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Interspecific chromosomal divergences in the genus *Characidium* (Teleostei: Characiformes: Crenuchidae)

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Karyotypes of seven fish species of the genus *Characidium*, three of them studied for the first time, were characterized using conventional cytogenetic techniques (Giemsa staining, Ag-NOR, and C-banding). All species presented a diploid number of 2n=50, with only metacentric and submetacentric chromosomes, as observed in all *Characidium* species studied. In two species cells with one to three B chromosomes were observed. All species analyzed have a single NOR-bearing chromosome pair with morphological differences among them. *Characidium* cf. *zebra* shows heterochromatic blocks restricted to the pericentromeric regions of all chromosomes denoting the absence of a sex chromosome system. On the other hand, the species *Characidium lanei*, *C. pterostictum*, *C. lauroi*, *C. oiticicai*, *C. schubarti*, and *Characidium* sp., besides presenting pericentromeric heterochromatic blocks, exhibited large interstitial and/or terminal heterochromatic blocks, and a ZZ/ZW sex chromosome system. The constitutive heterochromatin seems to play a relevant role in the chromosome differentiation process of the studied species, mainly in relation to the sex chromosomes. The geographical isolation of the rivers in which the species were sampled, associated with their way of life restricted to headwaters environments, may have favored the process of fixation of different karyotypes found in each of the analyzed species.

Os cariótipos de sete espécies de peixes do gênero *Characidium*, três estudadas pela primeira vez, foram caracterizados com o uso das técnicas citogenéticas convencionais (Giemsa, Ag-RONs e bandamento-C). Todas as espécies apresentaram número diplóide de 2n=50 cromossomos, com predominância de cromossomos dos tipos meta e submetacêntricos. Nesse estudo foi também observada a presença de até três cromossomos B em células de duas espécies, *C. oiticicai* e *C. pterostictum*. O bandamento C e o tratamento com nitrato de prata revelaram significativas diferenças nos cariótipos das espécies analisadas. A espécie *Characidium* cf. *zebra* apresenta heterocromatina restrita às regiões pericentroméricas dos cromossomos e ausência de heteromorfismos cromossômicos relacionados à diferenciação sexual, enquanto as espécies *Characidium lanei*, *C. pterostictum*, *C. lauroi*, *C. oiticicai*, *C. schubarti* e *Characidium* sp., evidenciaram, além de blocos pericentroméricos também observados em *Characidium* cf. *zebra*, grandes blocos heterocromáticos intersticiais e/ou terminais e sistema cromossômico de diferenciação sexual do tipo ZZ-ZW. A heterocromatina constitutiva parece exercer papel relevante no processo de diferenciação cromossômica destas espécies, principalmente em relação à diferenciação de cromossomos sexuais. O isolamento geográfico dos rios em que essas espécies foram amostradas, bem como o seu modo de vida restrito às regiões de cabeceira, podem ter favorecido o processo de diferenciação cromossômica e a fixação dos cariótipos particulares encontrados em cada uma das espécies analisadas.

Key words: Karyotypic evolution, Sex chromosomes, B-chromosomes, Ag-NORs.

Introduction

Fish represent the sister group to modern vertebrates and most species are gonochoristic and do not present differentiated sex chromosomes (Devlin & Nagahama, 2002) or are hermaphrodite. Polygenic sex determination may occur in most species of this group of organisms and is considered a basal mechanism among the sex determination systems (Ohno, 1974). However, fish species that present morphologically differentiated sex chromosomes, mainly those found in the Neotropical region (Oliveira *et al*., 2007), reveal a great variability of systems. The differentiated sex chromosomes may have developed independently in several lineages of Neotropical fishes, because their occurrence is sporadic and probably recent in certain groups. In some families, such as Erythrinidae and Parodontidae, different heteromorphic sex chromosome systems were identified among their representatives (Moreira-Filho *et al*., 1993; Born & Bertollo, 2000).

Characidium, the most specious genus of the subfamily

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Characidiinae (Crenuchidae), comprises about 50 species with a wide distribution in the freshwaters of the Neotropical region located between Panama and Argentina (Buckup, 2003). The phylogenetic position of this group has recently changed, since Characidiinae was considered a member of Hemiodontidae, a subfamily of Characidae, and also an independent family. A cladistic analysis suggested that Characidiinae and Crenuchinae belong to the monophyletic family Crenuchidae (Buckup, 1998).

Cytogenetic studies in this group are scarce, recent, and limited to some species of the genus *Characidium* (Miyazawa & Galetti Jr., 1994; Maistro *et al*., 1998, 2004; Centofante *et al*., 2001, 2003; Silva & Maistro, 2006; Vicari *et al*., 2008; Noleto *et al*., 2009). The analyzed species present a conserved karyotypic macrostructure, showing a constant diploid number of 2n=50 chromosomes and a predominance of metacentric and submetacentric chromosomes. On the other hand, the identification of a sex determination system of the ZZ/ZW type (Maistro *et al*., 1998, 2004), the occurrence of supernumerary chromosomes (Maistro *et al*., 1998, 2004), the presence of NORs (nucleolus organizing region) in different chromosomes pairs (Maistro *et al*., 1998, 2004), and even the occurrence of triploid individuals (Centofante *et al*., 2001) ascertain the great karyotypic variability of *Characidium*.

