RESEARCH NOTE

Physical mapping of $(GATA)_n$ and $(TTAGGG)_n$ sequences in species of *Hypostomus* (Siluriformes, Loricariidae)

JOSIANE BACCARIN TRALDI[1]*, DANIEL RODRIGUES BLANCO[1], MARCELO RICARDO VICARI[2], JULIANA DE FÁTIMA MARTINEZ[1], ROBERTO LARIDONDO LUI[1], ROBERTO FERREIRA ARTONI[2] and ORLANDO MOREIRA-FILHO[1]

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

[Traldi J. B., Blanco D. R., Vicari M. R., Martinez J. F., Lui R. L., Artoni R. F. and Moreira-Filho O. 2013 Physical mapping of $(GATA)_n$ and $(TTAGGG)_n$ sequences in species of *Hypostomus* (Siluriformes, Loricariidae). *J. Genet.* **92**, 127–130]

Introduction

Hypostomus, a genus of Loricariidae, is one of the most diversified and widely distributed genera among fishes of South America (Weber 2003). It is the dominant genus of armoured catfish in Brazilian rivers (Weber 2003). Chromosomally, this group is highly diversified and studies of repetitive DNAs in these species are still in preliminary stages. *Hypostomus* karyotypes range from 54 chromosomes in *H. plecostomus* (Muramoto *et al.* 1968) to 84 chromosomes in *Hypostomus* sp. 2 (Cereali *et al.* 2008). Artoni and Bertollo (2001) consider the diploid number of 54 chromosomes as basal for Hypostominae, using Trichomycteridae as outgroup, suggesting that the chromosome evolution of *Hypostomus* occurred through centric fissions. Recently, Bueno *et al.* (2012) showed that other chromosomal rearrangements, such as inversions, deletions, duplications and heterochromatinization, could contribute to the chromosomal differentiation within the genus.

Repetitive DNAs arranged in tandem are important tools in studies of taxonomic and evolutionary problems. These sequences could be associated with chromosomal rearrangement events (Wichman *et al*. 1991; Rosa *et al.* 2012; and others). According to Wichman *et al.* (1991), satellite DNAs rapidly diverge during evolution. Thereby, they are important for solving the taxonomic and evolutionary problems among related species.

The banded krait minor (Bkm) satellite DNA, one such simple sequence repeat, is highly conserved in eukaryotes. The major component of this satellite DNA is a tetranucleotide repeat of GATA which has been implicated in differentiation of sex chromosomes and speciation (Singh *et al.* 1984). Subramanian *et al.* (2003) propose that this sequence has a predominant association with sex chromosomes and a potential role in higher-order chromatin organization and a function in gene regulation of the human Y chromosome. In addition, it is proposed that GATA proteins are members of a zinc finger subfamily of DNA-binding proteins that recognize the consensus motif (T/A)GATA(A/G) (Orkin 1992). Six GATA family members have been identified in vertebrates with gene regulation function in numerous issues (for a review see Pedone *et al.* 1997).

The telomeres $(TTAGGG)_n$ are defined as a class of repetitive DNA which constitutes the terminal portion of linear chromosomes and are required for the replication and chromosome stability. Meyne *et al.* (1990) suggest that terminal and interstitial telomeric sites (ITS) can provide important information about the evolutionary status of the species. In Loricariidae, use of $(TTAGGG)_n$ sequence has contributed to the detection of chromosomal fusions and understanding of the large numerical and structural variation of the group (Rosa *et al.* 2012).

Given the high chromosomal diversity of the genus *Hypostomus* in Brazilian rivers, and the efficiency of the analysis of repetitive sequences on chromosome studies, this work aimed at contributing to a better understanding of *Hypostomus* chromosome diversification, using the distribution of $(GATA)_n$ and $(TTAGGG)_n$ sequences in species of this genus.

*For correspondence. E-mail: jositraldi@hotmail.com.

Keywords. repetitive DNA; Hypostominae; interstitial telomeric site; chromosomal diversification.

