Chromosomal divergence and maintenance of sympatric *Characidium* fish species (Crenuchidae, Characidiinae)

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Cytogenetic studies were performed in two syntopic species of *Characidium*, *C. lauroi* and *Characidium* sp. cf. *C. alipioi*, from Ribeirão Grande, Paraíba do Sul river basin. Both species have diploid number 2n = 50 chromosomes, but differ in chromosome shape, C-banding pattern and location of nucleolar organizing regions. In *Characidium* sp. cf. *C. alipioi* a new type of ZW sex chromosome system composed of equal sized metacentric chromosomes is reported for the first time in the genus *Characidium*. Species of *Characidium* with a sex chromosome system form a monophyletic group. Variations in this system are interpreted as resulting from geographic isolation among allopatric species.

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The neotropical Characidiinae comprise a group of relatively small (usually less than 10 cm standard length) fishes of the order Characiformes widely distributed in freshwater systems between Panama and eastern Argentina. The group was recently removed from the Characidae along with the Crenuchinae, and included in a separate, monophyletic family Crenuchidae (BUCKUP 1998). The group is a monophyletic unit (BUCKUP 1993a) including 80 nominal species, of which 71 are currently considered valid. A phylogenetic hypothesis of relationships is available for most taxa (BUCKUP 1993c). The hypothesis includes representatives of 8 genera, but recently described taxa still require detailed phylogenetic evaluation (e.g. *Skiotocharax*, PRESSWELL et al. 2000; *Geryichthys*, ZARSKE 1997). *Characidium* is the most diverse and widespread characidiin genus including 59 nominal taxa, of which 48 are regarded as valid species, usually occurring in small headwater streams. The genus is poorly diagnosed (BUCKUP 1993c), and several species are still undescribed or only recently described (ZARSKE and GÉRY 2001; MELO and BUCKUP 2002).

Cytogenetic studies of *Characidium* were initiated in the last decade. According to these studies, *Characidium* has a constant diploid number of chromosomes (2n = 50), but the karyotype structure is variable regarding chromosome shape, presence of B-chromosomes, sex chromosome systems, and natural occurrence of triploidy (MIYAZAUA and GALETTI JR. 1994; MAISTRO et al. 1998; CENTOFANTE et al. 2001).

Here we present chromosome data for two sympatric species of *Characidium* from the southeastern slope of Serra da Mantiqueira in southeastern Brazil. One of the species has been identified as *C. lauroi* Travassos, and the other may represent an undescribed species, possibly related to *C. alipioi* Travassos. The latter is here labeled as *Characidium* sp. cf. *C. alipioi*. The karotypes of these species are described, including a newly discovered form of ZZ/ZW sex chromosome system found in *Characidium* sp. cf. *C. alipioi*. The evolution of chromosome characters is discussed relative to their geographic distribution and available phylogenetic evidence.

MATERIAL AND METHODS

Chromosome analysis was carried out on 930 metaphases of *Characidium* sp. cf. *C. alipioi* (43 specimens: 26 females and 17 males), and 620 metaphases of *C. lauroi* (22 specimens: 14 females and 8 males). The specimens were collected at córrego Ribeirão Grande, a tributary of the Paraíba do Sul river drainage, at Fazenda Vera Cruz (22°46'2.98"S e 45°26'7.05"W), in the municipality of Pindamonhangaba, State of São Paulo, Brazil. Voucher specimens are deposited at the fish collection of the Museu Nacional (MNRJ), Rio de Janeiro, under catalog numbers MNRJ 22211 (*Characidium* sp. cf. *C. alipioi*, individuals 14683, 14684, 14792, 14794, 14797, 14798, 14802, 14829), and MNRJ 22212 (*C. lauroi*, individuals 14686, 14795, 14801, 14803, 14804, 14805, 14806, 15081, 15078, 15079).

