



$X_1X_1X_2X_2/X_1X_2Y$ sex chromosome systems in the Neotropical Gymnotiformes electric fish of the genus *Brachyhypopomus*

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Abstract

Several types of sex chromosome systems have been recorded among Gymnotiformes, including male and female heterogamety, simple and multiple sex chromosomes, and different mechanisms of origin and evolution. The $X_1X_1X_2X_2/X_1X_2Y$ systems identified in three species of this order are considered homoplastic for the group. In the genus *Brachyhypopomus*, only *B. gauderio* presented this type of system. Herein we describe the karyotypes of *Brachyhypopomus pinnicaudatus* and *B. n. sp. FLAV*, which have an $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome system that evolved via fusion between an autosome and the Y chromosome. The morphology of the chromosomes and the meiotic pairing suggest that the sex chromosomes of *B. gauderio* and *B. pinnicaudatus* have a common origin, whereas in *B. n. sp. FLAV* the sex chromosome system evolved independently. However, we cannot discard the possibility of common origin followed by distinct processes of differentiation. The identification of two new karyotypes with an $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome system in Gymnotiformes makes it the most common among the karyotyped species of the group. Comparisons of these karyotypes and the evolutionary history of the taxa indicate independent origins for their sex chromosomes systems. The recurrent emergence of the $X_1X_1X_2X_2/X_1X_2Y$ system may represent sex chromosomes turnover events in Gymnotiformes.

Keywords: sex chromosome fusion, sexual trivalent, meiosis.

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Introduction

Sex determination mechanisms in vertebrates are varied and may involve environmental and genetics factors. Among the species with genetic sex determination, there are variations that go from those that have the implication of only one locus to those having a morphological sex chromosome system (Ezaz *et al.*, 2006).

Fish is the most interesting group to study sex chromosomes because they have a large diversity of systems, including XY and ZW and their derivatives, with several stages of differentiation (Devlin and Nagahama, 2002). This evidences multiple origins of these systems in the group, even among closely related species (Ezaz *et al.*, 2006). Among the multiple sex chromosome systems registered in fish, the $X_1X_1X_2X_2/X_1X_2Y$ is the most common (Devlin and Nagahama, 2002; Kitano and Peichel, 2012). This type of system arises as a consequence of rearrangements involving autosomes and sex chromosomes or fission of a sex chromosome (White, 1973; Kitano and Peichel, 2012).

Gymnotiformes is the order that includes the electric fishes of the Neotropical region. These species have a high karyotypic variability that is reflected in diploid numbers (2n), karyotypic formula (KF), number and distribution of the nucleolar organizer regions, occurrence of B chromosomes and sex chromosome systems (Almeida-Toledo *et al.*, 1984, 1993, 2000a,b, 2001, 2002; Fernandes-Matioli *et al.*, 1998; Fernandes *et al.*, 2005; Silva and Margarido, 2005; Fonteles *et al.*, 2008; Milhomem *et al.*, 2007, 2008, 2012a,b; Silva *et al.*, 2009; Cardoso *et al.*, 2011; Scacchetti *et al.*, 2011; Mendes *et al.*, 2012; Silva *et al.*, 2013). The group presents simple and multiple sex chromosome systems and heterogamety of males and females (Table 1). This diversity evidences different origins and allows the identification of alternative mechanisms involved in the evolution of the sex chromosomes in this group (Henning *et al.*, 2008, 2011; Almeida-Toledo *et al.*, 2002; Silva *et al.*, 2009).

Brachyhypopomus Mago-Leccia 1994 is one the most diverse genera of Gymnotiformes (Crampton, 2011), but cytogenetic data are known only for two populations of *Brachyhypopomus gauderio* from the Paraná river basin (Almeida-Toledo *et al.*, 2000b; Mendes *et al.*, 2012). In the present work we describe the karyotypes of two species of electric fish of this genus, which have an $X_1X_1X_2X_2/X_1X_2Y$

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Table 1 - Sex chromosome systems in the Order Gymnotiformes.