Materials and methods

We analysed specimens of *H. ancistroides* (11 females and 10 males), *H. iheringii* (10 females and 11 males) and *H. nigromaculatus* (13 females and 13 males) from the Córrego da Lapa, Ipeúna, São Paulo, Brazil, and specimens of *H. tapijara* (26 females and 14 males) from Ribeira de Iguape river, Registro, São Paulo, Brazil. The animals were identified and deposited in the Museum of Zoology at the University of São Paulo (MZUSP) under voucher numbers MZUSP 110802, MZUSP 106769, MZUSP 110801 and MZUSP 109785 respectively.

The mitotic chromosome were prepared according to Foresti *et al.* (1993). We used $(GATA)_n$ and $(TTAGGG)_n$, obtained by PCR performed with biotin-11-dUTP (Roche Applied Science, Penzberg, Germany) in the absence of DNA templates. The primers $(GATA)_7/(TATC)_7$ and $(TTAGGG)_5/(CCCTAA)_5$ were used for amplification of these repetitive sequences according to the DNA amplification procedure described by Ijdo *et al.* (1991). The PCR reaction mix comprised 2.5 mM of $MgCl_2$; 40 μM of dCTP, dATP and dGTP; 28 μM of dTTP; 12 μM of biotin-11-dUTP (Roche Applied Science, Penzberg, Germany); 100 μM of each primer and 2 U of Platinum® *Taq* DNA polymerase (Invitrogen, Carlsbad, USA). The conditions of the PCR cycle were 94°C/5 min; 9 cycles of 94°C/1 min, 55°C/30 s, 72°C/1 min; 29 cycles of 94°C/1 min, 60°C/30 s, 72°C/90 s; with final at 72°C/5 min.

Fluorescence *in situ* hybridizations (FISH) were performed following the protocol described by Pinkel *et al.* (1986), under stringency conditions of 77% (200–500 ng of each probe, 50% of formamide, 10% of dextran sulphate 2× saline sodium citrate (SSC), pH 7.0–7.2 at 37°C over 16 h). After hybridization, the slides were washed in 15% formamide/0.2× SSC at 42°C for 20 min and in 4× SSC/0.05% Tween 20 at room temperature for 10 min. The last step was performed in two 5-min washes. The detection and amplification of the hybridization signal were carried out using conjugated avidin-FITC and anti-avidin biotin complex (Sigma, St Louis, USA). The chromosomes were counterstained with a solution of anti-fading/DAPI (40 μL of anti-fading + 1 μL of DAPI 0.2 mg/mL) and analysed under an Olympus BX51 epifluorescence microscope and images were captured by the camera system DP72 (Olympus, Tokyo, Japan).

