The karyotypes and evolution of ZZ/ZW sex chromosomes in the genus Characidium (Characiformes, Crenuchidae)

Marcela Baer Pucci¹, Viviane Nogaroto², Luiz Antonio Carlos Bertollo¹, Orlando Moreira-Filho¹, Marcelo Ricardo Vicari²

¹ Departamento de Genética e Evolução, Universidade Federal de São Carlos, Rodovia Washington Luís, Km 235, 13565-905, São Carlos, São Paulo State, Brazil ² Departamento de Biologia Estrutural, Molecular e Genética, Universidade Estadual de Ponta Grossa, Av. Carlos Cavalcanti, 4748, 84030-900, Ponta Grossa, Paraná State, Brazil

Corresponding author: Marcelo Ricardo Vicari (vicarimr@uepg.br)

Abstract
Available data on cytotaxonomy of the genus Characidium Reinhardt, 1867, which contains the greatest number of species in the Characidiinae (Crenuchidae), with 64 species widely distributed throughout the Neotropical region, were summarized and reviewed. Most Characidium species have uniform diploid chromosome number (2n) = 50 and karyotype with 32 metacentric (m) and 18 submetacentric (sm) chromosomes. The maintenance of the 2n and karyotypic formula in Characidium implies that their genomes did not experience large chromosomal rearrangements during species diversification. In contrast, the internal chromosomal organization shows a dynamic differentiation among their genomes. Available data indicated the role of repeated DNA sequences in the chromosomal constitution of the Characidium species, particularly, in sex chromosome differentiation. Karyotypes of the most Characidium species exhibit a heteromorphic ZZ/ZW sex chromosome system. The W chromosome is characterized by high rates of repetitive DNA accumulation, including satellite, microsatellite, and transposable elements (TEs), with a varied degree of diversification among species. In the current review, the main Characidium cytogenetic data are presented, highlighting the major features of its karyotype and sex chromosome evolution. Despite the conserved karyotypic macrostructure with prevalent 2n = 50 chromosomes in Characidium, herein we grouped the main cytogenetic information which led to chromosomal diversification in this Neotropical fish group.
Keywords
Chromosomal differentiation, Cryptic species, Repetitive DNA, Speciation genes

Introduction

Crenuchidae (Teleostei: Characiformes) include 18 genera and 95 species (Eschmeyer et al. 2018), grouped in Crenuchinae and Characidiinae (Buckup 1999). Characidium Reinhardt, 1867 is the most species-rich genus of Characidiinae, containing 64 valid species, which are morphologically very similar (Buckup 1993), and broadly distributed across the Neotropical region (Eschmeyer et al. 2018). These fishes are small-sized, reaching 15 cm of length at adulthood (Buckup 1999), and some are commercially used in aquarium hobbies. They usually live in streams and can be found in both lentic and lotic habitats (Buckup 1999). Their elongated body shape and ventrally extended pectoral and pelvic fins enable them to attach tightly to the substrate, allowing them to resist to the water flow and capture food (Aranha et al. 2000). Characidium can be classified as autochthonous and insectivorous (Aranha et al. 2000, Bastos et al. 2013, Fernandes et al. 2017) and usually do not exhibit morphological sexual dimorphism (Buckup 1999). Characidium satoi Melo & Oyakawa, 2015 is an exception, where males develop a seasonal darker and uniform pigmentation of the body and head vs. the vertical bars exhibited in females (Melo and Oyakawa 2015).

Phylogenetic analysis removed these fishes from the Characidae along with the Crenuchinae, and this group was organized in a new monophyletic family, the Crenuchidae (Buckup 1998). Phylogenetic relationships are available for most taxa in this family (Buckup 1993). According to available molecular and morphological data, Characidium is a monophyletic group, and its most recent common ancestor (Crenuchidae) likely originated during the Eocene, approximately 50.2 Mya. The geological events during this period boosted South American ichthyofauna diversity (Poveda-Martínez et al. 2016).

Based on morphological data, Characidium zebra Eigenmann, 1909 is the most ancestral species of the genus as well as also of Characidiinae (Buckup 1993). An integrative study using cytogenetic data combined to partial Cytochrome oxidase C subunit 1 (COI) and Cytochrome B sequences (Cyt B) for molecular phylogenetic analyses was applied in some Characidium species (Pansonato-Alves et al. 2014). This analysis proposed Characidium into two main groups of species: i) those which do not exhibit sex chromosomes heteromorphism; and ii) those with a ZZ/ZW sex chromosome heteromorphism with a partial or total heterochromatinization of the W chromosome (Pansonato-Alves et al. 2014). In addition, these data suggested: i) that the origin of sex chromosomes in analyzed Characidium species was unique and considered an apomorphic state and; ii) that B chromosomes present in some Characidium species presumably showed independent origins (Pansonato-Alves et al. 2014).

Another common characteristic in cytogenetic data of Characidium is the occurrence of cryptic species (Vicari et al. 2008, Machado et al. 2011, Pucci et al. 2014). This is suggested to be due to some populations of the same nominal taxa carrying the
Chromosome evolution in Characidium

Z and W chromosomes at different stages of differentiation and apparent flow gene isolation (Vicari et al. 2008). Hence, new Characidium species are frequently described in the scientific literature (Melo and Oyakawa 2015, Zanata and Camelier 2015, Zanata and Ohara 2015) and, the genus needs a critical revision.

General chromosomal characteristics in Characidium

Table 1 summarizes the recognized Characidium individuals/populations with cytogenetic data. The first cytogenetic investigation of this genus was performed by Miyazawa and Galetti (1994), who analyzed four species and some populations of C. cf. zebra, Characidium sp., Characidium cf. lagosantensis Travassos, 1947 and Characidium pterostictum Gomes, 1947, all of which had 2n = 50 chromosomes (Table 1). In fact, phylogenetically basal C. zebra, already possesses such chromosomal plesiomorphic features in the genus (2n = 50; 32m + 18sm), including the absence of heteromorphic sex chromosomes (Vicari et al. 2008, Machado et al. 2011, Pazian et al. 2013). This karyotype pattern occurs in most Characidium species (Table 1, Fig. 1), although rare spontaneous triploids have been detected among specimens of Characidium gomesi Travassos, 1956 (Centofante et al. 2001) and C. cf. zebra (Pansonato-Alves et al. 2011a). The evolutionary history of this genus revealed no large chromosomal rearrangements (Machado et al. 2011, Pucci et al. 2014, Scacchetti et al. 2015a, 2015b). However, occasional changes in the karyotypic formula can be found due to differences in the autosome morphology (Table 1).

