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Original Article

Highly Similar Morphologies Between Chromosomes Bearing U2 snRNA Gene Clusters in the Group *Astyanax* Baird and Girard, 1854 (Characiformes, Characidae): An Evolutionary Approach in Species with 2n = 36, 46, 48, and 50

Diovani Piscor,[1] Liano Centofante,[2] and Patricia Pasquali Parise-Maltempi[1]

Abstract

Repetitive sequences and their chromosomal locations have been widely studied in species of the *Astyanax* genus. However, the chromosomal organization of U2 snDNA remains largely unknown. The aims of this study were to examine the chromosomal contexts of U2 snRNA and 5S rRNA genes in *Astyanax* species and determine the degree of chromosome morphological similarity between species with different diploid numbers. Clusters of U2 snDNA and 5S rDNA were determined in nine species of *Astyanax*, including two karyomorphs of *Astyanax fasciatus* Cuvier, 1819. All species exhibited U2 snDNA clusters on two chromosome pairs, except *Astyanax mexicanus* De Filippi, 1853 (one pair). The 5S rDNA clusters were located on one chromosome pair in *Astyanax altiparanae* Garutti and Britski, 2000, and *Astyanax marionae* Eigenmann, 1911, two pairs in *Astyanax abramis* Jenyns, 1842, *Astyanax asuncionensis* Géry, 1972, *Astyanax bockmanni* Vari and Castro, 2007, *Astyanax eigenmanniorum* Cope, 1894, *A. fasciatus* (karyomorphs I and II), and *Astyanax schubarti* Britski, 1964, and four pairs in *A. mexicanus*. The relationships between the repetitive sequences in different species suggest that *A. schubarti* and *A. mexicanus* exhibit an unusual U2 snDNA chromosomal format as a result of events occurring in the evolutionary history of the *Astyanax* group.

Introduction

THE *ASTYANAX* GENUS contains numerous species (about 140 species),[1] many of which have not been examined cytogenetically. Diploid number ranges from $2n = 36$ chromosomes (e.g., *A. schubarti*)[2] to $2n = 50$ chromosomes (most species) as in *A. altiparanae*[3] and *A. bockmanni*.[4] Intermediate diploid numbers are also observed in species such as *A. fasciatus* ($2n = 46$)[5] and *Astyanax scabripinnis* Jenyns, 1842 ($2n = 48$).[6] However, the latter two species are considered to be species complexes that present variable diploid numbers (karyomorphs). For example, different karyomorphs of *A. fasciatus* were found under sympatric conditions.[7] *Astyanax* is therefore a promising model for studies of karyotype evolution.

The chromosomal locations of many repetitive sequences are well characterized in *Astyanax*, nevertheless some repetitives show variations related to cluster numbers, as the locations of 18S ribosomal DNA (18S rDNA). For instance, Fernandes and Martins-Santos[8] described four and seven sites of 18S rDNA in different *A. altiparanae* populations from the Paraná River Basin (Paraná state—PR, Brazil), whereas Peres *et al.*[9] described only a single 18S rDNA site in a population of *A. altiparanae* from the upper Paraná River Basin (São Paulo state—SP, Brazil). Conversely, fluorescent labeling showed that 5S rDNA location was conserved to the same chromosome pair in these *A. altiparanae* populations.

The chromosomal locations of histone and U small nuclear RNA (U snRNA) sequences are conserved.[10–13] More recently, Piscor and Parise-Maltempi[14] studied eight species of *Astyanax* and demonstrated that the chromosomal locations of H3 histone gene clusters were highly conserved in *A. abramis*, *A. asuncionensis*, *A. altiparanae*, *A. bockmanni*, *A. eigenmanniorum*, and *A. fasciatus*. Silva *et al.*[13] observed that the U1 and U2 snDNA clusters were located at different chromosomal sites in different *Astyanax* species but exhibited strong conservation in the number of sites per genome.

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Considering *Astyanax* a group with wide distribution and distinct cytogenetic features, the aims of this article were to compare the chromosomal organization of U2 snDNA and 5S rDNA in nine species, including those with different diploid numbers, and determine parameters underlying the chromosome evolution of U2 snRNA genes in the *Astyanax* genus.

Materials and Methods

Sampling and classical cytogenetics

Astyanax specimens 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), six *A. marionae* specimens from the Rio Claro stream 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 state (MS), two *A.* aff. *fasciatus* specimens (karyomorph I) from the Corumbataí River tributary (SP), five *A. fasciatus* specimens (karyomorph II) from the Ribeirão Claro River (SP), and three *A. mexicanus* and three *A. eigenmanniorum* specimens from aquariophiles in Brazil. Chromosomes were obtained as described by Foresti *et al.*,[15] and chromosome morphologies were determined according to the arm ratios (the most frequently used classification system for fish chromosomes in Brazil), as cited by Piscor *et al.*[16]

