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ARTICLE

Chromosomal mapping of H3 histone and 5S rRNA genes in eight species of *Astyanax* (Pisces, Characiformes) with different diploid numbers: syntenic conservation of repetitive genes

Diovani Piscor and Patricia Pasquali Parise-Maltempi

Abstract: The genus *Astyanax* is widely distributed from the southern United States to northern Patagonia, Argentina. While cytogenetic studies have been performed for this genus, little is known about the histone gene families. The aim of this study was to examine the chromosomal relationships among the different species of *Astyanax*. The chromosomal locations of the 5S rRNA and H3 histone genes were determined in *A. abramis*, *A. asuncionensis*, *A. altiparanae*, *A. bockmanni*, *A. eigenmanniorum*, *A. mexicanus* (all $2n = 50$), *A. fasciatus* ($2n = 46$), and *A. schubarti* ($2n = 36$). All eight species exhibited H3 histone clusters on two chromosome pairs. In six species (*A. abramis*, *A. asuncionensis*, *A. altiparanae*, *A. bockmanni*, *A. eigenmanniorum*, and *A. fasciatus*), syntenic clusters of H3 histone and 5S rDNA were observed on metacentric (m) or submetacentric (sm) chromosomes. In seven species, clusters of 5S rDNA sequences were located on one or two chromosome pairs. In *A. mexicanus*, 5S rDNA clusters were located on four chromosome pairs. This study demonstrates that H3 histone clusters are conserved on two chromosome pairs in the genus *Astyanax*, and specific chromosomal features may contribute to the genomic organization of the H3 histone and 5S rRNA genes.

Key words: repetitive DNAs, karyotype evolution, FISH, Mexican blind cavefish, *Astyanax schubarti*.

Résumé : Le genre *Astyanax* présente une large aire de distribution allant du sud des Etats-Unis au nord de la Patagonie en Argentine. Bien que des études cytogénétiques aient été réalisées chez ce genre, peu de choses sont connues au sujet des familles de gènes codant pour les histones. Le but de cette étude était d'examiner les relations chromosomiques au sein des différentes espèces d'*Astyanax*. Les positions chromosomiques des gènes codant pour les ARNr 5S et les histones H3 ont été déterminées chez *A. abramis*, *A. asuncionensis*, *A. altiparanae*, *A. bockmanni*, *A. eigenmanniorum*, *A. mexicanus* (toutes à $2n = 50$), *A. fasciatus* ($2n = 46$) et *A. schubarti* ($2n = 36$). Chez les huit espèces, les gènes d'histones H3 étaient groupés sur deux paires de chromosomes. Chez six espèces (*A. abramis*, *A. asuncionensis*, *A. altiparanae*, *A. bockmanni*, *A. eigenmanniorum*, et *A. fasciatus*), les amas de gènes codant pour les histones H3 et l'ADNr 5S ont été observés sur des chromosomes métacentriques (m) ou submétacentriques (sm). Chez sept espèces, les amas de séquences d'ADNr 5S étaient situés sur une ou deux paires de chromosomes. Chez l'*A. mexicanus*, les ADNr 5S étaient répartis sur quatre paires de chromosomes. Cette étude montre que les amas de gènes codant pour les histones H3 sont conservés sur deux paires de chromosomes au sein du genre *Astyanax* et que des caractéristiques chromosomiques spécifiques pourraient contribuer à l'organisation génomique des gènes codant pour les histones H3 et les ARNr 5S. [Traduit par la Rédaction]

Mots-clés : ADN répétés, évolution caryotypique, FISH, tétra aveugle, *Astyanax schubarti*.

Introduction

Ribosomal genes, which constitute one of the major multigene families, are studied extensively in fish. These genes can characterize groups being located in one chromosome pair as in the genus *Apareiodon* (Parodontidae) (Vicari et al. 2006; Rosa et al. 2006; Bellafronte et al. 2009, 2011), or distributed in one or more pairs, ranging numerically depending on the population studied, as shown in *Astyanax scabripinnis* (Characidae) (Ferro et al. 2001; Almeida-Toledo et al. 2002; Santos et al. 2012). The chromosomal locations of 5S ribosomal DNA (rDNA) sequences are well conserved in species of the genus *Astyanax*; this contrasts with 45S rDNA locations, which are remarkably variable (Piscor et al. 2015).