The purpose of the present study was to enhance the knowledge of the cytogenetic structures of species of the genus *Characidium*, describing and comparing the karyotypic constitution of seven species occurring in the Southern and Southeastern Brazil. The data obtained are used to propose a new hypothesis about karyotypic differentiation and diversification processes within this fish group.

Material and Methods

Seven fish species belonging to the genus *Characidium*, captured in different river basins in Southern and Southeastern Brazil, were analyzed (Fig. 1, Table 1). The individuals were fixed in 10% formaldehyde, conserved in 70% Ethanol, and after identification, deposited in the fish collection of Laboratório de Biologia e Genética de Peixes (LBP), UNESP-Botucatu, São Paulo, Brazil (Table 1).

Mitotic chromosome preparations were obtained from renal tissue and gills using the "air-drying" technique (Foresti *et al*., 1981). Metaphase chromosomes were analyzed under an optical photomicroscope (Olympus BX61) and the images were captured by Image Pro Plus, 6.0 software (MediaCybernetics). The chromosome morphology was determined according to the arm ratio proposed by Levan *et al*. (1964). Chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a) and arranged in the karyotype by type in decreasing order of size. The analysis of NORs by silver nitrate staining followed the technique proposed by Howell & Black (1980) and C-banding was accomplished following the protocol described by Sumner (1972).

Characidium zebra appears as a widespread and polymorphic species usually identified in the literature by the incorrect name of *C. fasciatum* (Buckup, 2003). The type locality of *C. zebra* is the Maripicru Creek, a branch of the Ireng River, Guyana, and there is no study defining the limits of its occurrence (Buckup & Reis, 1997). In the present study, individuals morphologically similar to *Characidium zebra* were provisionally called *Characidium* cf. *zebra*.

Results

The cytogenetic analysis of *Characidium* showed a conserved diploid number of 2n=50, with a predominance of metacentric and submetacentric chromosomes (Figs. 2-5). In *Characidium* cf. *zebra*, the first metacentric chromosome pair was the largest among the karyotypes analyzed, showing a significative difference in size when compared with the second pair. Other species analyzed presented the first two metacentric pairs with similar size, and *C. pterostictum* also presented a pair of acrocentric chromosomes. Except for *C.* cf. *zebra*, all the species presented heteromorphisms involving the chromosomes of pair 2 in females, while in males, this chromosome pair was homomorphic. Two species presented B chromosomes that ranged from zero to three in *C. oiticicai*, and from zero to two in *C. pterostictum* (Figs. 2c and 3), which showed to be consistent within each individual.

C-banding revealed significant differences in heterochromatin patterns among the species. *Characidium* cf. *zebra* presented a small amount of constitutive heterochromatin restricted to the pericentromeric areas of all chromosomes (Fig. 2b). Besides the pericentromeric heterochromatin observed in *C.* cf. *zebra*, the other six species presented large interstitial and/or terminal blocks and a sex differentiation chromosome system of the ZZ/ZW type, represented by the heteromorphic chromosome pair number

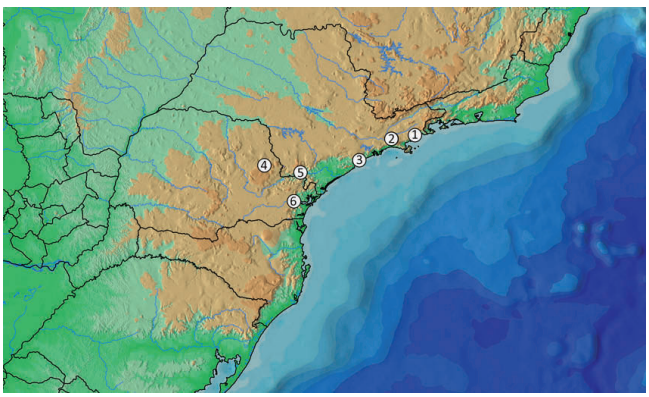

Fig. 1. Partial map of South America showing the *Characidium* species collection sites. 1 = Ubatuba, SP (*Characidium lauroi*), 2 = Salesópolis, SP (*Characidium oiticicai* and *Characidium* cf. *zebra*), 3 = Itanhaém, SP (*Characidium* sp.), 4 = Jaguariaíva, PR (*Characidium schubarti*), 5 = Apiaí, SP (*Characidium pterostictum*), 6 = Morretes, PR (*Characidium lanei*).

Table 1. Specimens of *Characidium* analyzed in this study. LBP = catalog number of vouchers; F = female; M = male; SP = São Paulo State; MG = Minas Gerais State; PR = Paraná State.

Species	Map Point	LBP	Sample Localities	F	M	Coordinates
C. lauroi	1	8741	tributary of rio Grande, Ubatuba, SP	4	1	23°23'42"S 45°07'17"W
C. oiticicai	2	8703	rio Paraitinga, Salesópolis, SP	15	5	23°30'40"S 45°51'32"W
C. cf. *zebra*	2	8704	rio Paraitinga, Salesópolis, SP	12	7	23°30'40"S 45°51'32"W
Characidium sp.	3	6818	tributary of rio Preto, Itanhaém, SP	18	8	24°10'39"S 46°50'56"W
C. schubarti	4	8702	rio Cinco Réis, Jaguariaiva, PR	10	7	25°17'46"S 49°44'56"W
C. pterostictum	5	7367	rio Betari, Apiaí, SP	15	5	24°33'03"S 48°40'49"W
C. lanei	6	8700	córrego Cari, Morretes, PR	11	7	25°26'29"S 48°32'28"W

