369–383.
- , — and — 1993. Karyotype evolution in Curimatidae (Teleostei, Characiformes) of the Amazon region. II. Centric fissions in the genus *Potamorhina*. *Genome* **36**: 372–376.
- Galetti Jr., P. M. and Foresti, F. 1986. Evolution of the ZZ/ZW system in *Leporinus* (Pisces, Anostomidae). *Cytogenet. Cell Genet.* **43**: 43–46.
- and — 1987. Two new cases of ZZ/ZW heterogamety in *Leporinus* (Anostomidae, Characiformes) and their relation-

- ships in the phylogeny of the group. *Braz. J. Genet.* **10**: 135–140.
- , —, Bertollo, L.A.C. and Moreira-Filho, O. 1981a. Karyotypic similarity in three genera (*Leporinus*, *Leporellus* and *Schizodon*) of the family Anostomidae (Pisces, Teleostei). *Braz. J. Genet.* **4**: 11–15.
- , —, — and — 1981b. Heteromorphic sex chromosomes in three species of the genus *Leporinus* (Pisces, Anostomidae). *Cytogenet. Cell Genet.* **29**: 138–142.
- , —, — and — 1984. Characterization of eight species of Anostomidae (Cypriniformes) fish on the basis of the nucleolar organizing region. *Caryologia* **37**: 401–406.
- , Mestriner, C. A. and Foresti, F. 1991a. Heterochromatin and karyotype reorganization in fish of the family Anostomidae (Characiformes). *Cytogenet. Cell Genet.* **56**: 116–121.
- , Cesar, A. C. G. and Venere, P. C. 1991b. Heterochromatin and NORs variability in *Leporinus* fish (Anostomidae, Characiformes). *Caryologia* **44**: 287–292.
- , Bertollo, L. A. C. and Moreira-Filho, O. 1994. Trends in chromosome evolution of neotropical Characiform fishes. *Caryologia* **47**: 289–297.
- , Lima, N. R. W. and Venere, P. C. 1995a. A monophyletic ZW chromosome system in *Leporinus* (Anostomidae, Characiformes). *Cytologia* **60**: 375–382.
- , Mestriner, C. A., Monaco, P. J. and Rasch, E. M. 1995b. Post-zygotic modifications and intra- and inter-individual nucleolar organizing region variations in fish: report of a case involving *Leporinus friderici*. *Chrom. Res.* **3**: 285–290.
- Géry, J. 1977. *Characoids of the World*. T. F. H. Publications, Neptune City.
- Greenwood, P. H., Rosen, D. E., Weitzman, S. H. and Myers, G. S. 1966. Phyletic studies of teleostean fishes with a provisional classification of living forms. *Bull. Am. Mus. Nat. Hist.* **131**: 339–456.
- Howell, W. M. and Black, D. A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: A 1-step method. *Experientia* **36**: 1014–1015.
- Jesus, C. M., Bertollo, L. A. C. and Moreira-Filho, O. 1999. Comparative cytogenetics in *Apareiodon affinis* (Pisces, Characiformes) and considerations regarding diversification of the group. *Genetica* **105**: 63–67.
- Krichanã, S. R. L. 1999. Contribuição ao estudo citogenético da família Anostomidae (Pisces, Characiformes) na região Amazônica. Master Thesis, Universidade Federal de São Carlos, SP, Brazil.
- Martins, C. and Galetti Jr., P. M. 1997. Narrow chromosome diversity in fishes of the genus *Schizodon* (Characiformes, Anostomidae). *Cytobios* **92**: 139–147.
- and — 1998. Karyotype similarity between two sympatric *Schizodon* fish species (Anostomidae, Characiformes) from the Paraguay river basin. *Genet. Mol. Biol.* **21**: 355–360.
- , Giuliano-Caetano, L. and Dias, A. L. 1996. Occurrence of a B chromosome in *Cyphocharax modesta* (Pisces, Curimatidae). *Cytobios* **85**: 247–253.
- Mestriner, C. A., Bertollo, L. A. C. and Galetti Jr., P. M. 1995. Chromosome banding and synaptonemal complexes in *Leporinus lacustris* (Pisces, Anostomidae): Analysis of a sex system. *Chrom. Res.* **3**: 440–443.