Unlike the rDNA sequences, histone genes are generally found on one or two chromosome pairs in vertebrates, including mammals (Graves et al. 1985; Tripputi et al. 1986), amphibian (Turner et al. 1988), and fish (Pendás et al. 1994*a*; Hashimoto et al. 2011, 2013; Silva et al. 2014; Costa et al. 2014). Few investigations of histone

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gene locations in fish have been described in the literature. The research presented in this paper comprises the most comprehensive study to date of the relationships between H3 histone and 5S rRNA genes in the genus *Astyanax*.

Chromosomal mapping of histone genes in fish was first described by Pendás et al. (1994*a*) in a study of gene distribution in three species (brown trout, Atlantic salmon, and rainbow trout). In the genus *Astyanax*, Hashimoto et al. (2011) mapped the locations of the H1 histone and 5S rRNA genes in the chromosomes of three species (*A. altiparanae*, *A. fasciatus*, and *A. bockmanni*). Analysis of the gene sequences and their chromosomal locations in these species (two chromosome pairs with H1 histone cluster and one chromosome pair with 5S rDNA synteny in all three species) suggested that *A. fasciatus* and *A. bockmanni* were closely related. In the fish *Rachycentron canadum* (Perciformes), H3 histone genes mapped to terminal regions on the short and long arms of all chromosomes, and genes co-located with rDNAs on chromosome pair 2 (18S rDNA) and pairs 3 and 13 (5S rDNA) (Costa et al. 2014).

This paper aims to compare the degree of conservation of the locations of repetitive sequences (mainly H3 histone genes) among species of *Astyanax*, enhance our understanding of the chromosomal organization of the H3 histone and 5S rRNA genes, and determine the parameters underlying chromosome evolution in the *Astyanax* group.

Materials and methods

Ethical standards

The international guidelines for the care and use of laboratory animals follows the Guide to the Care and Use of Experimental Animals (Vol. 1, 2nd ed., 1993, and Vol. 2, 1984). The animals were captured with permission from Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio (number 43497-1), and the use for laboratory experiments was approved by the Animal Experimental Ethics Committee from Universidade Estadual Paulista – UNESP (protocol number: 2335).

Sampling and cytogenetic preparations

Twenty-seven specimens of *Astyanax* were obtained from locations in Brazil as follows: three *A. abramis* and four *A. asuncionensis* specimens from the Bento Gomes River in Mato Grosso state (MT); five *A. altiparanae* specimens and one *A. schubarti* specimen from the Piracicaba River in São Paulo state (SP); three *A. bockmanni* specimens from the Iguatemi River in Mato Grosso do Sul (MS); five *A. fasciatus* specimens from the Ribeirão Claro River in São Paulo state (SP); and three *A. mexicanus* and three *A. eigenmanniorum* specimens from aquariophiles in Brazil. Chromosomes were obtained as described by Foresti et al. (1981). Chromosome morphologies were determined according to the ratio of the arms (the most frequently used classification system for fish chromosomes). Briefly, the length of the long arm (q) was divided by the length of the short arm (p). Chromosomes with two arms and an arm ratio of 1–1.7 were classified as metacentric (m), those with two arms and an arm ratio of 1.71–3 were classified as submetacentric (sm), and those with two arms and an arm ratio of 3.01–7 were classified as subtelocentric (st). Chromosomes with a single arm (arm ratio > 7) were considered to be acrocentric (a).

