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Original Article

Chromosomal Mapping of Transposable Elements of the *Rex* Family in the Bristlenose Catfish, *Ancistrus* (Siluriformes, Loricariidae), from the Amazonian Region

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Abstract

Repetitive DNA sequences are present in the genome of basically every known organism, and transposable elements (TE) are one of the most representative sequences involved in chromosomal rearrangements and the genomic evolution of eukaryotes. In fish, the non-LTR retrotransposon TEs, *Rex*1, *Rex*3, and *Rex*6, are widely distributed in fish genomes and are the best-characterized TEs in several species. In the current study, three of these retroelements were physically mapped, through fluorescent in situ hybridization (FISH), in 7 species (71 specimens) of the genus *Ancistrus*, known as bristlenose catfish: *Ancistrus ranunculus*, *Ancistrus* sp. 1 "Purus," *Ancistrus* sp. 2 "Catalão," *Ancistrus dolichopterus*, *Ancistrus maximus, Ancistrus* aff. *dolichopterus*, and *Ancistrus dubius*. *Rex*1, *Rex*3, and *Rex*6 showed a cluster distribution, mainly in the terminal and pericentromeric portions, in heterochromatic and euchromatic regions, and did not occur in sexual chromosomes; however, the number and position of the clusters varied between species. This TE distribution suggests its implication in the karyotypic evolution of these species, without affecting the rise of sexual chromosome systems in *Ancistrus*, in view of their chromosomal variation.

Subject areas: Conservation genetics and biodiversity; Genomics and gene mapping
Key words: chromosomal evolution, fish, Loricariidae, repetitive DNA, retrotransposons

Introduction

Among the diverse repetitive DNA sequences that are present in eukaryote genome, the transposable elements (TEs) are the most representative. They are defined as repetitive DNA sequences that are capable of moving along the chromosome or of transposing between nonhomologous sites in a genome (Capy et al. 1998). This ability to transpose can cause structural changes in the chromosomes, such as duplications and deletions, which lead to polymorphisms as a

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result of the variation in the number of copies of these elements in the species, thereby inducing alterations in gene expression (Capy et al. 1998). According to Blass et al. (2012), these elements have the potential to increase biodiversity, molecular domestication and evolutionary transitions, through gene mutations, alteration in the regulation process and by generating new genes.

TEs can be divided in 2 classes, based on their structure and the transposition mechanism in the genome. The class I is represented by the retrotransposons, which transpose through the use of the reverse transcriptase, an enzyme that promotes the synthesis of a DNA strand from a RNA one. Each complete replication cycle produces one new copy (Charlesworth et al. 1994; Wicker et al. 2007). The class II are the transposons, whose transposing mechanism acts through cutting and inserting sequences in the DNA without a RNA intermediate and contains 2 subclasses, which are distinguished by the number of DNA strands that are cut during transposition, but neither moves via an RNA intermediate (Charlesworth et al. 1994; Wicker et al. 2007; Böhne et al. 2008).

It is currently known that TEs are very important in eukaryotic genome evolution, being involved in chromosomal rearrangement processes (Raskina et al. 2008), expression and genes regulation (Medstrand et al. 2005; Shapiro and von Sternberg 2005), replication of DNA (Li et al. 2002) and sexual chromosome differentiation (Harvey et al. 2002; Steinemann and Steinemann 2005; Pokorná et al. 2011).

In regards to fishes, TEs are directly related to their genome evolution, with all types of described retroelements having already been found in the genome of these animals (Okada et al. 1997; Poulter et al. 1999; Volff et al. 1999; Aparicio et al. 2002). In this group of vertebrates, TEs of the non-LTR retrotransposon type, *Rex*1, *Rex*3, and *Rex*6, are those which have a better characterization so far, being active in the evolution of this group and widely distributed in their genome (Volff et al. 1999, 2000, 2001).

Some studies presented a diversity of distribution patterns for these elements in several groups of fishes, such as Cichlidae (Mazzuchelli and Martins 2009; Gross et al. 2009; Valente et al. 2011; Schneider et al. 2013), Tetraodontidae (da Silva et al. 2002; Bouneau et al. 2003; Fischer et al. 2004) and Loricariidae (Ferreira et al. 2011).