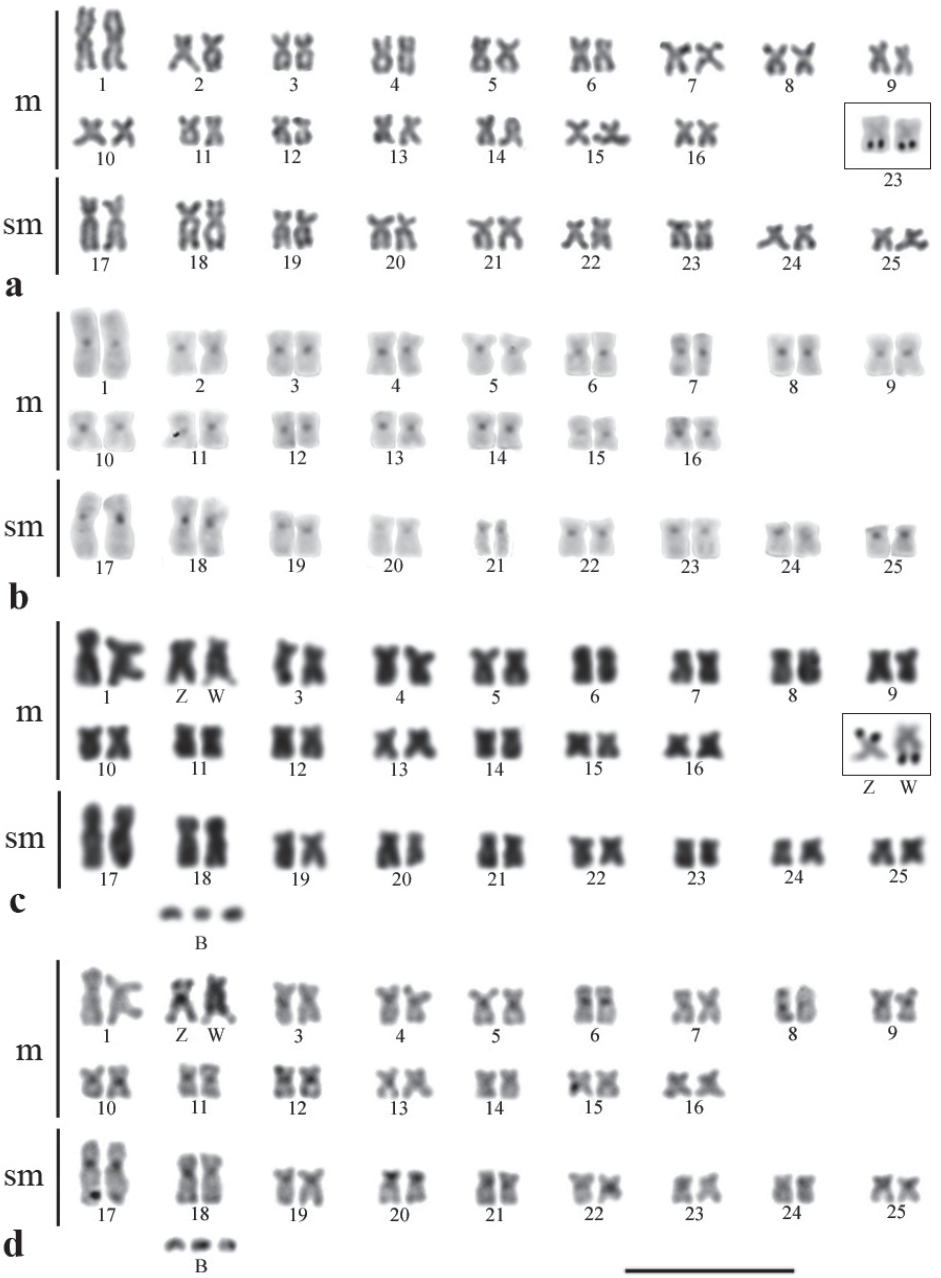

Fig. 2. Karyotypes of female specimens of *Characidium* cf. *zebra* and *Characidium oiticicai* from Salesópolis, SP, after conventional Giemsa staining (a, c) and C-banding (b, d), respectively. Note the differential patterns of heterochromatic blocks on the chromosomes and the differentiated sex chromosomes and heterochromatic B-chromosomes in *Characidium oiticicai*. In the box, the NOR-bearing chromosomes. Scale bar = 10 μm.