Interstitial telomeric sites (ITS), which are usually correlated with chromosomal fusions, were identified in the karyotypes of Characidium schubarti Travassos, 1955, Characidium lanei Travassos, 1967, Characidium lauroi Travassos, 1949, Characidium timbuiense Travassos, 1946, Characidium serrano Buckup & Reis, 1997, and two populations of C. pterostictum (Scacchetti et al. 2015c). The varied locations of ITS regions in the karyotypes were ascribed to their probable association with satellite DNA through transposition events and ectopic recombinations (Scacchetti et al. 2015c).

Generally, the constitutive heterochromatin has a preferential distribution in the pericentromeric regions in the most Characidium chromosomes, but some large interstitial and terminal blocks were also observed. Chromosomal mapping of 18S and 5S rDNAs showed varied autosomal positions among Characidium genomes, ranging from single to multiple sites (Table 1). Nucleolar organizing regions (NORs) were probably related to the origin of the ZZ/ZW sex chromosome system that characterizes many Characidium species (Table 1), as commented below.

Distribution of repetitive DNAs in the Characidium genome

In fishes, tandem or dispersed repetitive DNA sequences are relevant markers for clarifying karyotype evolution and sex chromosome differentiation (Schemberger et al. 2011, Barbosa et al. 2017, do Nascimento et al. 2018, Glugoski et al. 2018). Their
Table 1. Review of *Characidium* cytogenetic studies until 2018. The variation in the diploid number (2n) is due to the presence of B chromosomes. “Unknown” signifies that the data was not available in the original study. NOR: Nucleolar Organizer Region; M: Metacentric; SM: Submetacentric; ST: Subtelocentric; A: Acrocentric. * The chromosome pairs are not indicated in the original publication.