Valente et al. (2011) presented the chromosomal mapping of the retroelements *Rex*1, *Rex*3, and *Rex*6 in 8 species of cichlids and noticed a deposition of these elements in the pericentromeric region of the chromosomes. Other studies also show the preference of these retroelements for heterochromatic regions (da Silva et al. 2002; Fischer et al. 2004), regions that can usually accumulate more mutations without suffering major consequences.

Some authors also relate these transposable and retrotransposable elements with sexual chromosome differentiation in some fish groups such as Cyprinodontiformes (Volff et al. 2000; Böhne et al. 2012), Characiformes (Marreta et al. 2012; Terencio et al. 2012), and Beloniformes (Takehana et al. 2012). In *Semaprochilodus taeniurus,* Terencio et al. (2012) observed a significant increase in the size of the W chromosome due to repetitive DNA accumulation, and among these DNA sequences was *Rex*1.

In Loricariidae, the largest family of the Siluriformes with around 916 valid species (Eschmeyer and Fong 2016), there are only a few studies available covering transposable elements. Ferreira et al. (2011) performed a chromosomal mapping of the TEs *Rex*1 and *Rex*3 in 3 species from this family, where they observed small clusters dispersed throughout all the chromosomes, in both heterochromatin and euchromatin varieties.

One of the subfamilies with highest chromosomal variation in Loricariidae is Hypostominae, in which TEs studies are still scarce. The genus *Ancistrus*, allocated to this subfamily (tribe Ancistrini), is an example of such diversity, varying in regards to its diploid number, karyotypic formula, heterochromatic block distribution, 5S and 18S ribosomal DNA positions, and in having several sexual determination systems among the species analyzed so far (de Oliveira et al. 2007, 2008, 2009; Mariotto et al. 2011; Favarato et al. 2016).

In view of the karyotypic variation observed in *Ancistrus* and the TEs relation to genomic evolution already recorded for fishes, the current study is aimed at the chromosomal mapping of the retrotransposable elements *Rex*1, *Rex*3, and *Rex*6 in 7 species of the genus, so as to correlate these sequences distributional patterns with chromosomal evolution inside the genus.

Materials and Methods

Seventy-one specimens (males and females), from 7 species of *Ancistrus*, previously collected in 7 Amazonian localities (Figure 1), were analyzed. All specimens were identified by specialists, using the most current nomenclature that differs from the nomenclature of other studies, and voucher material was deposited in the fish collection of the *Instituto Nacional de Pesquisa da Amazônia* (Table 1). Cell suspensions were obtained from kidney tissue following the protocol of Bertollo et al. (1978). The 3 retroelements were isolated through the polymerase chain reaction (PCR). The following primer pairs were used: RTX1-F1 (5′-TTC TCC AGT GCC TTC AAC ACC-3′) and RTX1-R1 (5′- TCC CTC AGC AGA AAG AGT CTG CTC-3′) for *Rex*1 (Volff et al. 2000); RTX3-F3 (5′-CGG TGA YAA AGG GCA GCC CTG-3′) and RTX3-R3 (5′-TGG CAG ACN GGG GTG GTG GT-3′) for *Rex*3 (Volff et al. 1999); and *Rex*6-Medf1 (5′-TAA AGC ATA CAT GGA GCG CCA C-3′), and *Rex*6-Medr2 (5′-GGT CCT CTA CCA GAG GCC TGG G-3′) for *Rex*6 (Volff et al. 2001). PCR products were checked in 1% agarose gel, quantified in a NanoVue Plus spectrophotometer (GE Healthcare) and used as probes for the fluorescent in situ hybridization (FISH).

FISH was performed in accordance to the protocol of Pinkel et al. (1986), with 77% stringency. The PCR products of *Rex*1, *Rex*3, and *Rex*6 were stained through nick translation with digoxigenin-11-dUTP (Dig-Nick Translation mix; Roche), according to the protocol of the manufacturer's manual. Hybridization signal detection was performed with anti-digoxigenin-rhodamine (Roche Applied Science). Afterwards, the chromosomes were counterstained with DAPI, analyzed under an Olympus BX51 epifluorescence microscope and classified according to Levan et al. (1964).