Taxa	2n	SCS	References
Gymnotidae			
<i>Gymnotus pantanal</i>	39M/40F	X ₁ X ₁ X ₂ X ₂ /X ₁ X ₂ Y	1
Hypopomidae			
<i>Brachyhypopomus gauderio</i>	41M/42F	X ₁ X ₁ X ₂ X ₂ /X ₁ X ₂ Y	2, 3
<i>Brachyhypopomus pinnicaudatus</i>	41M/42F	X ₁ X ₁ X ₂ X ₂ /X ₁ X ₂ Y	4
<i>Brachyhypopomus</i> n. sp. FLAV	43M/44F	X ₁ X ₁ X ₂ X ₂ /X ₁ X ₂ Y	4
Rhamphichthyidae			
<i>Steatogenys elegans</i>	50	ZZ/ZW	5
Sternopygidae			
<i>Eigenmannia virescens</i>	38	ZZ/ZW*	6, 7
<i>Eigenmannia virescens</i>	38	XX/XY	8
<i>Eigenmannia</i> sp. 2	31M/32F	X ₁ X ₁ X ₂ X ₂ /X ₁ X ₂ Y	9

1. Silva and Margarido, 2005; 2. Almeida-Toledo *et al.*, 2000b; 3. Mendes *et al.*, 2012; 4. Present work; 5. Cardoso *et al.*, 2011; 6. Almeida-Toledo *et al.*, 2002; 7. Silva *et al.*, 2009; 8. Almeida-Toledo *et al.*, 2001; 9. Almeida-Toledo *et al.*, 1984. *Different populations have the same type of sex chromosome system but with different mechanisms of origin. SCS + sex chromosome system.

M: male. F: female.

sex chromosome system. We also discuss the process that may be involved in the evolution of these systems in Gymnotiformes.

Material and Methods

We analyzed 23 specimens of *Brachyhypopomus pinnicaudatus* Hopkins, 1991 (10 males and 13 females) and 12 specimens of *Brachyhypopomus* n. sp. FLAV (an undescribed species; Crampton, pers. Comm.; 6 males and 6 females; "FLAV" indicates the first four letters of the specific epithet that the species will receive), both from Reserva de Desenvolvimento Sustentável Mamirauá (Amazonas state, Brazil). All samples were identified by Dr. William G. R. Crampton and their collection was authorized by IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) permit 020/2005 (IBAMA Registration: 207419). The animals were deposited in the ichthyologic collection of Museu Paraense Emílio Goeldi (MPEG 22740, MPEG 22743, MPEG 27107, MPEG 27108, MPEG 27109) and in the ichthyologic collection of the Instituto de Desenvolvimento Sustentável Mamirauá (IDSMIctio000731, IDSMIctio000737, IDSMIctio000738, IDSMIctio000792). Eugenol was used to anesthetize the animals. Metaphase chromosomes were obtained following standard procedures (Bertollo *et al.*, 1978) and were analyzed after sequential C-banding (Sumner, 1972) and classified according to Guerra (1986). Meiotic chromosomes were obtained following standard procedures (Gross *et al.*, 2009).

Results

Males of *Brachyhypopomus pinnicaudatus* presented $2n = 41$ (1m-sm/40st-a), and females had $2n = 42$ (42st-a)

(Figure 1A,C). Males and females *Brachyhypopomus* n. sp. FLAV showed $2n = 43$ (1m-sm/42st-a) and $2n = 44$ (44st-a), respectively (Figure 2A,C). The reduced diploid number in males and the chromosomes morphologies strongly suggest a fusion between an autosome and the Y chromosome giving origin to an X₁X₁X₂X₂/X₁X₂Y sex chromosome system. In *B. pinnicaudatus*, the Y chromosome is metacentric and the X₁ and X₂ chromosomes are acrocentric with similar sizes. On the other hand, in *B. n. sp. FLAV*, the Y chromosome is submetacentric and the X₁ and X₂ chromosomes are acrocentric with different sizes.