| Species               | Localization                   | 2n     | Sex chromosome system | Karyotype formula | rDNA 18S         | rDNA 5S         | References                        |
|-----------------------|--------------------------------|--------|-----------------------|-------------------|-----------------|-----------------|-----------------------------------|
| *C. alipioi*          | Ribeirão Grande Stream, SP, Brazil | 50     | ZZ/ZW                | 30M+20SM          | Pair 16 (NOR)   | Unknown         | Centofante et al. (2003)          |
|                       | Ribeirão Grande Stream, SP, Brazil | 50–54  | ZZ/ZW                | 32M+18SM          | Pair 18         | Pair 20         | Serrano et al. (2017)             |
| *C. fasciatum*        | Rio São Francisco, MG, Brazil   | 50     | ZZ/ZW                | 32M+18SM          | Unknown         | Unknown         | Pazian et al. (2014)              |
| *C. cf. fasciatum*    | Parque Grande Stream, SP, Brazil | 50     | ZZ/ZW                | 32M+18SM          | Unknown         | Unknown         | Pazian et al. (2013)              |
|                       | Parapanema, SP, Brazil          | 50–54  | ZZ/ZW                | 32M+18SM          | Three autosomal pairs* | Unknown         | Maistro et al. (1998)            |
| *C. gomesi*           | Pardo River, SP, Brazil         | 50     | ZZ/ZW                | 32M+18SM          | Pair 7 and an additional chromosome (NOR) | Unknown         | Maistro et al. (2004), Serrano et al. (2016) |
|                       | Machado River, MG, Brazil       | 50     | ZZ/ZW                | 32M+18SM          | Pair 17 (NOR)   | Unknown         | da Silva and Maistro (2006)       |
|                       | Quebra Perna Stream, PR, Brazil | 50     | ZZ/ZW                | 32M+18SM          | One autosomal pair* | Pairs 4, 7 and 17 | Vicari et al. (2008), Pucci et al. (2014), Pucci et al. (2016) |
|                       | Alambari Stream, SP, Brazil     | 50     | ZZ/ZW                | 32M+18SM          | 31M+18SM+1ST    | Unknown         | Pizzato et al. (2011)            |
|                       | Nova River, SP, Brazil          | 50–54  | ZZ/ZW                | 32M+18SM          | Pair 18         | Pair 25         | Pansonato-Alves et al. (2011b, 2014) |
| *C. gomesi*           | Verde River, PR, Brazil         | 50     | ZZ/ZW                | 32M+18SM          | Pairs 17, 22 and in one of the homologs of the pairs 1 and 20 | Unknown         | Machado et al. (2011)            |
|                       | Rio da Cachoeira Stream, GO, Brazil | 50     | ZZ/ZW                | 32M+18SM          | Unknown         | Unknown         | Pazian et al. (2013, 2014)        |
|                       | Magdalena Stream, SP, Brazil    | 50–52  | ZZ/ZW                | 32M+18SM          | Unknown         | Unknown         | Pazian et al. (2014)              |
| *C. cf. gomesi*       | Grande River, SP, Brazil        | 50     | ZZ/ZW                | 32M+18SM          | Pair 17         | Unknown         | Machado et al. (2011)            |
|                       | Minhocca Stream, MG, Brazil     | 50     | ZZ/ZW                | 32M+18SM          | Pair 17         | Unknown         | Machado et al. (2011)            |
|                       | Tietê River, SP, Brazil         | 50     | ZZ/ZW                | 32M+18SM          | ZW              | Unknown         | Pansonato-Alves et al. (2014)     |
|                       | São Domingos River, MG, Brazil  | 50     | ZZ/ZW                | 32M+18SM          | Pair 17         | Unknown         | Pansonato-Alves et al. (2014)     |
|                       | Vermelho River, MT, Brazil      | 50     | ZZ/ZW                | 32M+18SM          | Pair 17         | Unknown         | Pansonato-Alves et al. (2014)     |
|                       | São João River, PR, Brazil      | 50     | ZZ/ZW                | 32M+18SM          | Pairs 10 and 17 | Unknown         | Pucci et al. (2016)              |
| Species | Localization | $2n$ | Sex chromosome system | Karyotype formula | rDNA 18S | rDNA 5S | References |
|---------|--------------|------|-----------------------|------------------|----------|---------|------------|
| *C. beirizontignata* da Graça & Pavaneli, 2008 | Barra Grande River, PR, Brazil | 50 | ZZ/ZW | 32M+18SM | Pair 4 | Pair 19 | Pucci et al. (2014) |
| *C. lagosantense* Travassos, 1947 | Amendoim Stream, MG, Brazil | 50 | Absent | Unknown | Unknown | Unknown | Pazian et al. (2013) |
| *C. cf. lagosantense* | Inferno Lagoon, SP, Brazil | 50 | Unknown | 32M+18SM | Unknown | Unknown | Miyazawa and Galetti (1994) |
| *C. lanei* Travassos, 1967 | Barroca River, PR, Brazil | 50 | ZZ/ZW | 32M+16SM+2A | ZW | One autosomal pair* | Noleto et al. (2009) |
| *C. laurii* Travassos, 1949 | Grande River, SP, Brazil | 50 | ZZ/ZW | $\overset{\circ}{32 M +18 SM}$ | $\overset{\circ}{31 M +18 SM +1ST}$ | ZW (NOR) | Centofante et al. (2003) Pansonato-Alves et al. (2010), Machado et al. (2011) |
| *C. oiticicai* Buckup & Reis, 1997 | Pairaitinguinha River, SP, Brazil | 50–53 | ZZ/ZW | 32M+18SM | ZW (NOR) | Unknown | Pansonato-Alves et al. (2010, 2014) |
| *C. orientale* Buckup & Reis, 1997 | Chasqueiro Stream, RS, Brazil | 50 | ZZ/ZW | 32M+18SM | ZW | Pairs 1, 3, 5, 6, 20 and W | Scacchetti et al. (2015a) |
| *C. pterostictum* Gomes, 1947 | Betari River, SP, Brazil | 50–53 | ZZ/ZW | 32M+16SM+2A | ZW | Unknown | Pansonato-Alves et al. (2010, 2014) |
| | Fati River, SP, Brazil | 50 | ZZ/ZW | 32M+16SM+2A | ZW | Unknown | Pansonato-Alves et al. (2014) |
| | Cari River, PR, Brazil | 50 | ZZ/ZW | 32M+16SM+2A | ZW | Unknown | Pansonato-Alves et al. (2014) |
| | Jacaré River, PR, Brazil | 50 | ZZ/ZW | 32M+16SM+2A | ZW | Unknown | Pansonato-Alves et al. (2014) |
| | Iapocu River, SC, Brazil | 50 | ZZ/ZW | 32M+16SM+2A | ZW | Unknown | Pansonato-Alves et al. (2014) |
| | Painiquera-Açú River, SP, Brazil | 50 | ZZ/ZW | 32M+16SM+2A | ZW | Pairs 9, 11 and 13 | Pucci et al. (2014) |
| | Iacuí River, RS, Brazil | 50 | ZZ/ZW | 32M+16SM+2A | ZW | Three autosomal pairs* | Scacchetti et al. (2015b) |
| | Iapêva Lagoon, RS, Brazil | 50 | ZZ/ZW | 32M+16SM+2A | Unknown | Unknown | Scacchetti et al. (2015c) |
| | Carlos Botelho Ecological Station, SP, Brazil | 50 | Unknown | 32M+16SM+2ST | Unknown | Unknown | Miyazawa and Galetti (1994) |
| *C. rachovii* Regan, 1913 | Cabeças Stream, RS, Brazil | 50 | ZZ/ZW | 32M+18SM | ZW | Pairs 1, 3, 5, 17, 20 and W | Scacchetti et al. (2015a) |
| *C. schubarti* Travassos, 1955 | Cinco Réis River, PR, Brazil | 50 | ZZ/ZW | 32M+18SM | ZW (NOR) | Unknown | Pansonato-Alves et al. (2010), Scacchetti et al. (2015c) |
| *C. serrano* Buckup & Reis, 1997 | Canoinha Stream, RJ, Brazil | 50 | ZZ/ZW | 32M+16SM+2A | Unknown | Unknown | Scacchetti et al. (2015c) |
| *C. stigmatum* Melo & Buckup, 2002 | Ave Maria River, GO, Brazil | 50 | Absent | 32M+18SM | Pair 23 | Pairs 1, 7 and 17 | Scacchetti et al. (2015a) |
| *C. temne* (Cope, 1894) | Chui Stream, SC, Brazil | 50 | Absent | 32M+18SM | Pair 23 | Pairs 1 and 7 | Scacchetti et al. (2015a) |
| *C. timbuiense* Travassos, 1946 | Vargusana Velha Stream, ES, Brazil | 50 | ZZ/ZW | 32M+16SM+2A | ZW | Three autosomal pairs* | Scacchetti et al. (2015b) |
| Species | Localization | 2n | Sex chromosome system | Karyotype formula | rDNA 18S | rDNA 5S | References |
|---------|--------------|----|----------------------|------------------|----------|---------|------------|
| C. vestigipinne | Caraguatá River, RS, Brazil | 50 | ZZZZW | 32M+18SM | ZW | Pairs 1, 17 and 20 | Scacchetti et al. (2015a) |
| C. vidali | Bananeiras Stream, RJ, Brazil | 50 | ZZZZW | 32M+18SM | One autosomal pair* | W' chromosome and in one autosomal pair* | Scacchetti et al. (2015b, c) |
| C. aff. vidali | Xingu River, MT, Brazil | 50–54 | ZZZZW | 32M+18SM | Pair 21 | Pairs 5, 12 and 20 | Scacchetti et al. (2015a) |
| C. xarantte da Graça, Pavanelli & Buckup, 2008 | Jataí Reservoir, SP, Brazil | 50 | Unknown | 32M+18SM | Pair 25 (NOR), with 1 to 2 additional pairs | Unknown | Miyazawa and Galetti (1994) |
| C. zebra | Jataí Reservoir, SP, Brazil | 50 | Unknown | 32M+18SM | Pair 23 | Pair 17 | Miyazawa and Galetti (1994) |
| | Piracicaba River, SP, Brazil | 50 | Unknown | 32M+18SM | Pair 25 (NOR) | Unknown | Miyazawa and Galetti (1994) |
| | Ribeirão Claro Stream, SP, Brazil | 50 | Absent | 32M+18SM | Pair 23 | Pairs 1, 6, and 17 | Pansonato-Alves et al. (2011a), Pucci et al. (2011a), Scacchetti et al. (2015b, 2015c) |
| | Pianaitinga River, SP, Brazil | 50 | Absent | 32M+18SM | Pair 23 | Pairs 1, 6, 9, 17 | Pansonato-Alves et al. (2011a), Pucci et al. (2011a) |
| C. cf. zebra | Paiol Grande Stream, SP, Brazil | 50 | Absent | 32M+18SM | Pair 23 (NOR) | Unknown | Centofante et al. (2001), Pucci et al. (2016) |
| | Machado River, MG, Brazil | 50 | Absent | 32M+18SM | Pair 23 (NOR) | Unknown | da Silva and Maistro (2006) |
| | Alambari River, SP, Brazil | 50 | Absent | 32M+18SM | Pair 23 | Pair 17 | Pansonato-Alves et al. (2011a) |
| | Novo River, SP, Brazil | 50 | Absent | 32M+18SM | Pair 23 | Pair 17 | Pansonato-Alves et al. (2011a) |
| | Aracuí River, SP, Brazil | 50 | Absent | 32M+18SM | Pair 23 | Pair 17 | Pansonato-Alves et al. (2011a) |
| | Duas Antas Stream, MT, Brazil | 50 | Absent | 32M+18SM | Pair 23 | Pairs 1 and 17 | Scacchetti et al. (2015a) |
| | Juba River, MT, Brazil | 50 | Absent | 32M+18SM | Pair 23 | Pairs 1, 6, 9, 17 and 18 | Pansonato-Alves et al. (2011a) |
| C. afl. zebra | Corredeira Stream, SP, Brazil | 50 | Absent | 32M+18SM | Pairs 4, 7 and 23 | Pair 17 | Pucci et al. (2014) |
| | Corredeira Stream, SP, Brazil | 50 | Absent | 32M+18SM | Pairs 2, 4, 7, 20, 23 and 17 | Pair 17 | Pucci et al. (2014) |
| Characidium sp. | Preto River, SP, Brazil | 50 | ZZZZW | 32M+18SM | ZW (NOR) | Unknown | Pansonato-Alves et al. (2010) |
| | Lagoon of the Corredeira Stream, SP, Brazil | 50 | ZZZZW | 32M+16SM+2A | ZW | Pairs 3, 7, 8, 23 and 24 | Pucci et al. (2014) |
| Characidium sp.2 | Vermelho River, MT, Brazil | 50 | ZZZZW | 32M+18SM | W and pair 7 | Pair 17 | Scacchetti et al. (2015a) |
| Characidium sp. | Formoso River, GO, Brazil | 50 | ZZZZW | 32M+18SM | Unknown | Unknown | Pansonato-Alves et al. (2013, 2014) |
| | Inferno Lagoon, SP, Brazil | 50 | Unknown | 32M+18SM | Unknown | Unknown | Miyazawa and Galetti (1994) |
accumulation is a key factor for the morphogenesis and the differentiation process of sex chromosomes, and the induction of gene erosion (Matsunaga 2009, Schemberger et al. 2014, Zienniczak et al. 2014).