Results

Cytogenetic Mapping of *Rex*1

Staining for retroelement *Rex*1 presented itself as conspicuous blocks in all species analyzed, with variation in the number of chromosomes stained and in the blocks' positions in the chromosomes. In *Ancistrus* sp. "Purus" and *Ancistrus maximus* this sequence was detected in the pericentromeric region of the chromosomes (Figure 2a, d), with less evident signs on the first species. In *A.* sp. "Purus" there were more conspicuous markings on pair 1, while in *A. maximus* they were on pairs 3, 4, 11, and 19. For the species *Ancistrus* sp. "Catalão", *Ancistrus dubius, Ancistrus ranunculus*, *Ancistrus dolichopterus*, *Ancistrus* aff. *dolichopterus* (Figure 2b, c, e, f, g, respectively) the *Rex*1 blocks were positioned mainly on terminal portions. *Ancistrus* sp. "Catalão" differed from the others in having diffuse markings and only on a few chromosomes, while on the other species the markings are much more intense and positioned

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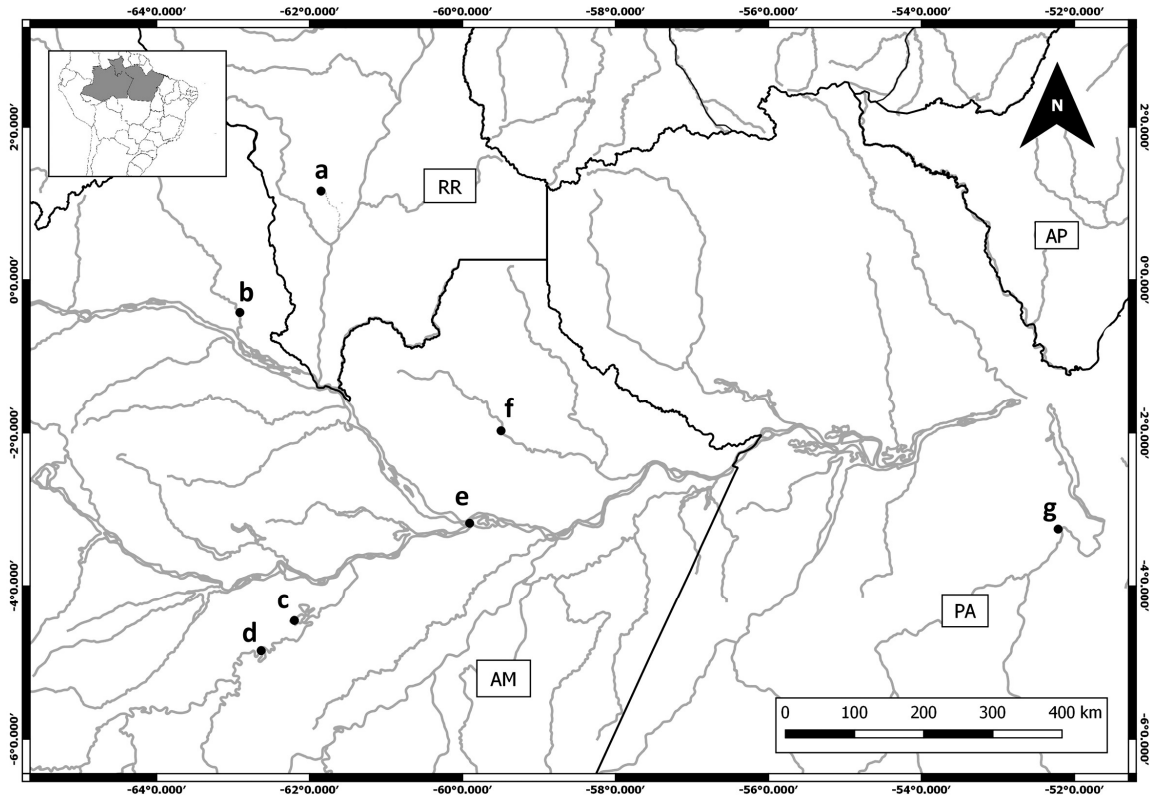

Figure 1. Map showing the collection sites for the 7 species of the genus *Ancistrus* in this study. (a) *Ancistrus* maximus; (b) *Ancistrus dolichopterus*; (c) *Ancistrus* sp. Purus; (d) *Ancistrus* aff. *dolichopterus*; (e) *Ancistrus* sp. Catalão; (f) *Ancistrus* dubius, and (g) *Ancistrus* ranunculus. In the box map of Brazil. AM, Amazonas; PA, Para; RR, Roraima.