DNA extraction, production of probes, and fluorescent in situ hybridization

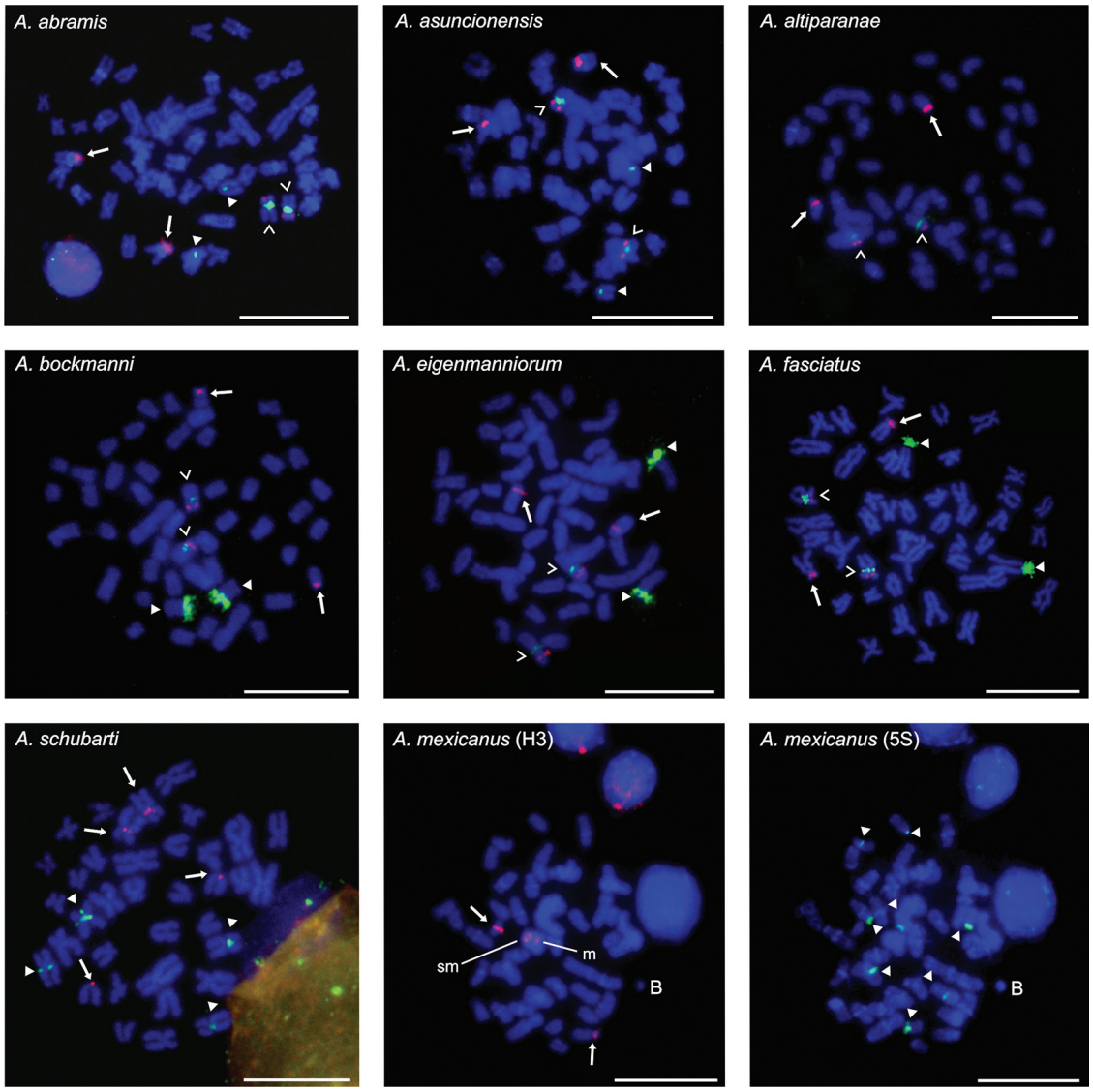
Genomic DNA was extracted from fin samples as described in Sambrook and Russell (2001). The 5S rDNA probe was prepared using polymerase chain reaction (PCR) with primers described by Pendás et al. (1994*b*) and Martins and Galetti (1999) (A, 5′-TACGCCCGATCTCGTCCGATC-3′, and B, 5′-CAGGCTGGTATGGCCGTAAGC-3′). The H3 histone gene probe was prepared using PCR with primers described by Cabral-de-Mello et al. (2010) (A, 5′-GGCNMGNACNAARCARAC-3′, and B, 5′-TGDATRTCYTTNGGCATDAT-3′). The 5S rDNA probe was labeled by PCR with biotin-14-dATP (Invitrogen, San Diego, Calif., USA) and digoxigenin-11-dUTP (Roche, Mannheim, Germany), and the H3 histone gene probe was labeled by PCR with digoxigenin-11-dUTP (Roche, Mannheim, Germany). Single, double, and sequential (on the same metaphase plate) fluorescent in situ hybridization (FISH) experiments were performed according to Pinkel et al. (1986) with modifications as described by Piscor et al. (2013). Chromosomes were counterstained with DAPI (4′,6-diamidino-2-phenylindole) and mounted using Vectashield Mounting Medium (Vector, Burlingame, Calif., USA). Chromosomes and fluorescent signals were visualized with an Olympus BX51 microscope coupled to a digital camera (Olympus model D71). Images were captured using the DP Controller software.