Despite the highly conserved karyotype structure, the genomes of Characidium species display a dynamic pattern of their internal chromosomal composition (Table 1, Fig. 2). Phylogenetics studies using mitochondrial DNA in Characidium were used to anchor a comparative cytogenetic analysis using telomeric DNA probe. This data indicated that the ITS signals found in genomes of some Characidium species (Fig. 2a) do not have relation with chromosome fusions but, on contrary, are associated with repetitive DNAs dispersion (Scacchetti et al. 2015c). Probably the ITS have origin in the evolutionary lineage of the genus in related hydrographic drainages (Scacchetti et al. 2015c), although some relationship species, such as C. zebra and C. gomesi, do not harbor such sequences. U2 small nuclear RNA (snRNA U2) had a highly conserved distribution in the first m pair in the most species (Fig. 2b), except for Characidium sp. aff. Characidium vidali Travassos, 1967, Characidium sp. 1 and Characidium alipioi Travassos, 1955, in which snRNA U2 site was located in the first submetacentric (sm) pair (Scacchetti et al. 2015b, Serrano et al. 2017).

Distinct microsatellites also had a wide distribution in autosomal pairs (Fig. 2c), probably due to their association with TEs (Scacchetti et al. 2015b, Pucci et al. 2016), such as Tc1-Mariner (Fig. 2d). This pattern was also corroborated by Serrano et al. (2017), evidencing (CA)$_{15}$ and (GA)$_{15}$ autosomal accumulation in the C. alipioi genome, as well as of several other microsatellites in C. zebra and C. gomesi. The molecular characterization and chromosome mapping of the histone genes H1, H3 and H4 were described for C. zebra and C. gomesi (Pucci et al. 2018). These three histone sequences appear to be associated with TEs and, in situ localization, revealed that they are dispersed throughout the autosomes, but they are not involved in the differentiation of the specific region of the W sex chromosome in C. gomesi (Pucci et al. 2018).

The available data point to the substantial role of repeated DNA sequences in the chromosomal constitution of Characidium species. However, due to the extension of the existing repetitive elements, additional investigations must address their significance in the evolutionary history of Characidium and, particularly, in sex chromosome differentiation.
Supernumerary and sex chromosomes in Characidium

Several Neotropical fish species are carriers of supernumerary or B chromosomes (Carvalho et al. 2008). Additionally, due to the variety of simple or multiple sex chromosome systems in these fishes, differentiated karyotypes exist between sexes (Moreira-Filho et al. 1993, Almeida-Toledo et al. 2001).

B chromosomes, ranging from one to four chromosomes, were described in several Characidium species (Table 1). They are hypothesized to have different and independ-
ent origins in evolutionary history of the species. To explain the origin, frequency and evolution of B chromosomes it was hypothesized that these elements are derivate from autosomes followed by gene silencing, heterochromatinization, and accumulation of repetitive DNA and transposons (Camacho et al. 2000, Vicari et al. 2011). In some species, B chromosomes are related to sex chromosomes due to share the same repetitive elements (Scacchetti et al. 2015a). In fact, genomes of C. gomesi, C. pterostictum and Characidium sp. aff. C. vidali displayed similar repetitive DNA sequences among B and sex chromosomes (Pansonato-Alves et al. 2014, Pazian et al. 2014, Scacchetti et al. 2015a, Serrano et al. 2016), while Characidium oticicai Travassos, 1967 and C. alipioi did not show such shared sequences (Pansonato-Alves et al. 2014, Serrano et al. 2017, respectively). Despite their molecular homology, it was demonstrated that B and W chromosomes do not form multivalent pairings during meiosis in male and female C. gomesi individuals.