Constitutive heterochromatin was detected in the centromeric region of all chromosomes of males and females of the two species and in the terminal region of pair 15 in males and females of *B. n. sp. FLAV* (Figure 2B,D). Furthermore, small heterochromatic bands can be seen in the interstitial and distal regions of pair 1 in males and females of *B. pinnicaudatus* (Figure 1B,D).

Analysis of meiosis allowed the identification of 19 bivalents and 1 trivalent in diplotene cells of *B. pinnicaudatus* (Figure 3A) and of 20 bivalents and 1 trivalent in *B. n. sp. FLAV* (Figure 3B). The trivalents showed two types of association: chiasmatic between X₂-Yq and end-to-end in the PAR region of X₁-Yp (Figure 3A,B).

Discussion

Our data indicate sex-related differences in the karyotypes of *Brachyhypopomus pinnicaudatus* and *B. n. sp. FLAV*. These differences result from the presence of a biarmed chromosome exclusively in the males' karyotypes, which is part of a trivalent during meiosis. The fact that it shows chiasmatic and end-to-end associations suggests that a rearrangement involving a sex chromosome occurred. If the large chromosome had resulted from the rearrangement

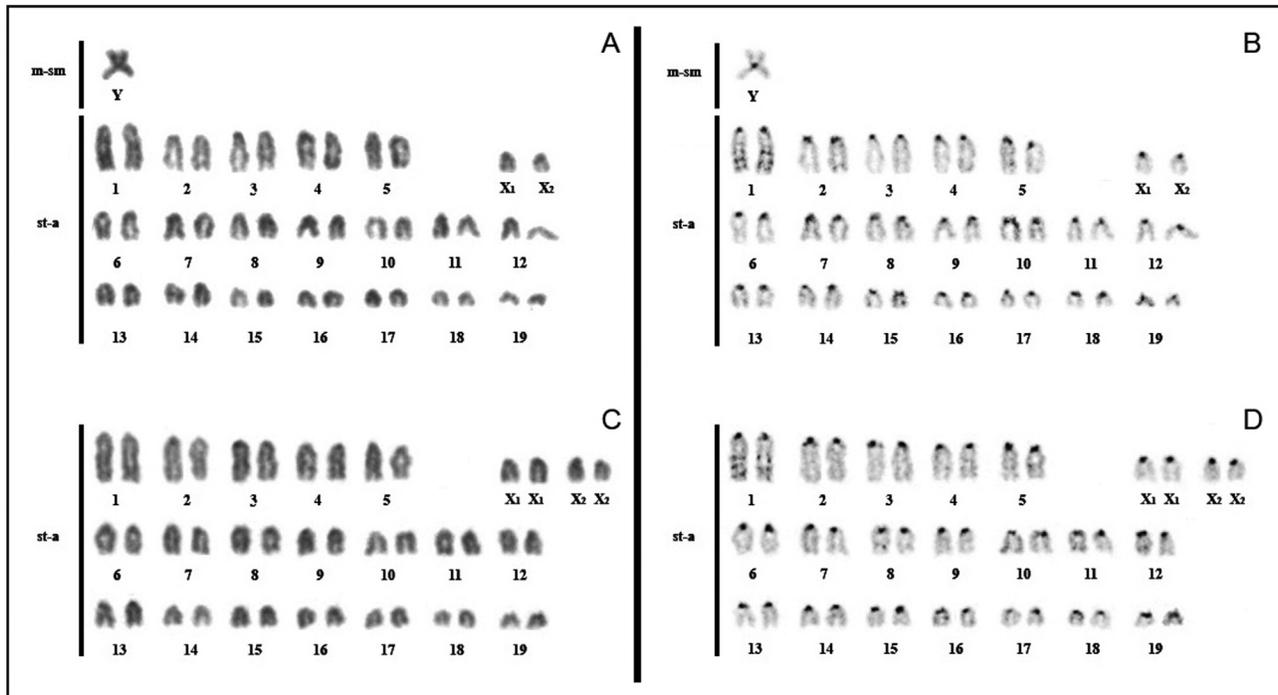


Figure 1 - Karyotypes of males (A, B) and females (C, D) of *Brachyhypopomus pinnicaudatus* after conventional staining (A, C) and C-banding (B, D).