Table 1. Species, number of specimens, collection sites, and deposit number in the fish collection of INPA

Names utilized by[a]	Corrected names	M	F	Locality/coordinates	City/state	Vouchers
Ancistrus ranunculus	*Ancistrus ranunculus*	3	2	River Xingu/03°15′21″S 52°12′45″W	Altamira/Pará	INPA 25624
Ancistrus sp. "Purus"	*Ancistrus* sp. "Purus"	5	7	River Purus/04°51′S 62°38′W	Amazonas	INPA 25625
Ancistrus sp. "Catalão"	*Ancistrus* sp. "Catalão"	5	7	Lake Catalão/03°10′45″S 59°54′25″W	Manaus/Amazonas	INPA 25626
Ancistrus sp. "Barcelos"	*Ancistrus dolichopterus*	7	4	River Demeni/00°25′19″S 62°54′42″W/ Negro river basin	Amazonas	INPA 25627
Ancistrus sp. "Macoari"	*Ancistrus maximus*	6	6	Igarapé Macoari/01°10′N 61°51′W/ Branco river affluent	Roraima	INPA 25629
Ancistrus sp. "Piagaçu"	*Ancistrus* aff. *dolichopterus*	3	4	Lake Aiapuá/04°27′26″S 62°11′56″W/ Purus river basin	Amazonas	INPA 25630
Ancistrus sp. "Balbina"	*Ancistrus dubius*	6	6	Igarapé Barretinho/ 01°58′20 ″S 59°29'48″W	Balbina/Amazonas	INPA 25633

INPA, Instituto Nacional de Pesquisa da Amazônia; M, males; F, females.
[a]de Oliveira (2006); de Oliveira et al. (2007, 2008, 2009).

in several chromosomes. Large blocks of *Rex*1 were observed in *A. dubius* (pairs 4, 5, 7, and 8), *A. ranunculus* (pairs 19 and 21), *A. dolichopterus* (pairs 2 and 3), and in *A.* aff. *dolichopterus* (pairs 1, 4, and 5). It is noteworthy that there were exclusive markings in *A. ranunculus*, with a pericentromeric marking on pair 20, and in *A.* aff. *dolichopterus*, with bitelomeric markings on pair 4.

Cytogenetic Mapping of *Rex*3

Overall, the distribution pattern of *Rex*3 obtained by FISH was similar to that of *Rex*1, with conspicuous markings on the terminal and pericentromeric regions of the chromosomes (Figure 3). In *A.* sp. "Purus," *A.* sp. "Catalão," and *A. maximus*, the markings were observed in the pericentromeric regions (Figure 3a, b, d). In these species the markings seem diffuse and on just a few chromosomes, visible only on pairs 1, 2, 3, 4, and 12 in *A.* sp. "Purus," on pair 12 in *A.* sp. "Catalão" and on pairs 2 and 19 in *A. maximus*.

In *A. dubius*, *A. ranunculus*, *A. dolichopterus*, and *A.* aff. *dolichopterus* the markings were more evident and predominant on the distal regions, varying in some cases between the long and short arm or in both arms. *Ancistrus dubius* showed terminal markings on pairs 3, 6, and 7, with bitelomeric markings on pair 6 (Figure 3c). *Ancistrus ranunculus* showed large terminal blocks on pairs 2, 4,