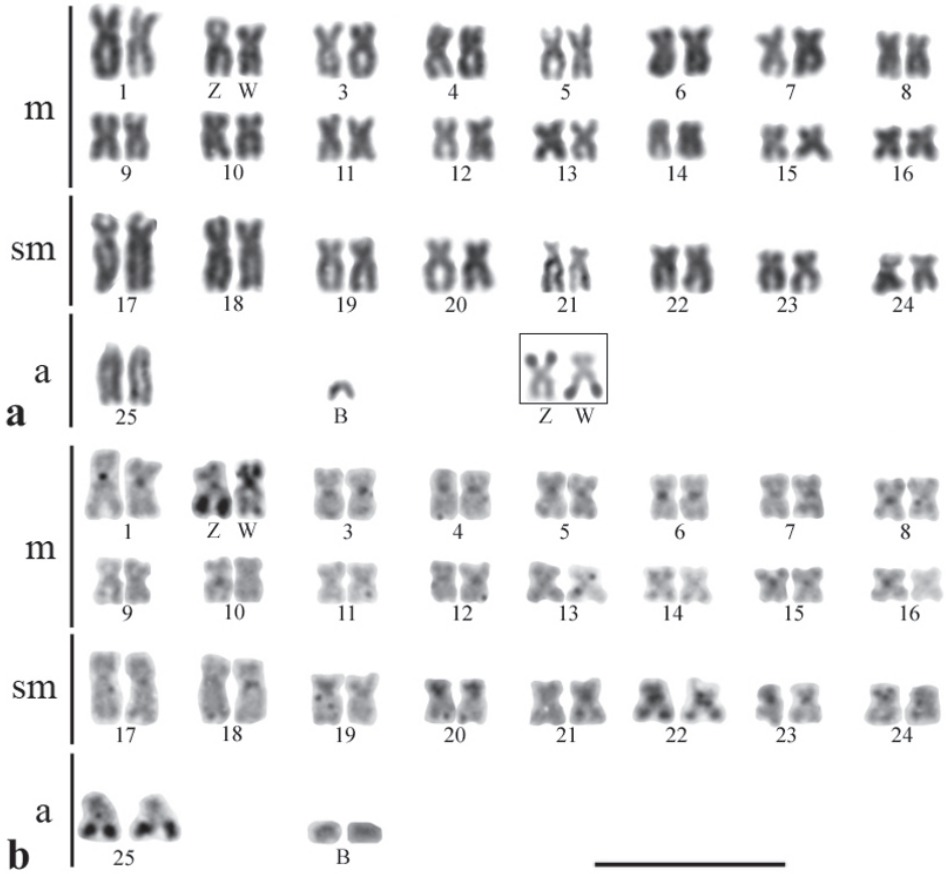

Fig. 3. Karyotypes of female specimens of *Characidium pterostictum* from Apiaí, SP, after conventional Giemsa staining (a) and C-banding (b). Note the differential patterns of heterochromatic blocks on the Z and W chromosomes and the heterochromatic B-chromosomes In the box, the Z and W chromosomes carrying the NORs in an inverted position. Scale bar = 10 μm.

2 observed in females of these species (Figs. 2c-d, Figs. 3-5).

The exclusive acrocentric chromosomes found in *C. pterostictum* showed large heterochromatic blocks in the terminal position (Fig. 3b). B chromosomes of *C. oiticicai* and *C. pterostictum* were shown to be partially heterochromatic (Figs. 2d and 3b).

The silver nitrate staining demonstrated that all the species of *Characidium* under analysis possess single NORs (Table 2; highlighted in Figs. 2-5). *Characidium* cf. *zebra* presented two marks at the final position on the long arm of pair 23 (Fig. 2a). The other species presented ribosomal sites associated with the sex chromosomes, Z chromosome has the NORs at the terminal position on the short arms, and the W chromosome has the NORs located at the terminal position on the long arms (Fig. 6).

Discussion

In the present study, the karyotypes of *C. oiticicai*, *C. schubarti*, and *Characidium* sp. were studied for the first time, increasing the number of species karyotyped in the genus to ten. The species studied presented a conserved karyotypic macrostructure, mainly in relation to their diploid number and the presence of metacentric and submetacentric chromosomes. Nevertheless, the presence of B chromosomes in *C. pterostictum* and *C. oiticicai*, the occurrence of sex chromosomes in most of the analyzed species, the occurrence of NORs associated with sex chromosomes, and the occurrence of a pair of acrocentric chromosomes in *C. pterostictum* (Table 2) reinforce the evidence already postulated for some species of *Characidium*, which suggested the existence of a great chromosome variability among the representatives of this genus (Maistro *et al*., 1998, 2004; Centofante *et al*., 2001, 2003; Vicari *et al*., 2008; Noleto *et al*., 2009).

The species *Characidium zebra* is considered morphologically basal in the phylogeny of *Characidium*, presenting several plesiomorphic characters (Buckup, 1993). In that sense, the karyotype of this species could also be characterized as basal for the group under study and chromosome variations observed in the other species could be explained by the occurrence of several structural chromosome rearrangements. Thus, the acrocentric pair found in *C. pterostictum* could have arisen from the event of a pericentric inversion in a submetacentric chromosome pair, followed by the accumulation of heterochromatin in the region