Specimens previously identified as *Characidium* cf. *fasciatum* in the study of MAISTRO et al. (1998) and subsequently donated to MNRJ were reidentified in the present study as *Characidium* sp. cf. *C. gomesi*, and *Characidium* sp. aff. *C. zebra*. The former are registered under catalog numbers MNRJ 24025 (specimens 3196 to 3201), MNRJ 24028 (specimens 4411, 4412, 4416–4418, 4426, 4427, 4429–4431, 4434–4437), and MNRJ 24031 (specimens 2747, 3103B, 3104, 3114, 3115). Two specimens from córrego da Quinta belong to a different species and are currently registered under MNRJ 24032 (specimens 3103A, 3113).

Mitotic chromosomes were obtained according to the technique described by BERTOLLO et al. (1978). Constitutive heterochromatin was detected using the method described by SUMNER (1972), and silver-staining nucleolar organizer regions (Ag-NORs) were detected with the technique described by HOWELL and BLACK (1980). Sequential banding preparations followed CENTOFANTE et al. (2002a). Chromosomal morphology was determined according to their arm ratios (LEVAN et al. 1964).

RESULTS

The karyotype of *Characidium* sp. cf. *C. alipioi* is composed of 30 metacentric and 20 submetacentric chromosomes (2n = 50). The fundamental number (number of chromosomal arms) is 100 (Fig. 1). Constitutive heterochromatin occurs in pericentromeric regions of all chromosomes in both sexes (Fig. 1b and d). However, males and females differ in the C-banding pattern of chromosome pair. In males both chromosomes of this pair 1 exhibit telomeric heterochromatin (Fig. 1d), while in females this is the case in only one of the two chromosomes, the other chromosome being completely heterochromatic (Fig. 1b). Such difference characterizes a ZZ/ZW sex chromosome system. Ag-NORs are located in a single chromosome pair, in the distal region of the long arm of pair 16 (Fig. 1-box), coinciding with an heterochromatic region (Fig. 1b and d).

The karyotype of *C. lauroi* is composed of 24 metacentric, 24 submetacentric, and 2 subtelocentric chromosomes (2n = 50). As in the previous species the fundamental number is 100 (Fig. 2). Constitutive heterochromatin occurs in pericentromeric regions of all chromosomes, and in the telomeric region of a few chromosome pairs (Fig. 2b). No sex heteromorphism was observed in this species. Ag-NORs are located on the telomeric region of the chromosomes of pairs 5 and 23, being present up to three chromosomes. Most often these ribosomal sites are present in two non-homologous chromosomes, as in the short arm of one chromosome 5 and in the long arm of one chromosome 23 (Fig. 2-box). As in the previous species the Ag-NORs are also coincident with small heterochromatic segments (Fig. 2b).

DISCUSSION

Both *C. lauroi* and *Characidium* sp. cf. *C. alipioi* have the same diploid number of chromosomes (2n = 50) and fundamental number (100). However, they differ in chromosome formula, location of ribosomal sites (Ag-NORs), and C-banding pattern. In *Characidium* sp. cf. *C. alipioi* a ZZ/ZW sex chromosome system is present, while no sex chrosome heteromorphism was detected in *C. lauroi*.

Heteromorphic sex chromosome systems are relatively rare in neotropical freshwater fish, being recorded in only 6 % of the species which have been studied cytogenetically (CENTOFANTE et al. 2002b). However, compared with other vertebrates, these systems are remarkably diverse, varying from simple systems such as the ZZ/ZW and XX/XY to multiple systems such as ZZ/ZW_1W_2, $X_1X_1X_2X_2/X_1X_2Y$, and XX/XY_1Y_2 (MOREIRA-FILHO et al. 1993). Female heterogamety is most frequent, corresponding to 65 % of the recorded cases (CENTOFANTE et al. 2002b). In the majority of simple systems there are marked differences in the size and shape of the Y or W chromosome. However, the number of systems with minor morphological differences may be underrepresented, because their detection may require special chromosome analyses (ALMEIDA-TOLEDO et al. 1984; HAAF and SCHMID 1984; NANDA et al. 1990; SOLA et al. 1990; MOREIRA-FILHO et al. 1993).