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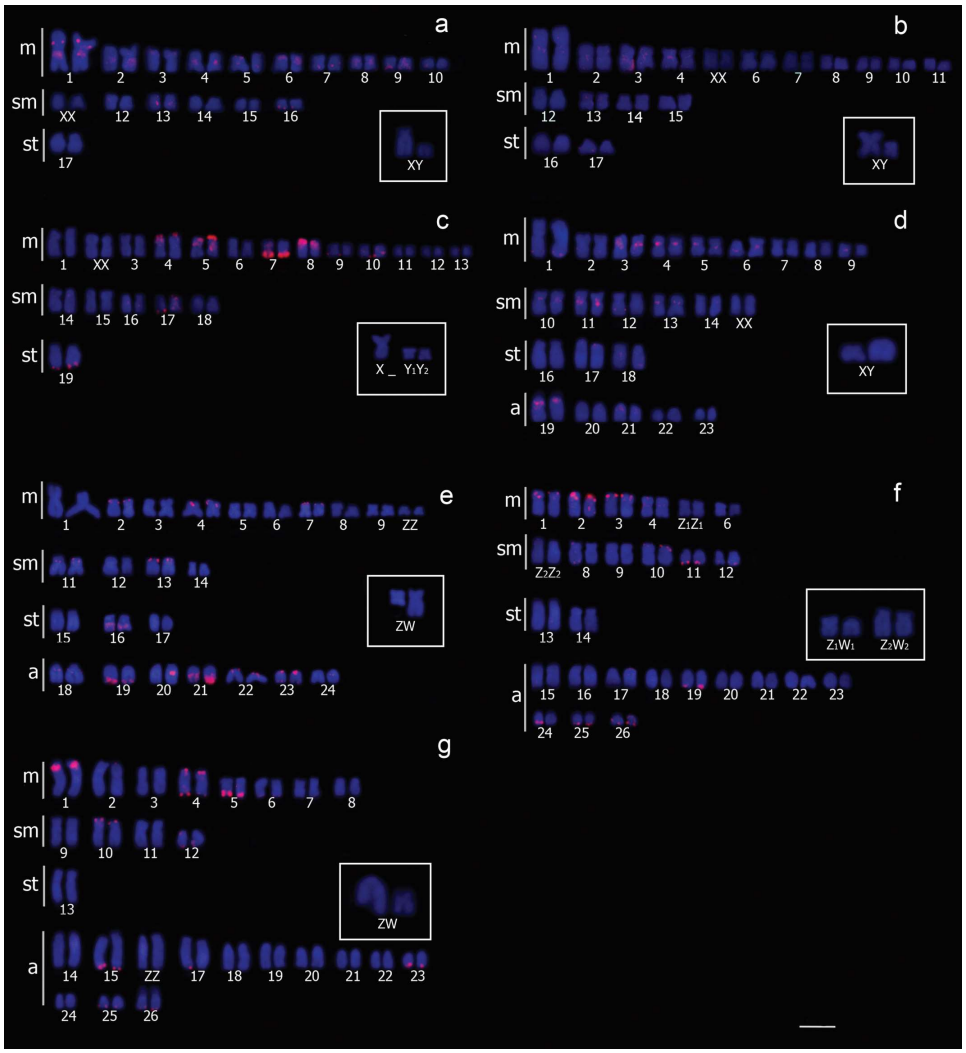

Figure 2. Karyotype of the 7 species of *Ancistrus* analyzed by FISH with retroelement *Rex1* (red) probes and counterstained with DAPI: (a) *Ancistrus* sp. Purus; (b) *Ancistrus* sp. Catalão; (c) *Ancistrus dubius*; (d) *Ancistrus maximus*; (e) *Ancistrus ranunculus*; (f) *Ancistrus dolichopterus*; (g) *Ancistrus* aff. *dolichopterus*. Bar = 10 μm.

18, 19, and 23, and pericentromeric markings on pairs 11 and 22 (Figure 3e). *Ancistrus dolichopterus* presented a similar pattern, with terminal markings on pairs 2 and 15, and pericentromeric ones on pairs 1, 8, and 10 (Figure 3f). In *A.* aff. *dolichopterus* there were only terminal markings on pairs 1, 3, 6, 15, and 20 (Figure 3g). The last 3 species had, in common, the presence of large blocks of *Rex*3 involving a whole chromosome arm, as seen on pairs 19 and 23 of *A. ranunculus*, on pair 15 of *A. dolichopterus* and *A.* aff. *dolichopterus*. In *A. dolichopterus*, there were also *Rex*3 markings through almost the whole extension of chromosomes 8 and 10.

Cytogenetic Mapping of *Rex*6

Retroelement *Rex*6 was mainly distributed on the terminal regions of the chromosomes of all species (Figure 4), however, some exceptions were observed. In *Ancistrus* sp. "Purus," the markings were observed in the pericentromeric region of pairs 1, 2, 3, 4, 5, and 13, and in *A. maximus* on pairs 4, 5, 10, and 19 (Figure 4a, d), while in *A.* sp. "Catalão" there were no visible markings for this retroelement (Figure 4b).

In *A. dubius*, terminal markings were observed on pairs 4, 6, 7, and 9, in *A. ranunculus* on pairs 5, 12, 16, 20, 21, and 24, in *A. dolichopterus* on pairs 1, 3, 9, 25, and 26, and in *A.* aff. *dolichopterus* on pairs 1, 2, 3, 5, 6, 7, 12, 20, 24, and 26 (Figure 4c, e, f, g). *Ancistrus ranunculus* had three pairs (16, 17, and 18) where the markings were positioned in the interstitial regions of the long arms (Figure 4e). Furthermore, *A. dolichopterus* and *A.* aff. *dolichopterus* presented a large block of *Rex*6 in pair 15, which extended into the pericentromeric region.