Results

The diploid number for six of the eight species of *Astyanax* was $2n = 50$ chromosomes (*A. abramis*, *A. asuncionensis*, *A. altiparanae*, *A. bockmanni*, *A. eigenmanniorum*, and *A. mexicanus*) (Fig. 1). All cells analyzed of *A. mexicanus* contained one acrocentric B microchromosome. *Astyanax fasciatus* had $2n = 46$ and *A. schubarti* had $2n = 36$ chromosomes (Fig. 1).

In six species of *Astyanax* (*A. abramis*, *A. asuncionensis*, *A. bockmanni*, *A. eigenmanniorum*, *A. fasciatus*, and *A. mexicanus*), the 5S rDNA was located on a metacentric pair (Figs. 1, 2A, 2B, 2D–2G). In *A. altiparanae*, the 5S rDNA was located on a submetacentric pair in the pericentromeric region (Figs. 1, 2C). Species with 5S rDNA on a metacentric pair also exhibited fluorescent signals in the pericentromeric regions on other chromosomes. Fluorescent 5S rDNA signal was noted on a subtelocentric pair in *A. abramis* (Figs. 1, 2A), an acrocentric pair in *A. asuncionensis* (Figs. 1, 2B), in *A. bockmanni* (Figs. 1, 2D), in *A. eigenmanniorum* (Figs. 1, 2E), in *A. fasciatus* (Figs. 1, 2F), and three pairs (one submetacentric and two acrocentrics) in *A. mexicanus* (Figs. 1, 2G). The species with lower diploid numbers,

Fig. 1. Location of 5S rDNA and H3 histone clusters on chromosomes of species of the genus *Astyanax*. The arrows indicate the H3 histone clusters, the arrowheads indicate the 5S rDNA clusters, and the slim arrowheads indicate chromosomes with 5S-H3 synteny. Note that in *A. mexicanus*, single FISH experiments (using 5S rDNA and H3 histone probes) were performed on the same metaphase plate. Scale bar = 10 μm.



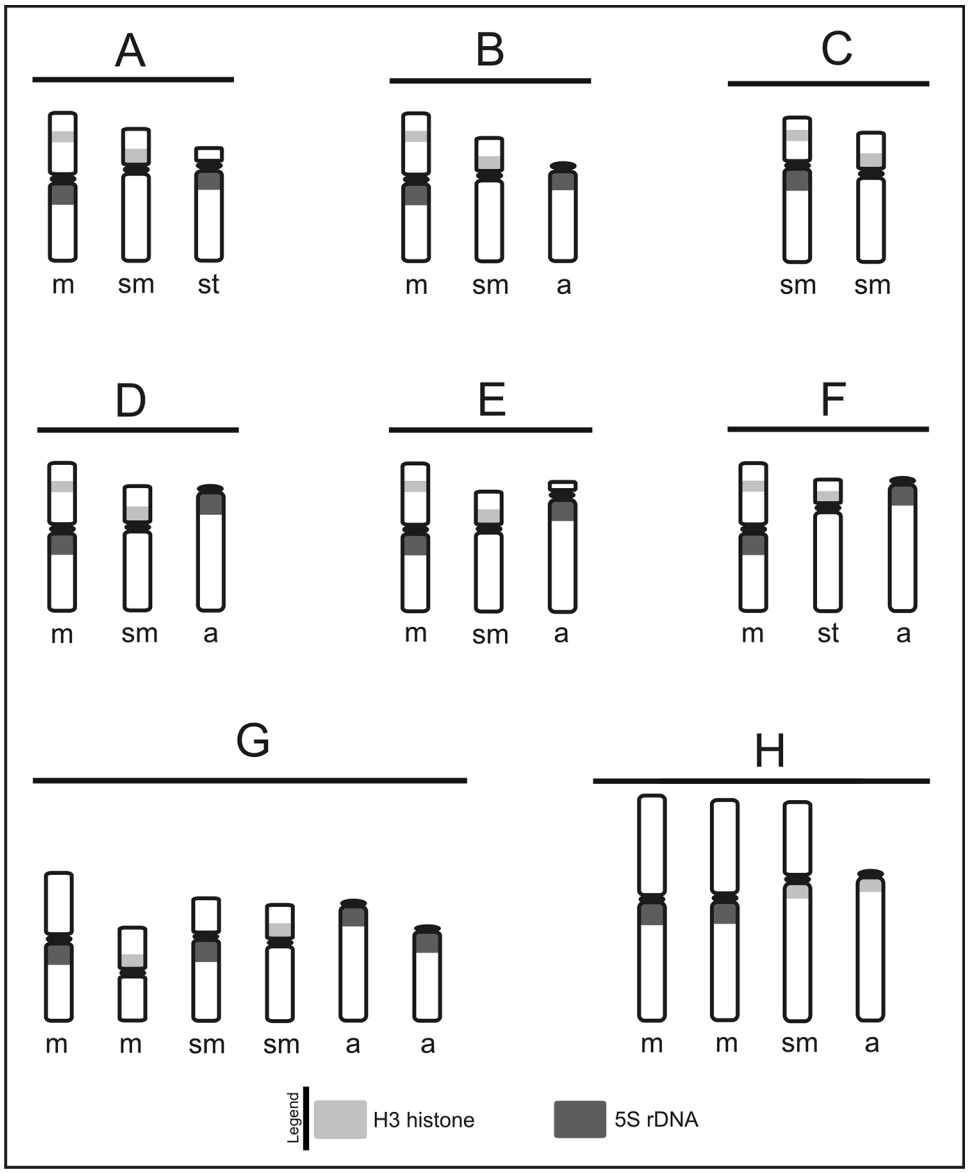
A. schubarti (2*n* = 36), harbored 5S rDNA clusters at pericentromeric regions on two metacentric pairs (Figs. 1, 2H).