Sex chromosome systems of the ZZ/ZW type have been described for 3 *Characidium* species. MAISTRO et al. (1998) first described a sex chromosome heteromorphism in two populations identified as "*Characidium* cf. *fasciatum*" from the Paranapanema basin (Paraná-Plata system). Based on external morphology we identify most of the specimens analysed by MAISTRO et al. (1998) as *Characidium* sp. cf. C. *gomesi* (see Matherial and Methods, above). In this species the sex chromosomes are represented by pair 19, and have the same shape (submetacentric) and size. In *Characidium gomesi* from the headwaters of the Rio Grande basin (Paraná-Plata system) on northwestern slope of the Serra da Mantiqueira, the Z chromosome is a metacentric member of pair 2, and the W chromosome is a smaller submetacentric chromosome (CENTOFANTE et al. 2001). In *Characidium* sp. cf. *C. alipioi* (current study) the Z and W chromosomes have also the same size and shape (metacentric), but correspond to pair 1. In these three cases the W chromosome is distinguished because it is completely heterochromatic after C-banding.

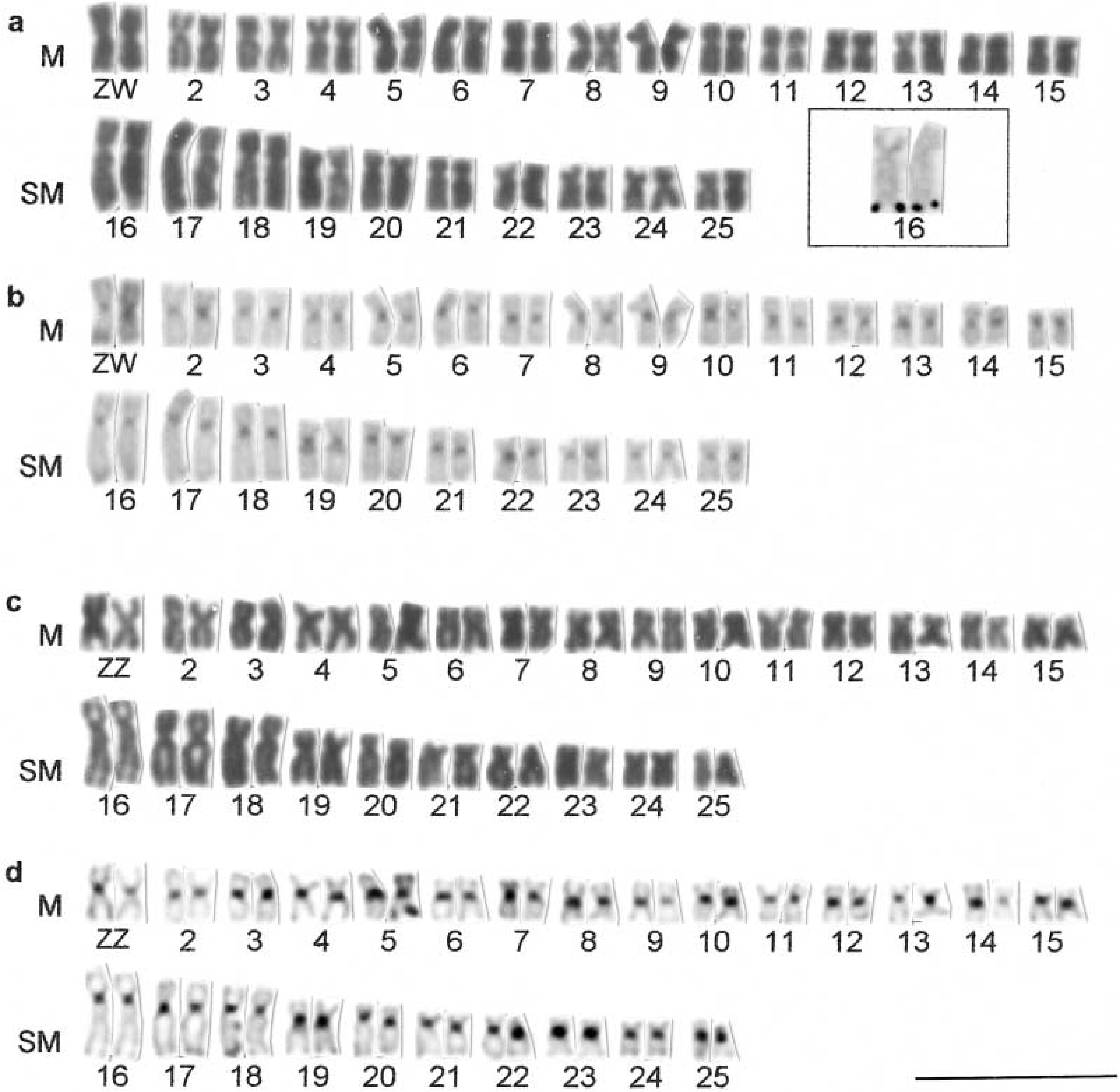