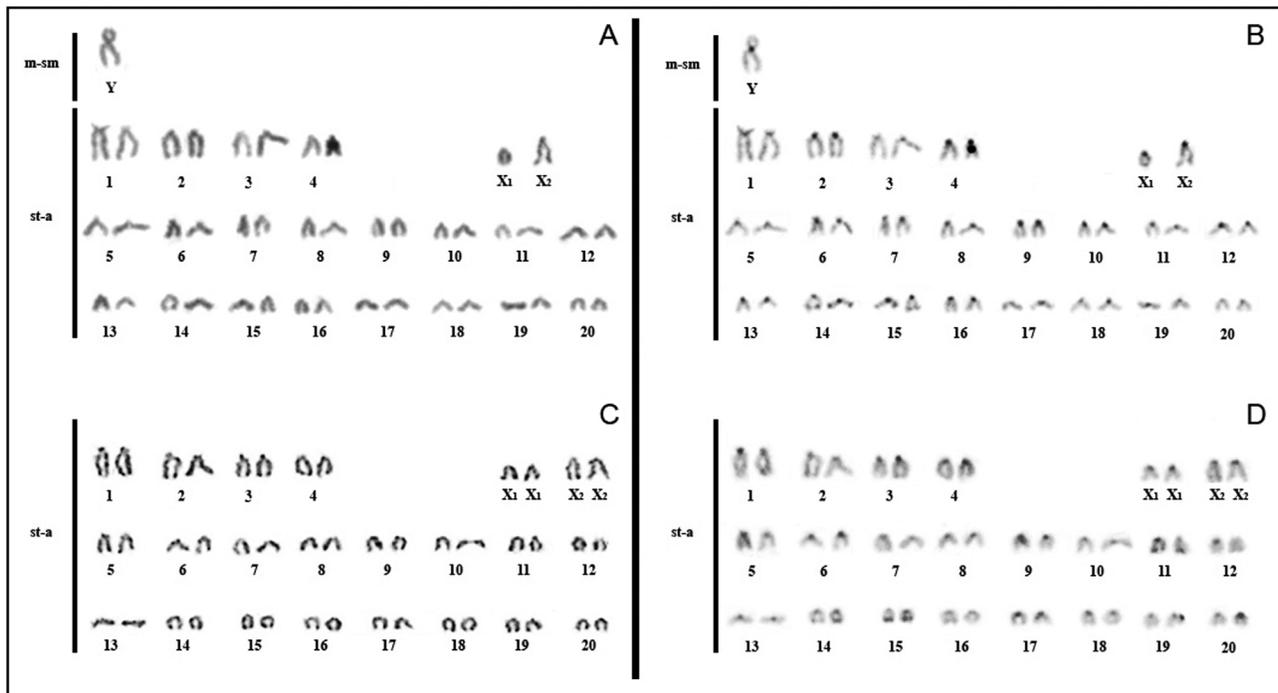


Figure 2 - Karyotypes of males (A, B) and females (C, D) of *Brachyhypopomus* n. sp. FLAV after conventional staining (A, C) and C-banding (B, D).

between two autosomes, only chiasmatic associations would be present (Ueno and Takai, 2008). The association of these variations with the sex and the involvement of a pair of autosomes plus the sex chromosomes indicate that

the two species have a $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome system.