Meiotic analyses revealed the bivalent pairing of the ZW chromosomes, as well as the bivalent plus one univalent formation in specimens carrying three B chromosomes (Serrano et al. 2016). Chromosome pairing does not always indicate complete homology between chromosomes (Ramsey and Schemske 2002). In fact, the Z and W sex chromosomes in Characidium species possesses differences in 45S rDNA chromosomal localization and in heterochromatin blocks extension (Fig. 3). Chromosomal localization differences of the repetitive sequences among Characidium species are also observed, such as in (TTA)$_{10}$, (GAG)$_{10}$, (CG)$_{15}$ and (GATA)$_{n}$ sequences (Scacchetti et al. 2015b, Pucci et al. 2016). In C. gomesi it was shown that the short arm of the W chromosome keeps homology with the terminal region of the Z chromosome in relation to the (CG)$_{15}$, (GATA)$_{n}$ and (TAA)$_{10}$ sequences (Pucci et al. 2016). (GATA)$_{n}$ and (TAA)$_{10}$ homology is also present in the centromeric region of the C. gomesi (Pucci et al. 2016). These data help to explain ZW chromosome pairing and its bivalent formation in Characidium species.
The occurrence of a ZZ/ZW sex chromosome system is another karyotypic characteristic of *Characidium* genomes. It was first described by Maistro et al. (1998) in *Characidium cf. fasciatum* Reinhardt, 1867 (Table 1), but it is also present in most *Characidium* species studied. The sex chromosomes in *Characidium* show a high degree of differentiation among species by chromosomal size, morphology, heterochromatin accumulation and presence or absence of rDNA sites (Maistro et al. 1998, 2004, Centofante et al. 2001, 2003, Vicari et al. 2008, Noleto et al. 2009, Pansonato-Alves et al. 2010, 2011b, 2014, Machado et al. 2011, Pazian et al. 2013, 2014, Pucci et al. 2014, 2016, Scacchetti et al. 2015a, 2015b, 2015c, Serrano et al. 2017), as exemplified in Fig. 3. Interestingly, the W chromosome can possess distinct cytotypes among *C. gomesi* populations, such as sm (Centofante et al. 2001, Pansonato-Alves et al. 2011b) or subtelocentric (Vicari et al. 2008, Pucci et al. 2014, 2016).

The majority of microsatellites sites were located in the terminal region of the Z chromosome and in the terminal/centromeric regions of W chromosome. The exception is (TTA)$_{10}$, which was widely distributed throughout the whole W chromosome, and (GAG)$_{10}$ which had a preferential accumulation in the W and B chromosomes of *C. alipioi* (Scacchetti et al. 2015b). (CG)$_{15}$ and (GATA)$_{n}$ sequences were mainly found on the short arm of W chromosome in genomes of *C. zebra* and *C. gomesi*. It was suggested that these regions are enriched with sex-specific genes (Pucci et al. 2016), since the (GATA)$_{n}$ sequences are known as a motif for sex- and tissue-specific GATA-binding proteins. However, this pattern was not found in *Characidium heirmostigmata* da Graça & Pavanelli, 2008 (Fig. 2).

18S rDNA sequences are also particular components of many *Characidium* sex chromosomes, occupying the short and the long arms of Z and W chromosomes, respectively, or the long arms of both sex chromosomes (Table 1, Fig. 3). These ribosomal sequences were likely associated with the origin of the protosex chromosome. It is likely that the NORs of the sm pair 23 (an ancestral pattern) were translocated to opposite arms of the second metacentric (m) pair (Machado et al. 2011, Pucci et al. 2014).

Later differentiations in such protosex chromosomes were gradually acquired by isolated populations, leading to deletions and duplications in the rearranged regions due to meiotic pairing failures. Thus, recombination suppression mechanisms (rearrangements, heterochromatinization, repeated DNA accumulation and gene erosion) were naturally selected, giving rise to distinct heteromorphic W chromosomes (Machado et al. 2011, Pucci et al. 2014). Such modifications also promoted the accumulation of the so-called “speciation genes”, particularly in linked Z chromosome loci (Pucci et al. 2014). These genes established meiotic barriers and post-zygotic isolation mechanisms, along with the morphological variations of W chromosome (Fig. 4).

The current sympatric occurrence of some *Characidium* species does not display hybridization events among them. Sympatric and syntopic pairs of *Characidium* species, with the presence or absence of sex chromosomes, had already been described, namely *C. alipioi* and *Characidium* sp. cf. *C. lauroi* (Centofante et al. 2003), and *C. cf. zebra* and *C. gomesi* (da Silva and Maistro 2006). Thus, it is likely that NOR displacements throughout the genome was a key factor linked to W chromosome differ-
Chromosome evolution in Characidium

Figure 3. Idiograms showing main characteristics already identified for the ZZ/ZW sex chromosome system in Characidium species. It was highlighted the position of the centromere, distribution of euchromatin and heterochromatin, W-specific probes, and rDNA sites. The a column detaches the species carrying 18S rDNA sites on the short and long arms of the Z and W chromosomes, respectively; the b column highlights the species bearing 18S rDNA sites on the long arms of both Z and W chromosomes; the c column shows the species that do not present 18S rDNA sequences on either Z or W chromosomes; the d column presents the species bearing Z and W chromosomes with unusual characteristics, including morphology, 18S and 5S rDNA sites, and W-specific probe distribution.
entiation in Crenuchidae. Usually, when the W chromosome is partially heterochromatic, it is still a NOR bearing chromosome; but in totally heterochromatic chromosomes, NORs are found in different autosomes (Table 1, Fig. 3). Restriction-site associated DNA sequencing (RAD-seq) was applied to study the sex chromosomes of *C. gomesi* (Utsonomia et al. 2017). This application identifies 26 female-specific RAD loci, putatively located on the W chromosome, as well as 148 sex-associated SNPs showing significant differentiation. The use of W markers validated for *in situ* localization in other populations and species of the genus *Characidium* suggested a rapid turnover of W-specific repetitive elements (Utsonomia et al. 2017). This finding corroborates the inference that modifications on sex chromosomes also promote the accumulation of the “speciation genes”, leading to chromosomal speciation mechanisms in *Characidium*.