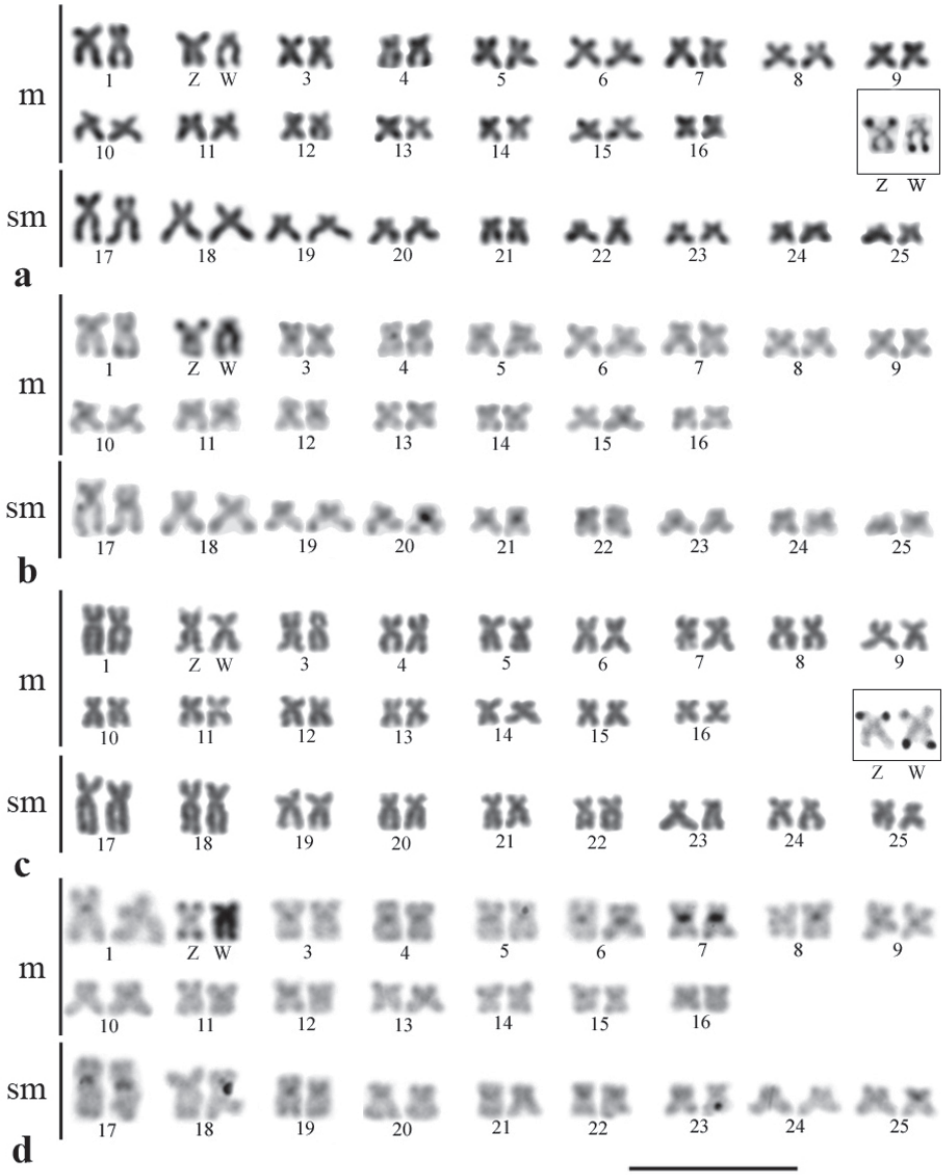

Fig. 4. Karyotypes of female specimens of *Characidium* sp. from Itanhaém, SP, and *Characidium schubarti* from Jaguariaiva, PR, after conventional Giemsa staining (a, c) and C-banding (b, d), respectively. Note conspicuous heterochromatic blocks in the centromeric region of some chromosome pairs and the differentiated sex chromosomes, being the W almost completely heterochromatic in both species. In the box, the Z and W chromosomes carrying the NORs in an inverted position. Scale bar = 10 μm.

where the rearrangement occurred. The size variation observed between pairs 1 and 2 of *Characidium* cf. *zebra,* which are not found in the remaining species, could be related to the formation of sex chromosomes identified as the second pair in those species, where the process of heterochromatinization of both Z and W resulted in an increase in size of these chromosomes.

Maistro *et al*. (1998, 2004) had previously described the occurrence of sex chromosome systems of the ZZ/ZW type in *Characidium* sp. aff. *C. gomesi*, cited as *Characidium* cf. *fasciatum*, collected in a tributary of the Paranapanema River. In this population, sex chromosomes were represented by submetacentric chromosomes of pair 19 and the Z chromosome, besides showing identical size and morphology, presented a pericentromeric heterochromatic block, while chromosome W was completely heterochromatic. Centofante *et al*. (2001) reported a second case of sex chromosome heteromorphism in *Characidium gomesi* among samples captured at the Paiol Grande Stream, a tributary of the rio Grande basin, in Serra da Mantiqueira. In this species, the Z chromosome was identified as a metacentric chromosome of pair number 2, with pericentromeric heterochromatin, while the W chromosome was described as a small, submetacentric, and completely heterochromatic chromosome. In