Fig. 1. Karyotypes of *Characidium* sp. cf. *C. alipioi*, with **a:** conventional Giemsa staining and **b:** C-banding of females and **c:** conventional Giemsa staining and **d:** C-banding of males. The box surrounds the NOR-carrying pair. The bar is equivalent to 5μm.

Characidium gomesi belongs to a relatively derived monophyletic subset of Characidiinae that also includes *C. fasciatum* (BUCKUP 1992, 1993b). *Characidium* sp. cf. *C. alipioi* is very similar to *C. gomesi*, differing only in minor morphometric and coloration details, and is likely to belong to the same monophyletic group. MAISTRO et al. (1998) referred to the two local populations from the Paranapanema as "*Characidium* cf. *fasciatum*", also implying membership in that group. The distribution of ZW sex chromosome systems with a completely heterochromatic W chromosome suggests that within the Characidiinae this kind of mechanism is restricted to a relatively small subset of species closely related to *C. gomesi*. On the other hand, that system is absent in other species of *Characidium* so far studied, including populations provisionally assigned to *C. zebra* (MIYAZAUA and GALETTI JR. 1994; MAISTRO et al. 1998; CENTOFANTE et al. 2001). Species with a fully scaled breast and isthmus area such as *C. zebra* are morphologically primitive and occupy a basal position in the phylogeny of *Characidium* (BUCKUP