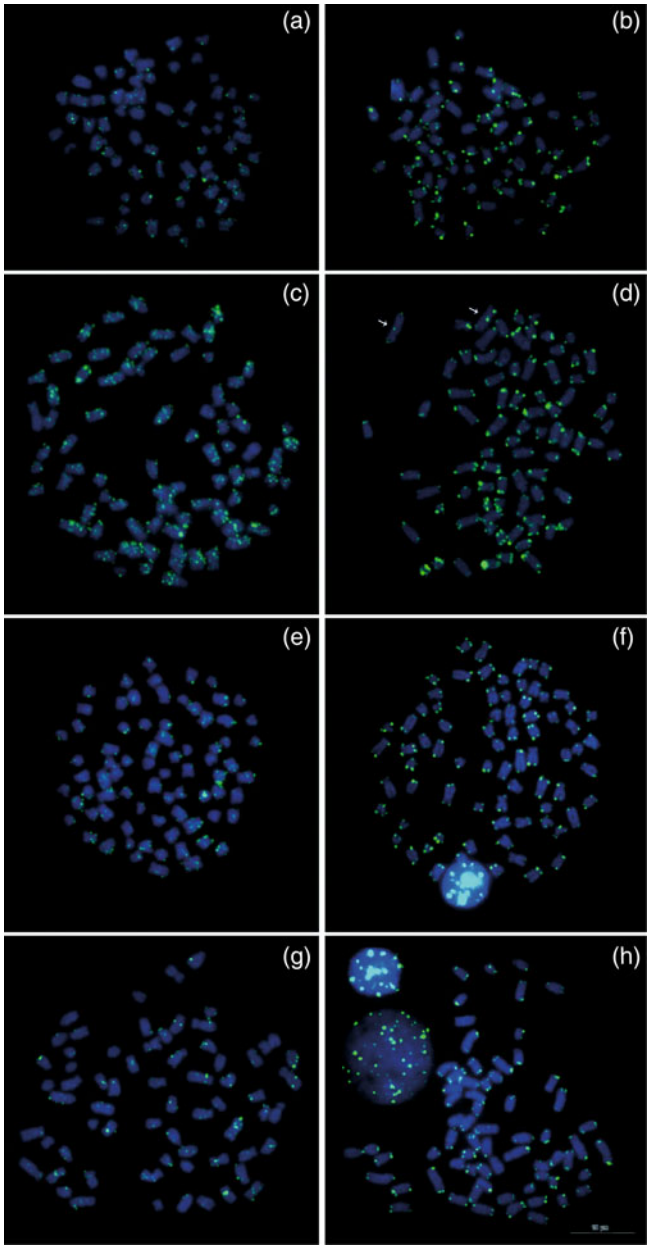

Figure 1. Metaphases of *H. ancistroides* (a, b), *H. iheringii* (c, d), *H. nigromaculatus* (e, f) and *H. tapijara* (g, h) subjected to fluorescence *in situ* hybridization with probes of $(GATA)_n$ sequence (a, c, e, g) and $(TTAGGG)_n$ sequence (b, d, f, h).

Results and discussion

FISH with $(GATA)_n$ probe revealed that this repetitive element is dispersed over the chromosomes, with small accumulations in interstitial and terminal portions of the chromosomes of the four studied species (figure 1, a,c,e&g). *H. iheringii* showed a higher accumulation of this sequence on the chromosomes (figure 1c) than the other species. In fishes, studies with GATA repeats showed that some species (Cross *et al.* 2006; Úbeda-Manzanaro *et al.* 2010) have a dispersed pattern of this sequence, with no preferential accumulation in some chromosomes, like the four species of *Hypostomus* that were analysed in the present work. Thus, it is observed that the recurrence of disperse pattern of the GATA repeats in the genomes of fishes. However, there are variations in the concentration and localization of this sequence among the species (Cross *et al.* 2006; Úbeda-Manzanaro *et al.* 2010; present work).

The dispersed pattern of $(GATA)_n$ sequence, observed in some fish species (Cross *et al.* 2006; Úbeda-Manzanaro *et al.* 2010; present work), might be a consequence of different

chromosomal rearrangements like translocation, paracentric and pericentric inversion, fusion, fission, duplication, deletion, genic conversion and heterochromatic amplification. In addition, these rearrangements might also be involved in different concentration and distribution of this sequence in the analysed species. Simple quadruplet repeats of GATA are always interspersed with other repetitive DNAs or single-copy sequences (Schäfer *et al.* 1986). This way, it is also possible to suppose that the dispersed pattern of (GATA)$_n$ sequence in fish is a consequence of transposition events of transposable elements associated to this sequence. In this context, the GATA repeats would be indirectly participating in the chromosomal diversification of the species that maintain this DNA repeat, as well in the four species of *Hypostomus* that were analysed in this work.

Subramanian *et al.* (2003) demonstrated in humans that most of the GATA repeats might be intergenic, suggesting that they may, in association with matrix-associated region (MAR), possess a function to regulate the entire domain that is defined by these sequences. Thus it is possible, that this sequence might have a functional role in fish genome and is maintained in these species by selective forces. Hence, GATA repeats might be participating in the evolution of these animals by the introduction of new genomic and evolutionary alternatives that influence the differentiation of these organisms.

Several studies demonstrate the existence of an association between GATA repeats and sex chromosomes, and the importance of this sequence in the evolution of these systems (Singh *et al.* 1984). The species analysed in this study do not show sex chromosome systems, however they display GATA repeats dispersed through several chromosomes of the karyotypes. In various species, the occurrence of this sequence in autosomes has been reported, but typically with a higher concentration of these sites in the sex chromosomes (Singh *et al.* 1984). But in *Ephestia kuehniella*, despite the existence of a well-defined sex chromosome system ZZ/ZW, GATA repeat sites were observed only in autosomes (Traut 1987). Thus, a direct link between sex chromosomes and the GATA repeats is not evident in species of different groups, as it was not verified in any of the four analysed species of *Hypostomus*.