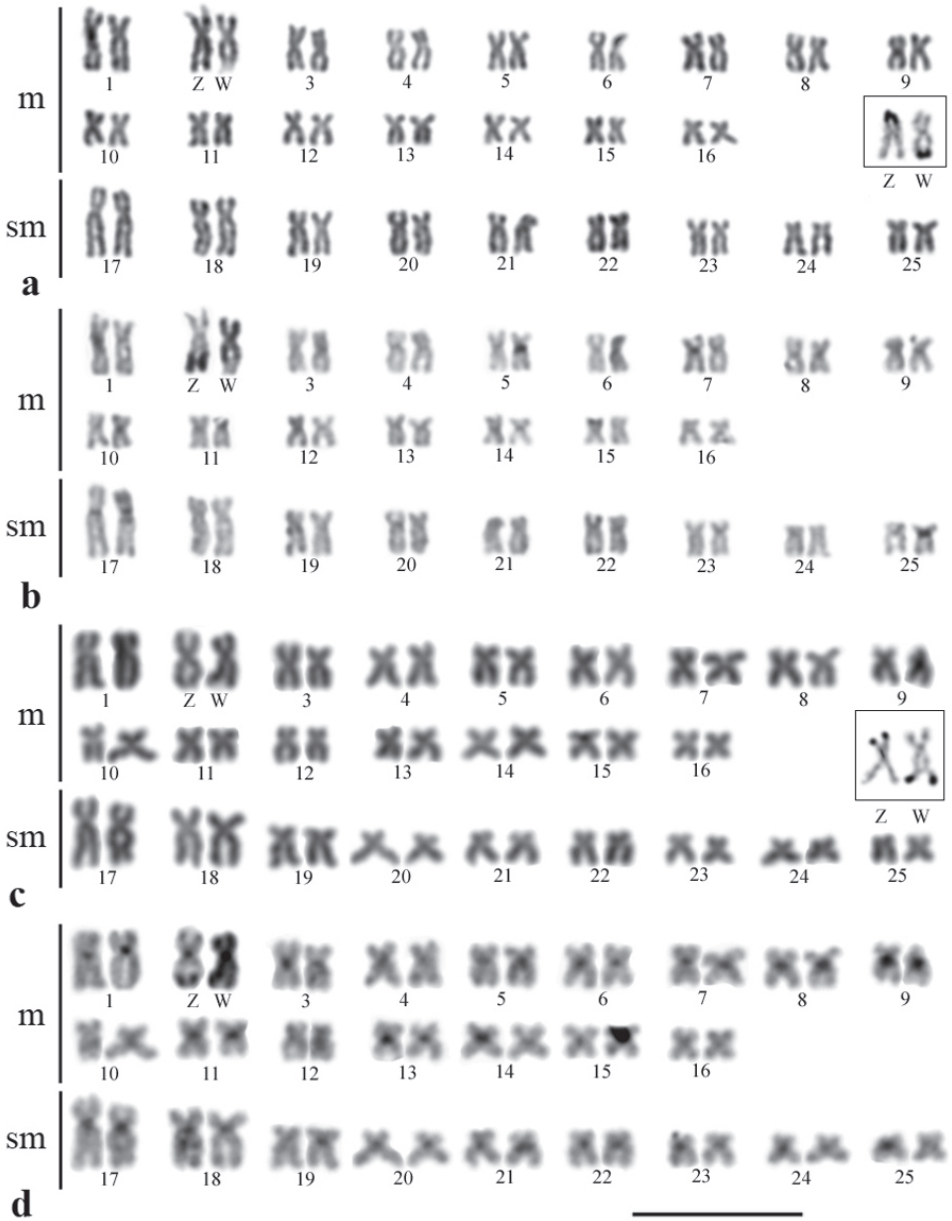

Fig. 5. Karyotypes of the female specimen of *Characidium lauroi* from Ubatuba, SP, and *Characidium lanei* from Morretes, PR, after conventional Giemsa staining (a, c) and C-banding (b, d), respectively. Note the different patterns of heterochromatic blocks on the chromosomes and the differentiated sex chromosomes, being the W almost completely heterochromatic in both species. In the box, the Z and W chromosomes carrying the NORs in an inverted position. Scale bar = 10 μm.

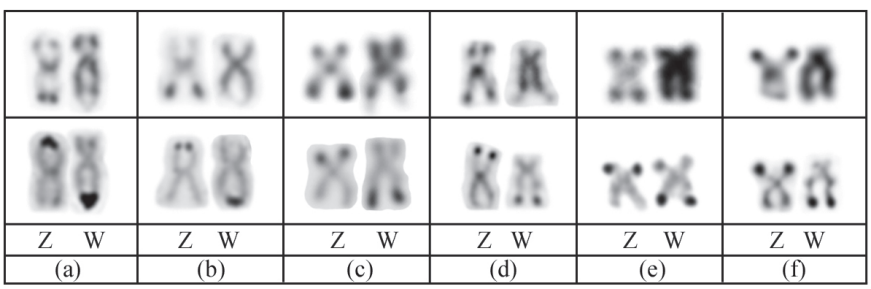

Fig. 6. Z an W sex chromosomes of the *Characidium* species analyzed in this study, after C-banding (first row) and silver nitrate staining (second row). Note the different distribution of the heterochromatin, mainly in the W chromosome in (a) *C. schubarti*, (b), *Characidium* sp., (c) *C. pterostictum*, (d) *C. oiticicai*, (e) *C. lanei*, and (f) *C. lauroi*.

Characidium sp. cf. *C. alipioi*, the Z chromosome presented pericentromeric and telomeric heterochromatin and the W chromosome was totally heterochromatic (Centofante *et al*., 2003). In this species, sex chromosomes also present the same size and morphology and correspond to the chromosomes of pair number 1 in the karyotype. The analysis of *Characidium* cf. *gomesi* from the Quebra Perna Stream, Tibagi River basin, conducted by Vicari *et al*. (2008) registered another occurrence of ZZ/ZW chromosomes, where Z was represented by a metacentric chromosome with pericentromeric heterochromatin and W by a subtelocentric chromosome totally heterochromatic.