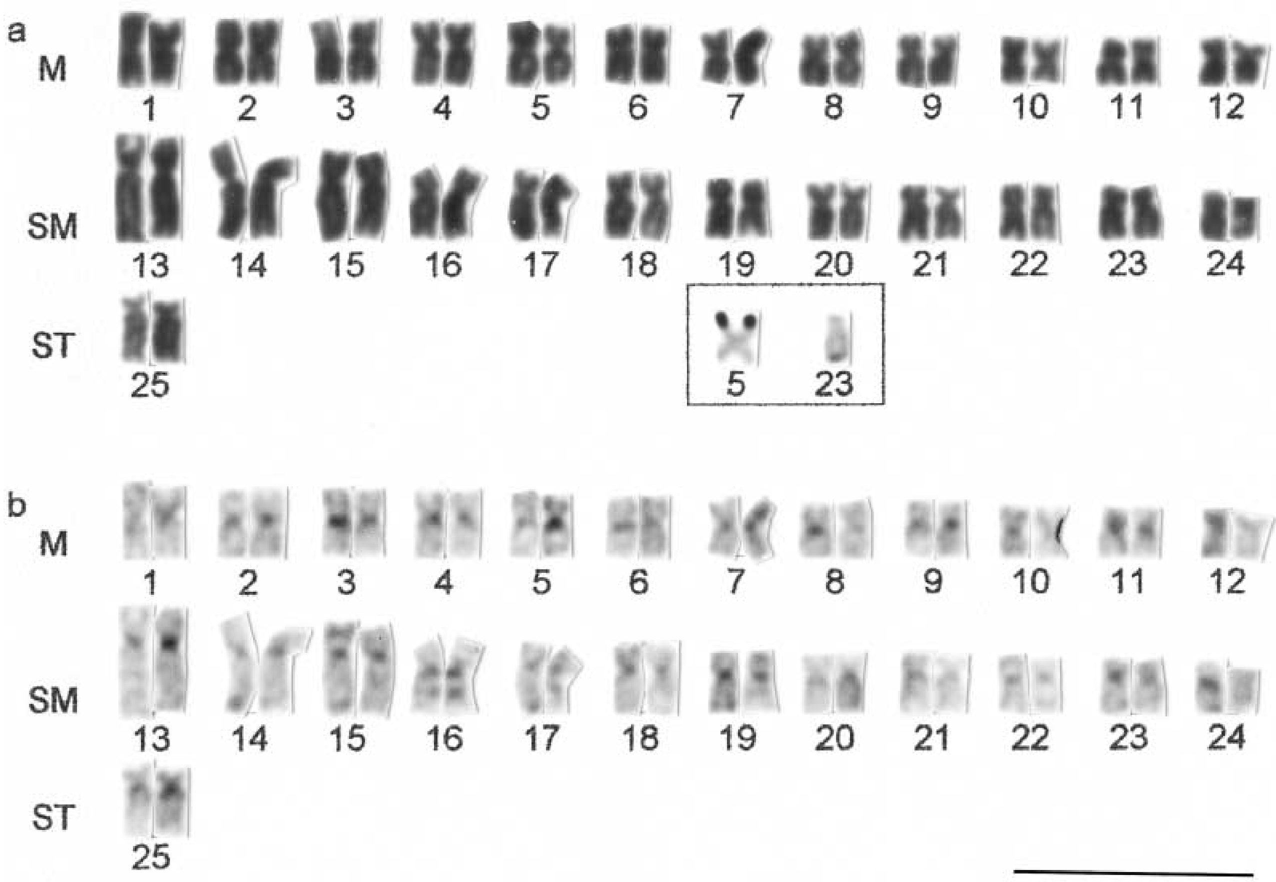

Fig. 2. Karyotypes of *Characidium lauroi* with **a:** conventional Giemsa staining and **b:** C-banding. The box surrounds the NOR-carrying chromosomes. The bar is equivalent to 5μm.

1993b). The lack of a differentiated sex chromosome system is therefore a plesiomorphic condition for the genus *Characidium*, while the ZW sex system seems to be a synapomorphy of a relatively restricted subset of species that includes at least some members of Clade 2 as diagnosed by BUCKUP (1993b).

Evolutionary differentiation in ZW sex chromosome systems may be associated with biogeographic barriers. The forms exhibiting a completely heterochromatic W chromosome are allopatric relative to each other. The population from the Paraíba do Sul drainage (present study) is currently isolated from the two populations from the La Plata basin by the Mantiqueira mountain range in the north and the Arujá rise in the south. The Paranapanema and Grande drainage both belong to the La Plata basin, but their headwaters are widely separated by intervening ridges and basins.

Female chromosome heteromorphism usually involves an increase in size of the W chromosome due to the accumulation of heterochromatin relative to the Z chromosome (CENTOFANTE et al. 2001). The W chromosome of *C. gomesi* from the Rio Grande basin (Paraná-Plata system), however, is considerably smaller than the corresponding Z chromosome (CENTOFANTE et al. 2001). Among neotropical fish such form of sex heteromorphism is known only in the characid genus *Triportheus*. In this genus all species studied exhibit a ZZ/ZW sex system and the size and shape of the W chromosome is quite variable among species when compared to the Z chromosome and the general karyotype macrostructure (BERTOLLO and CAVALLARO 1992; ARTONI et al. 2001). The difference in size between the sex chromosomes of *C. gomesi* in the sample from the Rio Grande basin (Paraná-Plata system) is more parsimoniously interpreted as an autapomorphy, because that species is unrelated phylogenetically to *Triportheus* (BUCKUP 1998), and *C. gomesi* is more closely related to populations with equal-sized sex chromosomes.