- Moreira-Filho, O., Bertollo, L. A. C. and Galetti Jr., P. M. 1984. Structure and variability of nucleolar organizing regions in Parodontidae fish. *Can. J. Genet. Cytol.* **26**: 564–568.
- , — and — 1985. Karyotypic studies of some species of family Parodontidae (Pisces, Cypriniformes). *Caryologia* **38**: 47–55.
- , — and — 1993. Distribution of sex chromosome mechanisms in neotropical fish and description of a ZZ/ZW system in *Parodon hilarii* (Parodontidae). *Caryologia* **38**: 47–55.
- Navarrete, M. C. and Júlio Jr., H. F. 1997. Cytogenetic analysis of four curimatids from the Paraguay Basin, Brazil (Pisces: Characiformes: Curimatidae). *Cytologia* **62**: 241–247.
- Pauls, E. and Bertollo, L. A. C. 1983. Evidence for a system of supernumerary chromosome in *Prochilodus scrofa* (Pisces, Prochilodontidae). *Caryologia* **36**: 307–314.
- and — 1990. Distribution of a supernumerary chromosome system and aspects of karyotypic evolution in the genus *Prochilodus* (Pisces, Prochilodontidae). *Genetica* **81**: 117–123.
- Porto, J. I. R., Feldberg, E., Falcão, J. N. and Nakayama, C. M. 1993. Cytogenetics studies in Hemiodontidae (Ostariophysi, Characiformes) fishes from central Amazon. *Cytologia* **58**: 397–402.
- Santos, G. M. and Jégui, M. 1987. Novas ocorrências de *Gnathodolus bidens*, *Synaptolaemus cingulatus* e descrição de duas espécies novas de Sartor (Characiformes, Anostomidae). *Amazoniana* **10**: 181–196.
- Schmid, M. 1980. Chromosome banding in Amphibia. IV. Differentiation of GC- and AT- rich chromosome regions in Anura. *Chromosoma* **77**: 83–103.
- and Guttenbach, M. 1988. Evolutionary diversity of reverse (R) fluorescent chromosome bands in vertebrates. *Chromosoma* **97**: 101–114.
- Schweizer, D., Loidl, L. and Hamilton, B. 1987. Heterochromatin and the Phenomena of Chromosome Banding. In: Hennig, W. (ed.) *Structure and Function of Eukaryotic Chromosomes*. Heidelberg Inc. pp. 235–250.
- Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. *Exptl. Cell Res.* **75**: 304–305.
- Vari, R. P. 1983. Phylogenetic relationships of the families Curimatidae, Prochilodontidae, Anostomidae and Chilodontidae

- (Pisces, Characiformes). *Smithsonian Contributions to Zoology* **378**: 1–60.
- , Castro, R. M. and Raredon, S. J. 1995. The neotropical fish family Chilodontidae (Teleostei: Characiformes): A phylogenetic study and a revision of *Caenotropus* Günther. *Smithsonian Contributions to Zoology* **577**: 1–32.
- Venere, P. C. 1991. Citogenética comparativa de peixes da família Curimatidae (Characiformes). Master Thesis. Universidade Federal de São Carlos, SP, Brazil.
- 1998. Diversificação cariotípica em peixes do médio rio Araguaia, com ênfase em Characiformes e Siluriformes (Teleostei, Ostariophysi). Doctoral Thesis. Universidade Federal de São Carlos, SP, Brazil.
- and Galetti Jr., P. M. 1989. Chromosome relationships of some neotropical Characiformes of the family Curimatidae. *Braz. J. Genet.* **12**: 17–25.
- , Miyazawa, C. S. and Galetti Jr., P. M. 1999. New cases of supernumerary chromosomes in characiform fishes. *Gen. Mol. Biol.* **22**: 345–349.
- White, M. J. D. 1978. Chain processes in chromosomal speciation. *Systematic Zoology* **27**: 285–298.
- Wilson, A. C., Bush, G. L., Case, S. M. and King, M. C. 1975. Social structuring of mammalian populations and rate of chromosomal evolution. *Proc. Nat. Acad. Sci.* **71**: 5061–5065.
- Winterbottom, R. 1980. Systematics, osteology and phylogenetic relationships of fishes of the Ostariophysian subfamily Anostominae (Characoidei, Anostomidae). *Royal Ontario Museum, Life Sciences Contribution* **123**: 1–112.