An $X_1X_1X_2X_2/X_1X_2Y$ system has also been previously identified in a population of *B. pinnicaudatus* from

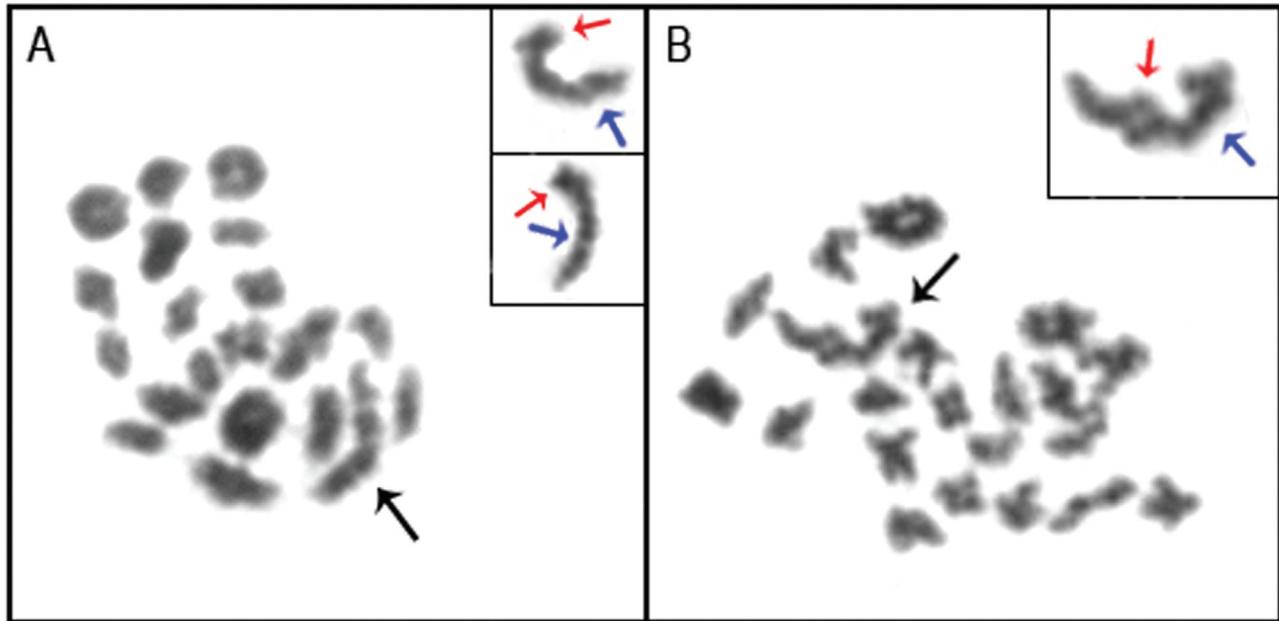


Figure 3 - Meiotic chromosomes of *Brachyhyppopomus pinnicaudatus* (A) and *B. n. sp. FLAV* (B) after conventional staining. Black arrows indicate the sexual trivalent. Boxes: red arrows indicate chiasmata; blue arrows indicate end-to-end pairing.

the Tietê river (Almeida-Toledo *et al.*, 2000b). This population was later identified as *B. gauderio* in a different report dealing with populations of *B. gauderio* from the upper Paraná river (Mendes *et al.*, 2012). However, in a report describing this species, the distribution area of *B. gauderio* did not include the region where the population of the Tietê river was found (Giora and Malabarba, 2009). According to Campos-da-Paz (pers. comm.), this species was introduced into the upper part of the Paraná river (including the Tietê river) (Graça and Pavanelli, 2007). Furthermore, Crampton (pers. comm.) analyzed samples of several localities and confirmed that the population of the Tietê river (Almeida-Toledo *et al.*, 2000B) is *B. gauderio* and that *B. pinnicaudatus* occurs only in the basins of the rivers of the Amazon and Guiana Shield, although *B. gauderio* is endemic to the Paraná-Paraguay system. Therefore, since the population from the Tietê river is not *B. pinnicaudatus* but *B. gauderio*, this is the first record of the X_1X_2Y sex chromosome system in *B. pinnicaudatus*, and also in *B. n. sp. FLAV*.