The occurrence of a simple sex determination system originating from a differential heterochromatinization process in one of the sex chromosomes is a common event among fishes (Oliveira *et al*., 2007). The heterochromatinization process, when observed in one of the chromosomes of the previously homomorphic pair in a region adjacent to the sexual determining sequence, enables the suppression of recombination events and favors the independent evolution of this chromosome and/or chromosome region (John, 1988; Rice, 1996; Steinemann & Steinemann, 1992, 2001). Vicari *et al*. (2008) consider the existence of a protosex chromosome pair for some *Characidium* species and, both the Z and W chromosomes would have undergone shape and size modifications, probably due to duplications, deletions and/or inversions, in relation to the putative ancestor condition. Thus, the heterochromatinization mechanism appears to play an important role in the differentiation of W chromosome from the protosex chromosome among populations/species

Table 2. Cytogenetic data available for the genus *Characidium* (2n=50 chromosomes). Ref. = 1. Miyazawa & Galetti Jr. (1994); 2. Maistro *et al*. (1998); 3. Silva & Maistro (2006); 4. Centofante *et al*. (2001); 5. Centofante *et al*. (2003); 6. Vicari *et al*. (2008); 7. Noleto *et al*. (2009); 8. Present study. SC = Sex Chromosomes System; NOR Position on Chromosomes T = terminal. I = interstitial; BC = Number of B Chromosomes; SP = São Paulo State; MG = Minas Gerais State; PR = Paraná State.

Species	Sample Localities	SC	Ag-NOR-bearing pair	Hydrographic system	BC	Karyotypic formula	Ref.
C. sp. cf. *C. alipioi*	córrego Ribeirão Grande, SP	ZZ/ZW	16 (T)	Paraíba do Sul River	-	males 30m+20sm females 30m+20sm	5
C. sp. aff. *C. gomesi*	rio Pardo, SP	ZZ/ZW	17 (T)	Paranapanema River	1-4	males 32m+18sm females 32m+18sm	2
C. sp. aff. *C. gomesi*	rio Quinta, SP	ZZ/ZW	17 (T)	Paranapanema River	1-4	males 32m+18sm females 32m+18sm	2
C. gomesi	rio Machado, MG	-	17 (T)	Grande River	-	males 32m+18sm females 32m+18sm	3
C. gomesi	córrego Paiol Grande, SP	ZZ/ZW	18 (I)	Grande River	-	males 32m+18sm females 31m+19sm	4
C. cf. *gomesi*	rio Quebra Perna, PR	ZZ/ZW	multiple	Tibagi River	-	males 32m+18sm females 31m+18sm+1st	6
C. lagosantensis	rio Mogi-Guaçu, SP	-	-	Mogi-Guaçu River	-	males 32m+18sm females 32m+18sm	1
C. lanei	rio Barroca	ZZ/ZW	2 (T)	coastal rivers		males 32m+16sm+2a females 31m+17sm+2a	7
C. lanei	córrego Cari, PR	ZZ/ZW	2 (T)	coastal rivers	-	males 32m+ 18sm females 31m+ 19sm	8
C. lauroi	córrego Ribeirão Grande, SP	-	5 and 23	Paraíba do Sul River	-	males 24m+24sm+2st females 24m+24sm+2st	5
C. lauroi	tributary of rio Grande, SP	ZZ/ZW	2 (T)	Tietê River	-	males 32m+18sm females 31m+18sm+1a	8
C. oiticicai	rio Paraitinga, SP	ZZ/ZW	2 (T)	coastal rivers	1-3	males 32m+18sm females 31m+19sm	8
C. pterostictum	Reserva Jataí, SP	-	-	Ribeira do Iguape River	-	males 32m+16sm+2st females 32m+16sm+2st	1
C. pterostictum	rio Betari, SP	ZZ/ZW	2 (T)	Ribeira do Iguape River	1-2	males 32m+16sm+2a females 32m+16sm+2a	8
C. schubarti	rio Cinco Réis, PR	ZZ/ZW	2 (T)	Paranapanema River	-	males 32m+18sm females 32m+18sm	8
C. cf. *zebra*	Reserva Jataí, SP	-	25 (T)	Ribeira do Iguape River	-	males 32m+18sm females 32m+18sm	1
C. cf. *zebra*	rio Passa Cinco, SP	-	25 (T)	Tietê River	1	males 32m+18sm females 32m+18sm	1
C.cf. *zebra*	rio Piracicaba, SP	-	25 (T)	Tietê River	-	males 32m+18sm females 32m+18sm	1
C. cf. *zebra*	rio Machado, MG	-	23 (T)	Grande River	-	males 32m+18sm females 32m+18sm	3
C.cf. *zebra*	córrego Paiol Grande, SP	-	23 (I)	Grande River	-	males 32m+18sm females 32m+18sm	4
C. cf. *zebra*	rio Paraitinga, SP	-	23 (I)	upper Tietê River	-	males 32m+18sm females 32m+18sm	8
Characidium sp.	tributary of rio Preto, SP	ZZ/ZW	2 (T)	coastal rivers	-	males 32m+18sm females 32m+18sm	8

in an independent pathway. In the present study, the identification and analysis of sexual chromosomes in *C. lanei*, *C. pterostictum*, *C. lauroi*, *C. oiticicai*, *C. schubarti*, and *Characidium* sp., which characterize Z and W heteromorphic chromosome systems in distinct stages of differentiation, supports the propositions postulated by Centofante *et al*. (2001, 2003) and Vicari *et al*. (2008) that heterochromatinization events could have occurred in the ancestors of these species and that sex chromosomes differentiated independently during the speciation process. This could explain the differences found among the level of heterochromatinization in some species analyzed, possibly reinforcing the hypothesis of a common origin for heteromorphic sex chromosomes followed by an independent process of differentiation in the *Characidium* species.