**Perspectives on Characidium investigations**

Fish cytogenetic and molecular studies have improved over the last few years, especially with regard to better identification of the karyotypic evolution and sex chromosome differentiation among different groups of fish, as well as genes or specific regions related to sex determination. W-specific repetitive probes were already constructed for *Characidium* using microdissection from female metaphase chromosomes and degenerate oligonucleotide-primed PCR (DOP-PCR) or whole genome amplification (WGA) protocols. These probes were later applied to chromosome painting in *Characidium* using a *C. gomesi* W-specific probe (Machado et al. 2011, Pazian et al. 2013, 2014, Pansonato-Alves et al. 2014, Pucci et al. 2014). This was followed by investigations of homologous regions between the sex pairs, B chromosomes and autosomes (Machado et al. 2011, Pazian et al. 2013, 2014, Pansonato-Alves et al. 2014, Pucci et al. 2014, 2016, Scacchetti et al. 2015a, 2015b, Serrano et al. 2016, 2017), and the cloning of a W-specific sequence that generated the CgW9 clone, which is similar to the zebrafish *Helitron* transposon (Pazian et al. 2014).

The ZZ/ZW sex chromosome system is well-known and described. The repeated DNA classes related to gene erosion and differentiation of W chromosome, as well as regions or genes implicated in sex determination and gonadal differentiation, have not yet been properly investigated in most species. It has been demonstrated that the repeated DNA sequences are closely related to the regulatory genes network, particularly TEs, in a process called molecular co-option or exaptation (Feschotte 2008). In this sense, future studies concerning the dynamics of mobile elements and molecular co-option in the regulatory system of *Characidium* will be relevant contributions to this research area. Sequencing and comparisons between male and female genomes of different *Characidium* species will contribute to highlighting the genic and/or repetitive sequences that are sex-restricted.

In other pathways, sequencing procedures of particular W fractions is needed for investigating specific genes related to sex determination and differentiation. Indeed, integrating cytogenetic, genomic, molecular, and bioinformatic tools will be essential for a better understanding of sex determination and differentiation processes in fishes, with applications in ecological and evolutionary studies.
**Conclusion**

Chromosomal diversification in *Characidium* here revised show a diversified karyotype microstructure despite its conserved karyotypic macrostructure with prevalent 2n of 50 chromosomes arranged in 32 m + 18 sm. Differences in the number of rDNA sites, in heterochromatin blocks, in B chromosomes number and, in sex chromosomes sizes, as well as an interesting dynamic of repetitive DNAs on the chromosomes are observed among species, leading to chromosomal diversification and speciation. The data showed that different microsatellite expansions are involved in the sex chromosome differentiation in *Characidium*. In addition, the microsatellite (TTA)$_{10}$ play an important role in gene degeneration and erosion on the W chromosome in some *Characidium* species. These data are important for the molecular characterization of the W and B chromosomes, to karyotype structures determination and comprehension of cryptic species. Future studies integrating cytogenetic, genomic and molecular data open perspectives to understand the sex determination, B chromosome composition and, “speciation genes” in *Characidium* genomes.
Acknowledgements

The authors are grateful to Instituto Chico Mendes de Conservação da Biodiversidade (protocol number SISBIO 15117) for authorizing the capture of specimens. This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Secretaria de Ciência e Tecnologia do Estado do Paraná (SETI), and Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Estado do Paraná (Fundação Araucária).

References

Almeida-Toledo LF, Foresti F, Pêquignot EV, Daniel-Silva MFZ (2001) XX:XY sex chromosome system with X heterochromatinization: an early stage of sex chromosome differentiation in the Neotropic electric eel *Eigenmannia virescens*. Cytogenetics and Cell Genetics 95: 73–78. https://doi.org/10.1159/000057020

Aranha JMR, Gomes JHC, Fogaça FNO (2000) Feeding of two sympatric species of *Characidium, C. lanei* and *C. pterostictum* (Characidiinae) in a coastal stream of Atlantic Forest (Southern Brazil). Brazilian Archives of Biology and Technology 43: 527–531. http://dx.doi.org/10.1590/S1516-89132000000500013

Barbosa P, Pucci MB, Nogaroto V, Almeida MC, Artoni RF, Vicari MR (2017) Karyotype analysis of three species of *Corydoras* (Siluriformes: Callichthyidae) from southern Brazil: rearranged karyotypes and cytotaxonomy. Neotropical Ichthyology 15: e160056. http://dx.doi.org/10.1590/1982-0224-20160056

Bastos RF, Miranda SF, Garcia AM (2013) Dieta e estratégia alimentar de *Characidium rachovii* (Characiformes, Crenuchidae) em riachos de planície costeira do sul do Brasil. Iheringia 103: 335–341. http://dx.doi.org/10.1590/S0073-47212013000400001

Buckup PA (1993) Phylogenetic interraletionships and reductive evolution in neotropical characiidn fishes (Characiformes, Ostariophysi). Cladistics 9: 305–341. https://doi.org/10.1111/j.1096-0031.1993.tb00227.x

Buckup PA (1998) Relationships of the Characidiinae and phylogeny of characiform fishes (Teleostei: Ostariophysi). In: Malabarba LR, Reis RE, Vari RP, et al. (Eds) Phylogeny and classification of neotropical fishes. Edipucrs, Porto Alegre, 123–144.

Buckup PA (1999) Ecologia de peixe de riachos. Série Oecologia Brasiliensis, vol. VI. PPGE-UFRJ. In: Caramaschi EP, Mazzoni R, Peres Neto PR (Eds) Chapter 3, Sistemática e Biogeografia de Peixes de Riachos. Rio de Janeiro, 91–138.