A review on the occurrence of $X_1X_1X_2X_2/X_1X_2Y$ systems in fishes suggested that a fusion between an autosome and the ancestral Y was the major mechanism for its origin (Kitano and Peichel, 2012). The same mechanism may explain the origin of the multiple sex chromosome systems identified in *B. pinnicaudatus*, *B. n. sp. FLAV* and *B. gauderio* (Almeida-Toledo *et al.*, 2000b; Mendes *et al.*, 2012). In the ancestral karyotype with a simple XX/XY sex chromosome system, the X and Y chromosomes were morphologically undifferentiated (but likely divergent in genetic content) and paired only through the pseudoauto-

somal region (PAR) during meiosis I. A centric fusion between the Y chromosome and an acrocentric autosome would have originated the $X_1X_1X_2X_2/X_1X_2Y$ system (Figure 4A), resulting in a meiotic trivalent involving the sex chromosomes and with two types of association: chiasmatic between X_2-Yq and end-to-end in the X_1-Yp PAR region (Figure 3 and 4B). The regions involved in the chiasmatic association are undifferentiated or poorly differentiated, similar to those found in autosomal bivalents (Figure 3). This suggests a recent origin for these multiple sex chromosome systems, differently from those that only present the end-to-end association, which would be more differentiated (Suzuki *et al.*, 1988).

A similar system was found in the spider *M. ferruginea*, in which an $X_1X_2X_3X_4X_5Y$ system arose through a rearrangement between the homomorphic sex chromosome pair and an autosome. The constitutive heterochromatin pattern of the original sex chromosomes did not differ from those of autosomes and the multiple X chromosomes were generated by non-disjunctions of the sex pair. The structural differentiation of the newly formed X chromosomes was facilitated by their heterochromatinization, observed in the sex chromosomes bivalents during prophase I of females (Kráľ, 2007).

The occurrence of $X_1X_1X_2X_2/X_1X_2Y$ systems in three species of the same genus suggests a common origin. However, whereas in *B. pinnicaudatus* and *B. gauderio* the neo-Y is metacentric and the X_1 and X_2 are acrocentric of similar size, in *B. n. sp. FLAV*, the neo-Y is submetacentric and the X_1 and X_2 are acrocentric of different sizes (Figure 5). Moreover, meiotic chromosome pairing allowed us to

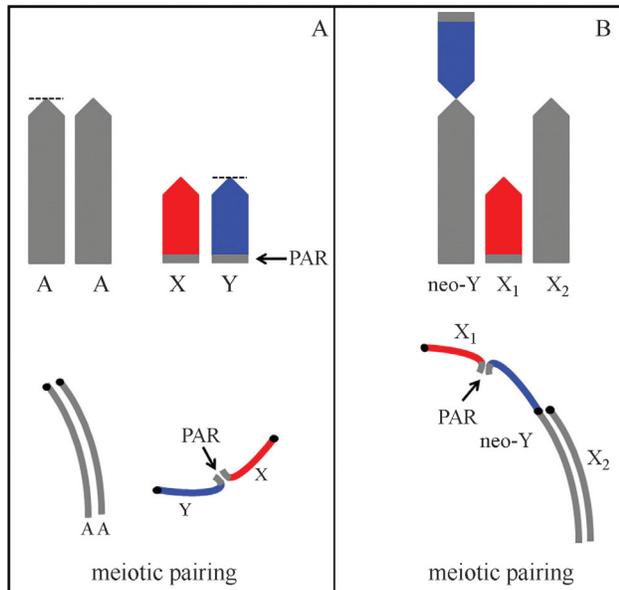


Figure 4 - Probable mechanism of evolution of the X_1X_2Y sex systems in *Brachyhypopomus*: an ancestral homomorphic XX/XY sex system (A) underwent a fusion between an autosome and the Y chromosome (B) originating a multiple X_1X_2Y system.