The Ag-NORs in species of *Characidium* with sex chromosomes occur in the terminal region of the long arm of a large submetacentric, presumably homeologous chromosome. However, in other species of *Characidium* the Ag-NORs are located in other types of chromosomes and in different arrangements. In populations of *C.* cf. *zebra*, the Ag-NORs are located in interstitial regions of the long arm of a small submetacentric chromosome pair (Miyazaua and Galetti Jr. 1994; Centofante et al. 2001). In *C. lauroi* there are multiple Ag-NORs located in small meta-submetacentric chromosomes (this study). These data are consistent with the hypothesis that species of *Characidium* with ZZ/ZW sex chromosome systems are more closely related among themselves than to species without such systems.

The occurrence of relatively closely related species with and without differentiated sex chromosomes living in syntopy or sympatry is suggestive that these systems may be an important mechanism in the maintenance of genetic isolation between coexisting species. Such occurrence has been observed in *Apareiodon* (Moreira-Filho et al. 1985), *Leporinus* (Galetti Jr. et al. 1995), *Hoplias* (Scavone et al. 1994; Bertollo et al. 2000), *Characidium* (Centofante et al. 2001; present study) and *Parodon* (Centofante et al. 2002b).

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REFERENCES

Almeida-Toledo, L. F., Foresti, F. and Toledo-Filho, S. A. 1984. Complex sex chromosome system in *Eigenmannia* sp. (Pisces, Gymnotyformes). – Genetica 64: 165–169.

Artoni, R. F., Falcão, J. N., Moreira-Filho, O. and Bertollo, L. A. C. 2001. An uncommon condition for a sex chromosome system in Characidae fish. Distribution and differentiation of ZZ/ZW system in *Triportheus*. – Chromosome Res. 9: 449–456.

Bertollo, L. A. C. and Cavallaro, Z. I. 1992. A highly differentiated ZZ/ZW sex chromosome system in a Characidae fish, *Triportheus guentheri*. – Cytogenet. Cell Genet. 60: 60–63.

Bertollo, L. A. C., Takahashi, C. S. and Moreira-Filho, O. 1978. Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). – Rev. Bras. Genet. 1: 103–120.

Bertollo, L. A. C., Born, G. G., Fenocchio, A. S. and Moreira-Filho, O. 2000. A biodiversity approach in neotropical Erythrinidae fish, *Hoplias malabaricus*. Karyotypic survey, geografic distribution of cytotypes and cytotaxonomic considerations. – Chromosome Res. 8: 603–613.

Buckup, P. A. 1992. Redescription of *Characidium fasciatum*, type species of the Characidiinae (Teleostei, Characiformes). – Copeia 4: 1066–1073.

Buckup, P. A. 1993a. The monophyly of Characidiinae, a neotropical group of Characiformes fishes (Teleostei, Ostariophysi). – Zool. J. Linn. Soc. 108: 225–245.

Buckup, P. A. 1993b. Phylogenetic interrelationships and reductive evolution in neotropical Characidiin fishes (Characiformes, Ostariophysi). – Cladistics 9: 305–341.

Buckup, P. A. 1993c. Review of characidiin fishes (Teleostei: Characiformes), with descriptions of four new genera and ten new species. – Ichthyol. Explor. Freshw. 4: 97–154.

Buckup, P. A. 1998. Relationships of the Characidiinae and phylogeny of characiform fishes (Teleostei: Ostariophysi). – In: Malabarba, L. R., Reis, R. E., Vari, R. P. et al. (eds), Phylogeny and classification of neotropical fishes. EDIPUCRS, Porto Alegre, pp. 123–144.

Centofante, L., Bertollo, L. A. C. and Moreira-Filho, O. 2001. Comparative cytogenetics among sympatric species of *Characidium* (Pisces, Characiformes). Diversity analysis with the description of a ZW sex chromosome system and natural triploidy. – Caryologia 55: 253–260.