Camacho JP, Sharbel TF, Beukeboom LW (2000) B-chromosome evolution. Philosophical Transactions of the Royal Society B: Biological Sciences 355: 163–178. https://doi.org/10.1098/rstb.2000.0556

Carvalho PA, Martins-Santos IC, Dias AL (2008) B-chromosomes: an update about its occurrence in freshwaters Neotropical fishes (Teleostei). Journal of Fish Biology 72: 1907–1932. https://doi.org/10.1111/j.1095-8649.2008.01835.x
Centofante L, Bertollo LAC, Buckup PA, Moreira-Filho O (2003) Chromosomal divergence and maintenance of sympatric Characidium fish species (Crenuchidae, Characidiinae). Hereditas 138: 213–218. https://doi.org/10.1034/j.1601-5223.2003.01714.x

Centofante L, Bertollo LAC, 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 54: 253–260. https://doi.org/10.1080/00087114.2001.10589233

da Silva AR, Maistro EL (2006) Cytogenetic divergence between two sympatric species of Characidium (Teleostei, Characiformes, Crenuchidae) from the Machado River, Minas Gerais, Brazil. Genetics and Molecular Biology 29: 459–463. http://dx.doi.org/10.1590/S1415-47572006000300010

do Nascimento VD, Coelho KA, Nogaroto V, Almeida RB, Ziemniczak K, Centofante L, Pavanelli CS, Torres RA, Moreira-Filho O, Vicari MR (2018) Do multiple karyomorphs and population genetics of freshwater darter characines (Apareiodon affinis) indicate chromosomal speciation? Zoologischer Anzeiger 272: 93–103. https://doi.org/10.1016/j.jcz.2017.12.006

Eschmeyer WN, Fricke R, van der Laan R (Eds) (2018) Catalog of fishes: genera, species, references [Internet]. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp [updated 2018 Apr 30, cited 2018 May 21]

Fernandes S, Leitão RF, Dary EP, Guerreiro AIC, Zuanon J, Bührnheim CM (2017) Diet of two sympatric species of Crenuchidae (Ostariophys: Characiformes) in an Amazonian rocky stream. Biota Neotropica 17(1): e20160281. http://dx.doi.org/10.1590/1676-0611-bn-2016-0281

Feschotte C (2008) Transposable elements and the evolution of regulatory networks. Nature Reviews Genetics 9: 397–405. http://doi.org/10.1038/nrg2337

Glugoski L, Giuliano-Caetano L, Moreira-Filho O, Vicari MR, Nogaroto V (2018) Co-located hAT transposable element and 5S rDNA in an interstitial telomeric sequence suggest the formation of Robertsonian fusion in armored catfish. Gene 650: 49–50. https://doi.org/10.1016/j.gene.2018.01.099

Machado TC, Pansonato-Alves JC, Pucci MB, Nogaroto V, Almeida MC, Oliveira C, Foresti F, Bertollo LAC, Moreira-Filho O, Vicari MR (2011) Chromosomal painting and ZW sex chromosomes differentiation in Characidium (Characiformes, Crenuchidae). BMC Genetics 12: 65. https://doi.org/10.1186/1471-2156-12-65

Maistro EL, de Jesus CM, Oliveira C, Moreira-Filho O, Foresti F (2004) Cytogenetic analysis of A-, B-chromosomes and ZZ/ZW sex chromosomes of Characidium gomesi (Teleostei, Characiformes, Crenuchidae). Cytologia 69: 181–186. https://doi.org/10.1508/cytologia.69.181

Maistro EL, Mata EP, Oliveira C, 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. https://doi.org/10.1023/A:1003242020259

Matsunaga S (2009) Junk DNA promotes sex chromosome evolution. Heredity 102: 525–526. https://doi.org/10.1038/hdy.2009.36
Melo MRS, Oyakawa OT (2015) A new species of Characidium Reinhardt (Characiformes, Crenuchidae) with a distinctively dimorphic male. Copeia 103: 281–289. https://doi.org/10.1643/CI-14-073

Miyazawa CS, Galetti Jr PM (1994) First cyto genetic studies in Characidium species (Pisces: Characiformes, Characidiinae). Cytologia 59: 73–79. https://doi.org/10.1508/cytologia.59.73

Moreira-Filho O, Bertollo LAC, Galetti Jr PM (1993) Distribution of sex chromosome mechanisms in Neotropical fish and description of a ZZ/ZW system in Parodon bilirii (Pardontidae). Caryologia 46: 115–125. https://doi.org/10.1080/00087114.1993.10797253

Noleto RB, Amorim AP, Vicari MR, Artoni RF, Cestari MM (2009) An unusual ZZ/ZW sex chromosome system in Characidium fishes (Crenuchidae, Characiformes) with the presence of rDNA sites. Journal of Fish Biology 75: 448–453. https://doi.org/10.1111/j.1095-8649.2009.02342.x

Pansonato-Alves JC, Oliveira C, Foresti F (2011a) Karyotypic conservatism in samples of Characidium cf. zebra (Teleostei, Characiformes, Crenuchidae): Physical mapping of ribosomal genes and natural triploidy. Genetics and Molecular Biology 34: 208–213. http://dx.doi.org/10.1590/S1415-47572011005000005

Pansonato-Alves JC, Paiva LRS, Oliveira C, Foresti F (2010) Interspecific chromosomal divergences in the genus Characidium (Teleostei: Characiformes: Crenuchidae). Neotropical Ichthyology 8: 77–86. http://dx.doi.org/10.1590/S1679-62252010000100010

Pansonato-Alves JC, Serrano EA, Utsunomia R, Camacho JPM, Silva GJC, Vicari MR, Artoni RF, Oliveira C, Foresti F (2014) Single origin of sex chromosomes and multiple origins of B chromosomes in fish genus Characidium. Plos One 9: e107169. https://doi.org/10.1371/journal.pone.0107169

Pansonato-Alves JC, Vicari MR, Oliveira C, Foresti F (2011b) Chromosomal diversification in populations of Characidium cf. gomesi (Teleostei, Crenuchidae). Journal of Fish Biology 78: 183–194. https://doi.org/10.1111/j.1095-8649.2010.02847.x

Pazian MF, Oliveira C, Foresti F (2014) Sex chromosome composition revealed in Characidium fishes (Characiformes: Crenuchidae) by molecular cytogenetic methods. Biologia 69: 1410–1416. https://doi.org/10.2478/s11756-014-0434-0

Pazian MF, Shimabukuro-Dias CK, Pansonato-Alves JC, Oliveira C, Foresti F (2013) Chromosome painting of Z and W sex chromosomes in Characidium (Characiformes, Crenuchidae). Genetica 141: 1–9. https://doi.org/10.1007/s10709-013-9701-1