H3 histone clusters were observed on two chromosome pairs in all eight examined species of *Astyanax* (Figs. 1, 2). One cluster was located in the interstitial region of the short arm on a metacentric pair in *A. abramis*, *A. asuncionensis*, *A. bockmanni*, *A. eigenmanniorum*, and *A. fasciatus* (Figs. 1, 2A, 2B, 2D, 2E, 2F, respectively) and submetacentric pair in *A. altiparanae* (Figs. 1, 2C). These same chromosome pairs exhibited synteny with the 5S rDNA sequences (Fig. 1: note the slim arrowheads). Additional H3 histone clusters were observed in the pericentromeric regions on a submetacentric pair in *A. abramis* (Figs. 1, 2A), *A. asuncionensis* (Figs. 1, 2B), *A. altiparanae* (Figs. 1, 2C), *A. bockmanni* (Figs. 1, 2D), *A. eigenmanniorum* (Figs. 1, 2E), and subtelocentric pair in *A. fasciatus* (Fig. 1, 2F). *Astyanax mexicanus* and *A. schubarti* did not display synteny between H3 histone and 5S rDNA clusters. In these species, H3 histone clusters were found in the pericentromeric regions of the short arm on a metacentric and submetacentric pairs (*A. mexicanus*; Figs. 1, 2G), and in pericentromeric regions of the long arm on a submetacentric and acrocentric pairs (*A. schubarti*; Figs. 1, 2H).

Discussion

The genus *Astyanax* displays variability among species with respect to chromosome structure, number, and morphology (see, for example, Morelli et al. 1983; Daniel-Silva and Almeida-Toledo 2001, 2005; Tenório et al. 2013). However, the current study showed that the H3 histone gene locations were conserved among species, particularly with

Fig. 2. Diagram showing chromosome pairs carrying 5S rDNA and H3 histone clusters in the eight species of *Astyanax* studied. (A) *A. abramis*, (B) *A. asuncionensis*, (C) *A. altiparanae*, (D) *A. bockmanni*, (E) *A. eigenmanniorum*, (F) *A. fasciatus*, (G) *A. mexicanus*, and (H) *A. schubarti*. Chromosome morphologies: m, metacentric; sm, submetacentric; st, subtelocentric; a, acrocentric.



respect to the pair numbers and morphologies of the chromosomes carrying the genes. The H3 histone genes were located on only two chromosome pairs in the eight species of *Astyanax* examined in this study. This corresponds with data from other populations of *Astyanax* as described by Hashimoto et al. (2011).