FISH with (TTAGGG)$_n$ probe revealed that this repetitive element is located at the terminal portion of both arms of all chromosome pairs in the four species analysed (figure 1, b,d,f&h). Only in *H. iheringii* was one ITS identified which was located in the proximal region of a submetacentric chromosome pair (figure 1d). This kind of sequence has an important role in detecting rearrangements involved in chromosome evolution (Meyne *et al.* 1990). In the present work, the verification of the telomere repeats are restricted to the terminal portion of the chromosomes in *H. ancistroides*, *H. nigromaculatus* and *H. tapijara* is an indication of the lack of chromosomal fusions and translocations in the process of the karyotype evolution of these species. This situation is consistent with the evolutionary hypothesis of Artoni and Bertollo (2001) for the genus *Hypostomus*. According to these authors, the process of chromosome evolution of the genus *Hypostomus* would have mainly occurred by successive centric fissions from the basal karyotype with $2n = 54$ chromosomes. Hence, the species of this genus present high diploid numbers and the absense of ITS in their karyotypes is expected. However, the possibility of loss of these sequences after the occurrence of the rearrangements cannot be ruled out.

In fishes, similar to other vertebrates, (TTAGGG)$_n$ sites are also found in interstitial chromosome portions (Rosa *et al.* 2012). The presence of ITS in a chromosome pair of the karyotype of *H. iheringii* suggests the occurrence of chromosomal fusions in the evolution of this species. In addition, rearrangements like translocations or inversions might also be involved in the formation of this ITS. In Loricariinae, Rosa *et al.* (2012) proposed, based on the presence of ITS sites, the occurrence of centric fusion to seal the unstable sites that were generated in the chromosomes by centric fission during the evolutionary history of the group. In this hypothesis, the unstable chromosome sites that were generated by centric fission (Meyne *et al.* 1990; Rosa *et al.* 2012) were sealed by fusion, and the chromosomal instability generated by ITS (Peitl *et al.* 2002) may lead to new chromosomal rearrangements. Hence, these mechanisms might be involved in the large chromosomal variation of Loricariidae, as well in the genus *Hypostomus*.

Thus, the evolutionary history of the genus *Hypostomus* is evident in the complex scenario of chromosomal rearrangements. In this context, (TTAGGG)$_n$ and (GATA)$_n$ repeats can be interesting markers for the study of chromosomal diversification of this group, implicating the possible chromosomal rearrangements in the evolution of these species. In addition, distribution of (GATA)$_n$ repeats might have a possible role in the genic regulation of the analysed species of *Hypostomus*.

Acknowledgements

The authors are grateful to Osvaldo Takeshi Oyakawa (MZUSP) and Claudio Henrique Zawadzki for the identification of the specimens, to the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA/MMA) for the authorization to collect the material, to Pedro L. Gallo and Luiz H. da Silva for collecting them. This study was financed by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), Fundação Araucária (Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Estado do Paraná) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

References

Artoni R. F. and Bertollo L. A. C. 2001 Trends in the karyotype evolution of Loricariidae fish (Siluriformes). *Hereditas* **134**, 201–210.

Bueno V., Zawadzki C. H. and Margarido V. P. 2012 Trends in chromosome evolution in the genus *Hypostomus* Lacépède, 1803

(Osteichthyes, Loricariidae): a new perspective about the correlation between diploid number and chromosomes types. *Rev. Fish Biol. Fish.* **22**, 241–250.

Cereali S. S., Pomini E., Rosa R., Zawadzki C. H., Froehlich O. and Giuliano-Caetano L. 2008 Karyotype description of two species of *Hypostomus* (Siluriformes, Loricariidae) of the Planalto da Bodoquena, Brazil. *Genet. Mol. Res.* **7**, 583–591.