Poveda-Martínez D, Sosa CC, Chacón-Vargas K, García-Merchán VH (2016) Historical biogeography of five Characidium fish species: Dispersal from the amazon paleobasin to southeastern South America. PLoS One 11: e0164902. https://doi.org/10.1371/journal.pone.0164902

Pucci MB, Barbosa P, Nogaroto V, Almeida MC, Artoni RF, Pansonato-Alves JC, Foresti F, Moreira-Filho O, Vicari MR (2014) Population differentiation and speciation in the genus Characidium (Characiformes: Crenuchidae): effects of reproductive and chromosomal barriers. Biological Journal of Linnean Society 111: 541–553. https://doi.org/10.1111/bij.12218
Chromosome evolution in Characidium

Pucci MB, Barbosa P, Nogaroto V, Almeida MC, Artoni RF, Pansonato-Alves JC, Scacchetti PC, Foresti F, Moreira-Filho O, Vicari MR (2016) Chromosomal spreading of microsatellites and (TTAGGG)_n sequences in the Characidium zebra and C. gomesi genomes (Characiformes: Crenuchidae). Cytogenetic and Genome Research 149: 182–190. https://doi.org/10.1159/000447959

Pucci MB, Nogaroto V, Moreira-Filho O, Vicari MR (2018) Dispersion of transposable elements and multigene families: Microstructural variation in Characidium (Characiformes: Crenuchidae) genomes. Genetics and Molecular Biology 41: 585–592. http://dx.doi.org/10.1590/1678-4685-GMB-2017-0121

Ramsey J, Schemske DW (2002) Neopolyploidy in flowering plants. Review of Ecology and Systematics 33(1): 589–639. https://doi.org/10.1146/annurev.ecolsys.33.010802.150437

Scacchetti PC, Utsunomia R, Pansonato-Alves JC, Vicari MR, Artoni RF, Oliveira C, Foresti F (2015b) Chromosomal mapping of repetitive DNAs in Characidium (Teleostei, Characiformes): Genomic organization and diversification of ZW sex chromosomes. Cytogenetic and Genome Research 146: 136–143. https://doi.org/10.1159/000437165

Scacchetti PC, Utsunomia R, Pansonato-Alves JC, Silva GJC, Vicari MR, Artoni RF, Oliveira C, Foresti F (2015a) Repetitive DNA sequences and evolution of ZZ/ZW sex chromosomes in Characidium (Teleostei: Characiformes). PLoS One 10: e0137231. https://doi.org/10.1371/journal.pone.0137231

Scacchetti PC, Utsunomia R, Pansonato-Alves JC, da Costa-Silva GJ, Oliveira C, Foresti F (2015c) Extensive spreading of interstitial telomeric sites in the chromosomes of Characidium (Teleostei, Characiformes). Genetica 143: 263–270. https://doi.org/10.1007/s10709-014-9812-3

Schemberger MO, Bellafronte E, Nogaroto V, Almeida MC, Schühli GS, Artoni RF, Moreira-Filho O, Vicari MR (2011) Differentiation of repetitive DNA sites and sex chromosome systems reveal closely related group in Parodontidae (Actinopterygii: Characiformes). Genetica 139: 1499–1508. https://doi.org/10.1007/s10709-012-9649-6

Schemberger MO, Oliveira JIN, Nogaroto V, Almeida MC, Artoni RF, Cestari MM, Moreira-Filho O, Vicari MR (2014) Construction and characterization of a repetitive DNA library in Parodontidae (Actinopterygii: Characiformes): A genomic and evolutionary approach to the degeneration of the W sex chromosome. Zebrafish 11: 518–527. https://doi.org/10.1089/zeb.2014.1013

Serrano EA, Araya-Jaime C, Suárez-Villota EY, Oliveira C, Foresti F (2016) Meiotic behavior and H3K4m distribution in B chromosomes of Characidium gomesi (Characiformes, Crenuchidae). Comparative Cytogenetics 10: 255–268. https://doi.org/10.3897/CompCytogen.v11i1.10886

Serrano EA, Utsunomia R, Scudeller OS, Oliveira C, Foresti F (2017) Origin of B chromosomes in Characidium alipioi (Characiformes, Crenuchidae) and its relationship with supernumerary chromosomes in other Characidium species. Comparative Cytogenetics 11: 81–95. https://doi.org/10.3897/CompCytogen.v11i1.10886

Utsunomia R, Scacchetti PC, Hermida M, Fernández-Cebrián R, Taboada X, Fernández C, Bekaeart M, Mendes NJ, Robledo D, Mank JE, Taggart JB, Oliveira C, Foresti F, Martínez P (2017)
Evolution and conservation of *Characidium* sex chromosomes. Heredity 119: 237–244. https://doi.org/10.1038/hdy.2017.43

Vicari MR, Artoni RF, Moreira-Filho O, Moreira-Filho O (2008) Diversification of a ZZ/ZW sex chromosome system in *Characidium* fish (Crenuchidae, Characiformes). Genetica 134: 311–317. https://doi.org/10.1007/s10709-007-9238-2

Vicari MR, Pistune HFM, Castro JP, Almeida MC, Bertollo LAC, Moreira-Filho O, Cama-cho JPM, Artoni RF (2011) New insights on the origin of B chromosomes in *Astyanax scabripinnis* obtained by chromosome painting and FISH. Genetica 139: 1073–1081. https://doi.org/10.1007/s10709-011-9611-z

Zanata AM, Camelier P (2015) Two new species of *Characidium* Reinhardt (Characiformes: Crenuchidae) from northeastern Brazilian coastal drainages. Neotropical Ichthyology 13: 487–498. http://dx.doi.org/10.1590/1982-0224-20140106

Zanata AM, Ohara WM (2015) A new species of *Characidium* Reinhardt (Ostariophysi: Char-aciformes: Crenuchidae) from headwaters of rio Pacaás Novos, rio Madeira basin, Rondônia, Brazil. Zootaxa 4021: 368–376. http://dx.doi.org/10.11646/zootaxa.4021.2.7

Ziemniczak K, Traldi JB, Nogaroto V, Almeida MC, Artoni RF, Moreira-Filho O, Vicari MR (2014) In situ localization of (GATA)\textsubscript{n} and (TTAGGG)\textsubscript{n} repeat DNAs and W sex chromosome differentiation in Parodontidae (Actinopterygii: Characiformes). Cytogenetic and Genome Research 144: 325–332. https://doi.org/10.1159/000370297