None of the 3 retroelements analyzed were located in the sexual chromosomes of the species studied.

Discussion

The physical mapping, through FISH, of the non-LTR retrotransposons *Rex*1, *Rex*3, and *Rex*6, has presented different organization patterns in fishes. However, they are found mainly in regions of heterochromatin or dispersed throughout the genome (Ferreira et al. 2011).

In agreement with previous studies involving all the current studied species, the heterochromatic blocks were distinct and positioned on centromeric, terminal, and interstitial regions of some of

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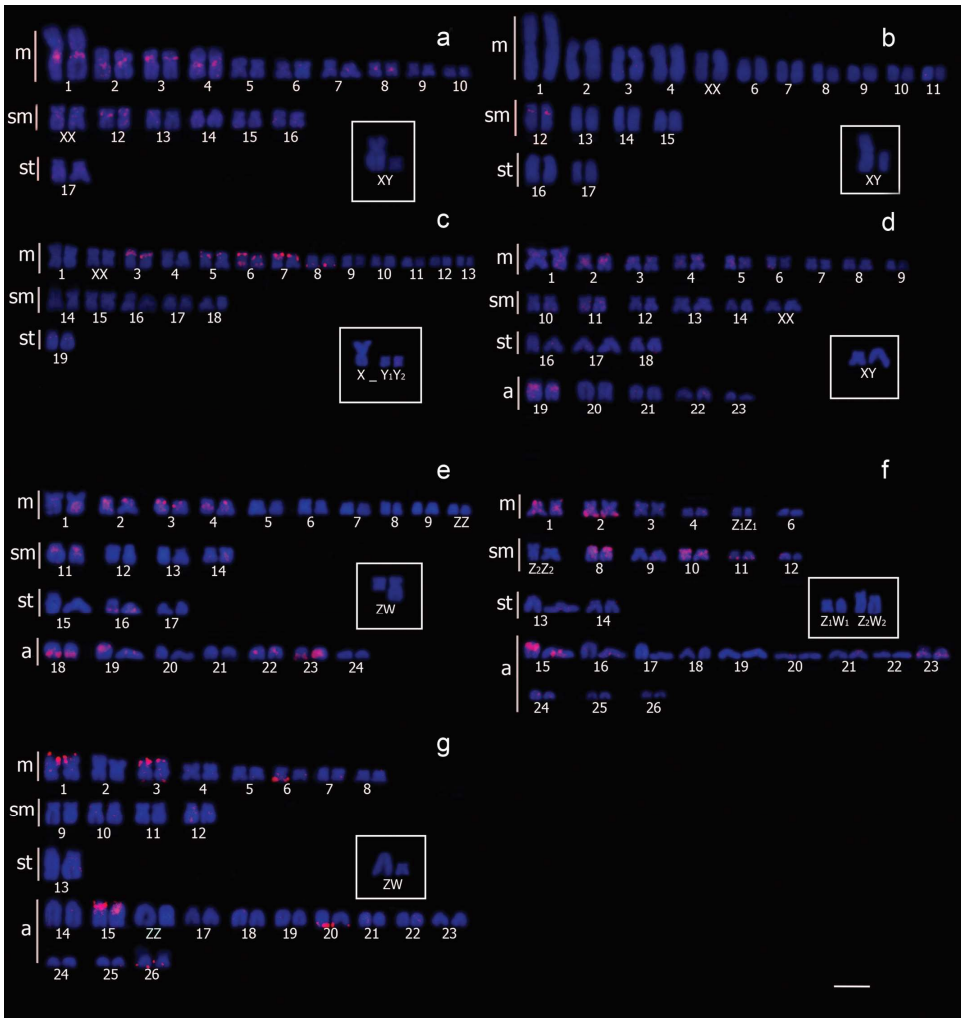

Figure 3. Karyotype of the 7 species of *Ancistrus* analyzed by FISH with retroelement *Rex3* (red) probes and counterstained with DAPI: (a) *Ancistrus* sp. Purus; (b) *Ancistrus* sp. Catalão; (c) *Ancistrus dubius*; (d) *Ancistrus maximus*; (e) *Ancistrus ranunculus*; (f) *Ancistrus dolichopterus*; (g) *Ancistrus* aff. *dolichopterus*. Bar = 10 μm.

the homologous chromosomes pair (de Oliveira 2006; de Oliveira et al. 2007, 2008, 2009). On the other hand, the mapping of the 3 *Rex* retrotransposons in these species showed them to be arranged in clusters, allocated mainly on the distal and pericentromeric regions in the majority of the chromosomes (Figures 2–4), being present both in heterochromatin as in euchromatin varieties.