Cross I., Merlo A., Manchado M., Infante C., Cañavate J. P. and Rebordinos L. 2006 Cytogenetic characterization of the *Solea senegalensis* (Teleostei: Pleurenectiformes. Soleidae): Ag-NOR, $(GATA)_n$, $(TTAGGG)_n$ and ribosomal genes by one-color and two-color FISH. *Genetica* **128**, 253–259.

Foresti F., Oliveira C. and Almeida-Toledo L. F. 1993 A method for chromosome preparations from large specimens of fishes using '*in vitro*' short treatment with colchicine. *Experientia* **49**, 810–813.

Ijdo J. W., Wells R. A., Baldini A. and Reeders S. T. 1991 Improved telomere detection using a telomere repeat probe $(TTAGGG)_n$ generated by PCR. *Nucleic Acids Res.* **19**, 4780.

Meyne J., Baker R. J., Hobart H. H., Hsu T. C., Ryder O. A., Ward O. G. *et al.* 1990 Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes. *Chromosoma* **99**, 3–10.

Muramoto J. I., Ohno S. and Atkin N. B. 1968 On the diploid state of the fish order Ostariophysi. *Chromosoma* **24**, 59–66.

Orkin S. H. 1992 GATA-binding transcription factors in hematopoietic cells. *Blood* **80**, 575–581.

Pedone P. V., Omichinski J. G., Nony P., Trainor C., Gronenborn A. M., Clore G. M. and Felsenfeld G. 1997 The N-terminal fingers of chicken GATA-2 and GATA-3 are independent sequence-specific DNA binding domains. *EMBO J.* **16**, 2874–2882.

Peitl P., Mello S. S., Camparoto M. L., Passos G. A. S, Hande M. P., Cardoso R. S. *et al.* 2002 Chromosomal rearrangements involving telomeric DNA sequences in Balb/3t3 cells transfected with the Ha-ras oncogene. *Mutagenesis* **17**, 67–72.

Pinkel D., Straume T. and Gray J. W. 1986 Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proc. Natl. Acad. Sci. USA* **83**, 2934–2938.

Rosa K. O., Ziemniczak K., Barros A. V., Nogaroto V., Almeida M. C., Cestari M. M. *et al.* 2012 Numeric and structural chromosome polymorphism in *Rineloricaria lima* (Siluriformes: Loricariidae): fusion points carrying 5S rDNA or telomere sequence vestiges. *Rev. Fish Biol. Fish.* **22**, 739–749.

Schäfer R., Ali S. and Epplen J. T. 1986 The organization of the evolutionarily conserved GATA/GACA repeats in the mouse genome. *Chromosoma* **93**, 502–510.

Singh L., Phillips C. and Jones K. W. 1984 The conserved nucleotide sequences of Bkm, which define Sxr in the mouse, are transcribed. *Cell* **36**, 111–120.

Subramanian S., Mishra R. K. and Singh L. 2003 Genome-wide analysis of Bkm sequences (GATA repeats): predominant association with sex chromosomes and potential role in higher order chromatin organization and function. *Bioinformatics* **19**, 681–685.

Traut W. 1987 Hypervariable Bkm DNA loci in a moth, *Ephestia kuehniella*: does transposition cause restriction fragment length polymorphism? *Genetics* **115**, 493–498.

Úbeda-Manzanaro M., Merlo M. A., Palazón J. L., Cross I., Sarasquete C. and Rebordinos L. 2010 Chromosomal mapping of the major and minor ribosomal genes, (GATA)n and U2 snRNA gene by double-colour FISH in species of the Batrachoididae family. *Genetica* **138**, 787–794.

Weber C. 2003 Subfamily Hypostominae. In *Checklist of the freshwater fishes of South and Central America* (ed. R. E. Reis, S. O. Kullander and C. J. Ferraris), pp. 351–372. Edipucrs, Porto Alegre, Brazil.

Wichman H. A., Payne C. T., Ryder O. A, Hamilton M. J., Maltbie M. and Baker R. J. 1991 Genomic distribution of heterochromatic sequences in equids: implications to rapid chromosomal evolution. *J. Hered.* **82**, 369–377.

Received 9 October 2012, in revised form 7 December 2012; accepted 9 January 2013
Published on the Web: 4 April 2013