The similarities in the chromosomal locations make the H3 histone one of the most highly conserved gene location found in *Astyanax*. When compared with rDNA repetitive clusters, the histone genes exhibit the highest level of conservation, followed by the 5S rDNA, and the least conserved gene is the 18S rRNA (for additional review see, for example, Mantovani et al. 2005; Fernandes and Martins-Santos 2006; Piscor et al. 2015).

The chromosomal locations housing the different repetitive sequences may explain why some sequences of *Astyanax* are found at divergent sites, as for 45S rDNA, and other sequences show conserved locations, as for 5S rDNA. According to Nakajima et al. (2012), interstitial or proximal chromosomal regions tend to be more stable than the terminal sections. Thus, the chromosome location of 18S rDNA in terminal regions, like in *A. scabripinnis* (Mantovani et al. 2005), could be subject to transposition events or chromosome rearrangements leading the dispersion of these sequences throughout the genome. The stability of proximal regions, as seen on the pair 5 in *A. altiparanae* in the present paper, may be responsible for the conserved locations of 5S rRNA genes exhibited by several populations of this species (see also Fernandes and Martins-Santos 2006; Domingues et al. 2007; Piscor et al. 2015). This would also apply to the conserved locations of the histone genes in only two chromosome pairs,

since these sequences occupy most regions "protected" in the chromosomes of *Astyanax*, as pericentromeric and interstitial regions.

Synteny of H3 histone and 5S rDNA clusters was prevalent in the studied species of *Astyanax*. Although the clusters were distributed non-syntenically in *A. mexicanus* and *A. schubarti*, synteny was found in one chromosome pair (submetacentric or metacentric) for the other examined species. Hashimoto et al. (2011) examined three species and also observed synteny of histone (H1 histone) and 5S rRNA genes. Syntenic features such as adjacent location or co-location of sequences from other multigene families have been observed in fish but at lower frequencies than for the H3-5S genes. For example, synteny between two classes of ribosomal DNA (5S rDNA and 45S rDNA) was reported in a population of *A. scabripinnis*, and one of the chromosomes carrying 5S rDNA also harbored nucleolar organizer regions (NORs) (Mantovani et al. 2005). Synteny was also observed in other groups, including *Bryconamericus* aff. *iheringii* (Characidae), in which 18S and 5S ribosomal genes were adjacent to one another (Piscor et al. 2013), and *Triportheus nematurus* (Characidae), in which 5S rDNA clusters were adjacent to NORs (Diniz et al. 2009).

Another feature that supported our conservation hypothesis for the H3 histone genes, in the genus *Astyanax*, was the presence of clusters on one m chromosome pair and one sm or st chromosome pair in all species, except *A. schubarti* and *A. mexicanus*. In *A. schubarti*, H3 histone genes were located on two chromosome pairs (sm and a); however, the sm chromosome was larger than the sm/st chromosomes of the other species in this study (Fig. 2). In *A. mexicanus*, H3 histone genes were located on one small m chromosome pair and one sm chromosome pair; here, the m chromosome was smaller than the m chromosomes of the other species. On the other hand, the size and morphologies of the sm/st pairs (st in *A. fasciatus* and sm in the other species) were similar, and it is therefore possible that these chromosomes may be homeologous. However, we cannot exclude the possibility that microrearrangements might have stimulated subtle morphological differences in the submetacentric and subtelocentric pairs in these species of *Astyanax*.

Our data suggest that, due to variation of the number of chromosomes presented to the genus *Astyanax* studied here (modal number of $2n = 36$, $2n = 46$, and $2n = 50$ chromosomes), only two chromosomes carrying the genes of H3 histone seem to be preserved for the genus as a whole. However, two notable features of H3 histone chromosomal distribution attracted our attention. First, the chromosomes carrying the H3 histone genes in *A. schubarti*, which has the lowest diploid number ($2n = 36$), were particularly large. Second, no H3-5S synteny was observed in *A. schubarti* and *A. mexicanus* (Mexican blind cavefish). Observations by Morelli et al. (1983) suggested that the lower diploid number ($2n = 36$) in *A. schubarti* might be of recent origin. The lack of synteny in this species may be related to the chromosomal rearrangements involved in the reduction of the diploid number. Lack of synteny in *A. mexicanus* may be due to the ancient separation of this particular species, millions of years ago, from the species from South American river basins.

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