Several studies have demonstrated the tendency of accumulation of retroelements in the heterochromatin regions in many groups of fishes (da Silva et al. 2002; Bouneau et al. 2003; Fischer et al. 2004; Ozouf-Costaz et al. 2004; Gross et al. 2009; Valente et al. 2011; Voltolin et al. 2013). However, this association was seen in very few chromosomes of the species analyzed here. Only *A.* sp. "Purus" and *A.* aff. *dolichopterus* presented some relationship between heterochromatic blocks and retroelement position. In the former, there were *Rex*1, *Rex*3, and *Rex*6 sites on pairs 1 and 2, and extra *Rex*3 sites, on pairs 3, 8, and 12, all coinciding with heterochromatic blocks. Whereas in the other species, just a heterochromatic block in the pair 1, was coincident with markings of the 3 retrotransposons.

Ferreira et al. (2011), while mapping the retroelements *Rex*1 and *Rex*3 in species from the subfamily Hypoptopomatinae, observed a similar positioning of these RTEs in the heterochromatin and euchromatin regions. This distribution was also recorded in other species from the Loricariidae family (Blanco 2012; Traldi et al. 2013), suggesting that this can be a characteristic of the family. The preferred position of *Rex*1, *Rex*3, and *Rex*6 in heterochromatic regions suggests an epigenetic mechanism regulating these elements to avoid an excessive propagation of them in the genome, since the presence of heterochromatin can regulate expression and dispersion of these sequences without altering the nucleotide sequence (Okamoto and Hirochika 2001; Richards et al. 2010). Since the induced variation of the TEs depends on their activity, much of the evolutionary potential of these retroelements is directed by its control and epigenetic regulation. Thus, TE regulation can have a significant relevance in some key mechanisms of genome evolution, including reactivation through induced stress and invasion by new genomes (Slotkin and Martienssen 2007). Although RTEs are silent most of the time, maintenance of these sequences implies a role in the maintenance of genetic variability (Mansour 2007) and thus TEs promote phenotypic and genetic variability among individuals due to their polymorphic position, causing phenomena known as mosaicism and variegation (Slotkin and Martienssen 2007). The TEs are common components in several epigenetic mechanisms and, according

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Figure 4. Karyotype of the 7 species of *Ancistrus* analyzed by FISH with retroelement *Rex6* (red) probes and counterstained with DAPI: (a) *Ancistrus* sp. Purus; (b) *Ancistrus* sp. Catalão; (c) *Ancistrus dubius*; (d) *Ancistrus maximus*; (e) *Ancistrus ranunculus*; (f) *Ancistrus dolichopterus*; (g) *Ancistrus* aff. *dolichopterus*. Bar = 10 μm.

to Slotkin and Martienssen (2007), epigenetic regulation of genetic expression correlated to chromosomal roles suggests that TEs were the original targets of this type of regulation. In fact, over the course of millions of years, these elements have reached a dynamic balance between negative effects at an individual level and positive effects at a genome level (Kazazian 2007).

Based on what was exposed above, the position of these transposable elements in euchromatic regions appears to have a major importance in the genomic evolution of *Ancistrus* species, since when they insert in euchromatic regions, which are rich in genes, these elements can generate mutations, affect levels of genetic expression and patterns of DNA recombination, and interfere in the organization of the genomic architecture (Kidwell and Lisch 1997; Kidwell 2002; Le Rouzic and Capy 2005). That would explain the large chromosomal variability observed in the species analyzed, which vary in regards to diploid number, karyotypic formula, presence of sexual chromosomes and types of sexual chromosome systems. Although the sequence positions were similar, the RTEs distribution in the species of the genus *Ancistrus* differed from the pattern already observed for other species of the family Loricariidae, in which these elements are dispersed through basically all the chromosomes, while in *Ancistrus* these elements were distributed in clusters only in some complement pairs (Figures 2–4).