The presence of rDNA segments in sex chromosomes is not a rare event and has been described in some groups of organisms. Cistrons of ribosomal genes 5.8S, 18S, and 28S that form the nucleolar organizer regions in animals and are usually stained with silver nitrate (Ag-NORs), were observed in sex chromosomes, including insects as *Drosophila* (Pelegrini *et al*., 1977), beetles (Juan *et al*., 1993) and flies (Parise-Maltempi & Avancini, 2001); mammals (Yonenaga-Yassuda *et al*., 1983; Oshida *et al*., 1999), and also plant chromosomes (Nakayama *et al*., 2001). Reports on the presence of Ag-NORs in fish sex chromosomes are restricted to *Fundulus diaphanus* (Howell & Black, 1979), *Salvelinus alpinus* (Reed & Phillips, 1997), *Hoplias malabaricus* (Born & Bertollo, 2000), *Triportheus guentheri* (Artoni & Bertollo, 2002), *Triportheus venezuelensis* (Nirchio *et al*., 2007) and *Characidium lanei* (Noleto *et al*., 2009). In the rainbow trout *Oncorhynchus mykiss*, which presents a sex determination system of the XX/XY type, the 5S ribosomal DNA was also observed on the X chromosome (Moran *et al*., 1996).

The occurrence of NORs in Z and W chromosomes of *C. lauroi*, *C. oiticicai*, *C. lanei*, *C. pterostictum*, *C. schubarti*, and *Characidium* sp. could possibly have occurred prior to the process of sexual chromosome differentiation in those species, and even preceded the heterochromatinization of these chromosomes, suggesting that all these sex chromosomes originated once in this group of *Characidium*. Among another group of species composed of *Characidium* sp. cf. *C. alipioi* (Centofante *et al*., 2003), *Characidium gomesi* (Centofante *et al*., 2001), *Characidium* sp. aff. *C. gomesi* (Maistro *et al*., 1998, 2004), and *Characidium* cf. *gomesi* (Vicari *et al*., 2008) Ag-NORs are not related to sex chromosomes and are usually located at the terminal position on the long arms of a large metacentric pair or in a multiple form distributed on several chromosomes.

The variation in the diploid number due to the presence of B chromosomes observed in *C. pterostictum* and *C. oiticicai* has been reported for other species of this genus. Miyazawa & Galetti Jr. (1994) reported the presence of one individual presenting one euchromatic acrocentric B chromosome in *Characidium* cf. *zebra*, among 28 individuals analyzed. Maistro *et al*. (1998, 2004) described the frequent presence of one to four entirely heterochromatic acrocentric B chromosomes in *Characidium* sp. aff. *C. gomesi*. B chromosomes identified in *C. pterostictum* and *C. oiticicai* in the present study showed an irregular constitutive heterochromatin pattern in their structure, which could characterize an intermediate heterochromatinization process or modifications that occurred in the chromosomes during the diversification process.

Different mechanisms have been proposed to explain the origin, differentiation, and maintenance of B chromosomes in different organisms (Jones & Rees, 1982). However, the existence of entirely heterochromatic B chromosomes seems to characterize the heterochromatinization process as a very common event, frequently found in most of the cases reported (Venere *et al*., 1999). Morphological differences and diverse heterochromatic patterns observed in B chromosomes of the *Characidium* species suggest an independent origin process for these chromosomes, followed by specific, internal modifications. Contrarily to the fully heterochromatic chromosomes found in *Characidium* sp. aff. *C. gomesi*, the partially heterochromatic B chromosomes found in *C. oiticicai* and *C. pterostictum* could have originated from recent events, *i.e*., from heterochromatic or euchromatic elements followed by modifications in the chromatin distribution patterns. On the other hand, the constitutive heterochromatin does not seem to have participated in the origin and development of the extra chromosome observed in *Characidium* cf. *zebra*, which is euchromatic (Venere *et al*., 1999).

Centofante *et al*. (2001, 2003) and Vicari *et al*. (1998) consider that the evolution and establishment of the sex chromosome systems in the genus *Characidium* might be intimately associated with the existence of biogeographic barriers. According to Weitzman *et al*. (1988), the main rivers of southern and southeastern Brazil are currently separated by barriers that hinder the dispersion of species and populations, thence favoring the occurrence of events that determine the isolation of the groups. In such context, exclusive karyotypic constitutions and different sex chromosome systems in fish could be fixed independently in different species of a genus, promoting intraspecific diversification and contributing to the speciation process of different groups. In the genus *Characidium*, which normally presents species with and without ZZ/ZW sex chromosome systems living in sympatry (Centofante *et al*., 2001, 2003), a plausible hypothesis is that the occurrence of headwaters capture events may have contributed to the present situation in the species diversification and distribution, once they usually occupy the same environment.

Although different events may have influenced the karyotypic diversification process in the genus *Characidium*, the constitutive heterochromatin seems to play an expressive role in the chromosome differentiation of the analyzed species, and the study of this chromatin portion using molecular techniques could bring new information to improve our understanding of the evolutionary relationships within this group.

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