Centofante, L., Porto, J. I. R. and Felberg, E. 2002a. Chromosomal polymorphism in *Serrasalmus spilopleura* Kner, 1858 (Characidae, Serrasalminae) from Central Amazon Basin. – Caryologia 55: 37–45.

Centofante, L., Bertollo, L. A. C. and Moreira-Filho, O. 2002b. A ZZ/ZW sex chromosome system in a new species of the genus *Parodon* (Pisces, Parodontidae). – Caryologia 55: 139–150.

Galetti Jr., P. M., Lima, N. R. W. and Venere, P. C. 1995. A monophyletic ZW sex chromosome system in *Leporinus* (Anostomidae, Characiformes). – Cytologia 60: 375–382.

Haaf, T. and Schmid, M. 1984. An early stage of ZZ/ZW sex chromosome differentiation in *Poecilia sphenops* var. *melanistica* (Poeciliidae, Cyprinodontiformes). – Chromosoma 89: 37–41.

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.

Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosomes. – Hereditas 52: 201–220.

Maistro, E. L., Mata, E. P., Oliveira, C. and Foresti, F. 1998. Unusual occurrence of a ZZ/ZW sex chromosome system and supernumerary chromosomes in *Characidium* cf. *fasciatum* (Pisces, Characiformes, Characidiinae). – Genetica 104: 1–7.

Melo, M. R. and Buckup, P. A. 2002. *Characidium stigmosum* (Characiformes: Crenuchidae): a new species of characidiin fish from central Brazil. – Copeia 4: 988–993.

Miyazaua, C. S. and Galetti Jr., P. M. 1994. First cytogenetical studies in *Characidium* species (Pisces, Characiformes, Characidiinae). – Cytologia 59: 73–79.

Moreira-Filho, O., Bertollo, L. A. C. and Galetti Jr., P. M. 1985. Karyotypic study of some species of family Parodontidae (Pisces, Cypriniformes). – Caryologia 38: 47–55.

Moreira-Filho, O., Bertollo, L. A. C. and Galetti Jr., P. M. 1993. Distribution of sex chromosome mechanism in neotropical fish and description of a ZZ/ZW system in *Parodon hilarii* (Parodontidae). – Caryologia 46: 115–125.

Nanda, I., Feichinger, W., Schmid, M. et al. 1990. Simple repetitive sequences are associated with differentiation of the sex chromosomes in guppy fish. – J. Mol. Evol. 30: 456–462.

Presswell, B., Weitzman, S. H. and Bergquist, T. 2000. *Skiotocharax meizon*, a new genus and species of fish from Guyana with a discussion of its relationships (Characiformes: Crenuchidae). – Ichthyol. Explor. Freshw. 11: 175–192.

Scavone, M. D., Bertollo, A. C. and Cavallini, M. M. 1994. Sympatric occurrence of two karyotypic forms of *Hoplias malabaricus* (Pisces, Erythrinidae). – Cytobios 80: 223–227.

Sola, L., Monaco, P. J. and Rasch, E. M. 1990. Cytogenetics of bisexual/unisexual species of *Poecilia*. I. C-bands, Ag-NORs polymorphisms and sex chromosomes in three populations of *Poecilia latipinna*. – Cytogenet. Cell Genet. 53: 148–154.

Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. – Exp. Cell Res. 75: 304–306.

Zarske, A. 1997. *Gerychthys sterbai* gen. Et spec. nov. und *Microcharacidium geryi* spec. nov.: Beschreibung einer neuen Gattung und zweier neuer Arten von Bodensalmlern aus dem Einzugsgebiet des rio Ucayali in Peru (Teleostei: Ostariophysi: Characiformes: Characidae). – Zool. Abh. Mus. Tierkd. 49: 157–172.

Zarske, A. and Géry, J. 2001. Beschreibung von drei neuen Arten der Gattung *Characidium* Reinhardt, 1866 aus Bolivien und Paraguay (Teleostei: Characiformes: Characidiinae). – Zool. Abh. Mus. Tierk. 51: 229–246.