Some studies have shown that transposable elements are involved in the processes of differentiation in sexual systems (Harvey et al. 2002; Steinemann and Steinemann 2005; Terencio et al. 2012). Although the seven species of the current study have different sexual chromosome systems, both simple and multiple, there was no apparent relation of the elements *Rex*1, *Rex*3, and *Rex6* in the rise and differentiation of these systems, since there were no clusters of these elements in the sexual chromosomes studied. Ozouf-Costaz et al. (2004), in studies with *Chionodraco hamatus*, showed the involvement of the retrotransposon *Rex*3 and the transposon Tc1-like in the structure of the sexual chromosomes of that species. In *Harttia carvalhoi* (Loricariidae), Blanco (2012) suggests that a deposition of retroelements *Rex*1, *Rex*3, and *Rex6* in the pericentromeric region of chromosome X might have influenced its fission, which culminated in the formation of the chromosomes Y_1 and Y_2.

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Although the accumulation of repetitive sequences, including mobile elements of the *Rex* family, has already been associated with sexual chromosome differentiation, the different sexual systems found in *Ancistrus* probably have different origins. Several studies associate the preferred presence of repetitive sequences to sexual chromosomes and heterochromatin regions (Terencio et al. 2012). However, it has already been observed that some non-LTR integrate into specific sites of the genome, such as the R1 and R2 of *Drosophila melanogaster* and *Bombix mori*, which show a preference for rDNA sites. Others, such as TRAS1 and SART1, prefer telomeric regions, as seen in *B. mori*. The elements Ty3 of *Saccharomyces cerevisae* show specificity for a few nucleotides in start sites for the transcription of RNA polymerase III and transcriptional factors, such as TFIIIB and TFIIIC (Kazazian 2004).

While observing the heterochromatin distribution in chromosomes of *Ancistrus*, it was noted that the sexual chromosomes are never heterochromatic. Only *A. ranunculus* has the long arm of the W chromosome completely heterochromatic (de Oliveira 2006; de Oliveira et al. 2007, 2008, 2009). Besides evidencing recent sexual systems, the prevalence of euchromatin in the sexual chromosomes of the species analyzed here explains the absence of blocks of *Rex*1, *Rex*3, and *Rex*6 on them, corroborating the hypothesis that the origin of sexual systems in this genus may have been influenced by other sequences, still unmapped in the group.

The RTEs have been reported as being co-positioned with ribosomal genes in several groups (Eickbush and Malik 2002; Kazazian 2004). Zhang et al. (2008) hypothesized that there is a distinct niche of the ribosomal genes that allows for a series of mobile elements to be maintained in them. These can have influence on the regulation of rRNA synthesis due to possible recombination events, or simply by the success of the parasitic role they have (Einckbush and Einckbush 2007). There was no retroelement *Rex* deposition on rDNA 45S sites in the *Ancistrus* species studied, although in *A. maximus* and *A. ranunculus* these flank the rDNA18S sites and are possibly co-positioned with rDNA 5S sites (Favarato et al. 2016).

Although still scarce, available data on transposable elements in fishes has shown a diverse organization pattern. In cichlids, for example, RTE mapping has shown a preference of these elements for regions of centromeric and telomeric heterochromatin in most chromosomes (Gross et al. 2009; Mazzuchelli and Martins 2009; Valente et al. 2011). In siluriforms the pattern is more disperse, as observed in *Harttia* (Blanco 2012), and is made up of small blocks distributed throughout all the chromosomes in species of Hypoptopomatinae (Ferreira et al. 2011).

However, when comparing the current results with others for the family Loricariidae, the genus *Ancistrus* is the taxon that presents the smallest amount of retroelements *Rex*1, *Rex*3, and *Rex*6 distributed in its genome. In the species currently analyzed, it is possible to state that the dispersion of *Rex*1, *Rex*3, and *Rex* 6 is related to the karyotypic evolution of the genus *Ancistrus* and the position of the ribosomal sequences 18S and 5S, but without acting on the rise of different sexual systems found in the group. Furthermore, the cluster distribution pattern of these elements, especially in the terminal portions of the chromosomes, is distinct. Although the transposable elements are important features of vertebrate species, reliable information regarding its evolutionary dynamic and its effect on the evolution and differentiation of fish genome is still scarce. If these repetitive sequences are still considered "junk DNA" it is a matter of dispute that it needs further study. However, it is unwarranted to deny the role TEs play in important events that lead to speciation and processes that allow for phenotypic plasticity and genomic malleability, as observed in the genus *Ancistrus*.

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