Species	X_1	X_2	neo-Y	Idiogram
<i>B. gauderio</i> *				
<i>B. pinnicaudatus</i>				
<i>B. n. sp. FLAV</i>				

Figure 5 - Morphology of the sex chromosomes in the genus *Brachyhypopomus*. *Species analyzed by Almeida-Toledo *et al.* (2000b).

conclude that in *B. n. sp. FLAV*, the X_1 is smaller than X_2 because the neo-Y has a chiasmatic association with the larger X chromosome and only an end-to-end association with the smaller X. Meiotic chromosome pairing also confirmed that the X_1 and X_2 have similar sizes in *B. pinnicaudatus*. These findings suggest that the ancestral sex chromosomes (XY) and the autosomes that evolved to form the $X_1X_1X_2X_2/X_1X_2Y$ systems in *B. pinnicaudatus* and *B. gauderio* had a similar size. On the other hand, in *B. n. sp. FLAV*, the sex chromosomes were small acrocentrics and the autosomes were large acrocentrics. These data indicate that *B. pinnicaudatus* and *B. gauderio* likely share a common ancestral sex chromosome system. This hypothesis is reinforced by the fact that they are considered sister-species (Crampton, 2011) and also because they likely

share the same karyotype (same $2n$ and KF). On the other hand, the sex chromosome system of *B. n. sp. FLAV* likely had an independent origin. Other differences in the karyotype of this species ($2n$ and KF) result from an event of fusion/fission involving autosomes. This may suggest that this species is less related with *B. pinnicaudatus* and *B. gauderio* than these two are between themselves, which may reinforce the hypothesis of an independent origin for the *B. n. sp. FLAV* sex chromosome system. Independent origins of sex chromosome systems in related species are well documented in fish (Devlin and Nagahama, 2002). In Gymnotiformes, several kinds of systems have been registered, and their disagreement with the phylogeny is consistent with this pattern (Silva and Margarido, 2005; Henning *et al.*, 2010). Despite the present evidence for the independent origins of $X_1X_1X_2X_2/X_1X_2Y$ systems in *Brachyhypopomus*, we cannot discard the possibility of a common origin followed by alternative processes of differentiation.

It is important to point out that the emergence of $X_1X_1X_2X_2/X_1X_2Y$ systems in Gymnotiformes is more recurrent than that of other types of systems (Table 1). This suggests an advantage in the fusion between the primitive Y and an autosome. Theoretical models suggest that sex chromosomes evolve by suppression of recombination in regions that harbor sexually antagonistic genes (genes with alleles that confer differential fitness in males and females) tightly linked to the sex determination locus, and that this sexual antagonism may be the driving force behind this process (Charlesworth *et al.*, 2005; van Doorn and Kirkpatrick, 2007; Kikuchi and Hamaguchi, 2013). In prot-simple sex systems (XY, ZW), inversions play an important role in the suppression of recombination and chromosome differentiation (Charlesworth *et al.*, 2005). On the other hand, when the sexually antagonistic gene is unlinked to the sex chromosomes, other mechanisms such as translocations or fusions involving an autosome and the sex chromosomes may create a linkage between them (multiple sex chromosome systems) and suppress crossing-over in the regions around the breakpoint in heterozygous (Charlesworth *et al.*, 2005; Kitano and Peichel, 2012). Moreover, in this last situation, the generation of neo-sex chromosomes as a neo-Y may avoid the degeneration of the ancestral Y caused by the suppression of recombination that promotes the accumulation of deleterious mutations and the expansion of the non-recombining segment, which may cause haplo-insufficiency (Volf *et al.*, 2007; Blaser *et al.*, 2012; Livernois *et al.*, 2012). The recurrent emergence of the $X_1X_1X_2X_2/X_1X_2Y$ system may represent events of sex chromosomes turnover that are driven by the process explained above. Future analyses aiming to detect possible closely related species with different sex chromosome systems and to understand the relationship between these systems may help to elucidate this question.

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