Isolation of repetitive DNA probes and fluorescence in situ hybridization

Genomic DNA was extracted from fin samples as described by Sambrook and Russell.[17] The 5S rDNA probe was prepared using polymerase chain reaction (PCR) with primers described by Pendás *et al.*[18] and Martins and Galetti[19] (A, 5′-TAC GCC CGA TCT CGT CCG ATC-3′; and B, 5′-CAG GCT GGT ATG GCC GTA AGC-3′). The U2 snDNA probe was prepared using PCR with primers described by Bueno *et al.*[20] (U2F, 5′-ATC GCT TCT CGG CCT TAT G-3′; and U2R, 5′-TCC CGG CGG TAC TGC AAT A-3′). The 5S rDNA probe was labeled by PCR with biotin-14-dATP (Invitrogen, San Diego, CA), and the U2 snDNA probe was labeled by PCR with digoxigenin-11-dUTP (Roche, Mannheim, Germany). Probes labeled with digoxigenin-11-dUTP were detected using antidigoxigenin–rhodamine (Roche), and probes labeled with biotin-14-dATP were detected using Alexa Fluor 488-conjugated streptavidin (Invitrogen). Single- and two-color fluorescence *in situ* hybridization (FISH) was performed using mitotic metaphasic chromosomes, according to Pinkel *et al.*[21] and with modifications as described by Cabral-de-Mello *et al.*[22] Chromosomes were counterstained with VECTASHIELD Mounting Medium (Vector, Burlingame, CA) containing DAPI (4′,6-diamidino-2-phenylindole). Chromosomes and fluorescent signals were visualized with an Olympus BX51 microscope coupled to a digital camera (Olympus model D71). Images were captured using DP Controller software.

Results

Species with $2n = 50$ chromosomes were *A. abramis*, *A. asuncionensis*, *A. altiparanae*, *A. bockmanni*, *A. eigenmanniorum*, *A. mexicanus* (Figs. 1A–F and Table 1), and *A.* aff. *fasciatus* (karyomorph I; the first described karyomorph for this population) (Fig. 2A and Table 1). All the examined *A. mexicanus* cells contained one acrocentric B chromosome (Fig. 1F, box). Species with smaller diploid numbers were *A. marionae* ($2n = 48$ chromosomes), *A. fasciatus* (karyomorph II; $2n = 46$), and *A. schubarti* ($2n = 36$) (Figs. 2B–D, respectively; Table 1).

The U2 snDNA clusters were observed on two chromosome pairs in eight of the nine *Astyanax* species (including the two *A. fasciatus* karyomorphs) (Figs. 1 and 2; Table 1). In *A. mexicanus*, U2 snDNA was observed on only one chromosome pair (Fig. 1F and Table 1). The U2 snDNA clusters were located on chromosome pairs 17 and 20 (sm) in *A. abramis* (Fig. 1A), pairs 11 and 13 (sm) in *A. asuncionensis* (Fig. 1B), pairs 10 and 13 (sm) in *A. altiparanae* (Fig. 1C), pairs 5 and 11 (sm) in *A. bockmanni* (Fig. 1D), pairs 15 (sm) and 16 (st) in *A. eigenmanniorum* (Fig. 1E), pair 8 (sm) in *A. mexicanus* (Fig. 1F), pairs 13 (sm) and 15 (st) in *A.* aff. *fasciatus* (karyomorph I) (Fig. 2A), pairs 5 and 9 (sm) in *A. marionae* (Fig. 2B), pairs 7 and 10 (sm) in *A. fasciatus* (karyomorph II) (Fig. 2C), and pairs 7 and 14 (sm) in *A. schubarti* (Fig. 2D). All fluorescent signals were located in the pericentromeric regions, with the exception of an interstitial signal observed on pair 7 in *A. schubarti* (Fig. 2D).

In five of the seven species with $2n = 50$ chromosomes (*A. abramis*, *A. asuncionensis*, *A. bockmanni*, *A. eigenmanniorum*, and *A. mexicanus*), the 5S rDNA was located on pair 2 (m) (Figs. 1A, B, D–F, respectively). In *A. altiparanae*, the 5S rDNA was located on pair 5 (sm) (Fig. 1C), and in *A.* aff. *fasciatus* (karyomorph I), the 5S rDNA was observed on pairs 3 (m) and 21 (a) (Fig. 2A). Species with 5S rDNA on pair 2 (m) also exhibited signals in pericentromeric regions on other chromosomes. Pericentromeric fluorescent signals were noted on pair 23 (st) in *A. abramis* (Fig. 1A), pair 25 (a) in *A. asuncionensis* (Fig. 1B), pair 19 (a) in *A. bockmanni* (Fig. 1D), pair 22 (a) in *A. eigenmanniorum* (Fig. 1E), and pairs 15 (sm), 22 (a), and 25 (a) in *A. mexicanus* (Fig. 1F). The three species with smaller diploid numbers, *A. marionae* ($2n = 48$), *A. fasciatus* karyomorph II ($2n = 46$), and *A. schubarti* ($2n = 36$), harbored 5S rDNA clusters at pericentromeric regions on pair 22 (a) (*A. marionae*; Fig. 2B), pairs 3 (m) and 22 (a) (*A. fasciatus* karyomorph II; Fig. 2C), and pairs 3 and 4 (m) (*A. schubarti*; Fig. 2D).

A summary diagram indicating the chromosomal locations of the U2 snDNA clusters in the nine *Astyanax* species is shown in Figure 3. Note that three groups were formed: the first group with two chromosome pairs bearing U2 snDNA clusters shared by several species (Fig. 3A), the second group with only one pair (Fig. 3B), and the third group with two pairs but the first pair with interstitial clusters (Fig. 3C).

Discussion

As demonstrated by previous cytogenetic observations, the most common diploid number in the *Astyanax* genus is $2n = 50$ chromosomes. This is consistent with the majority of species in the family Characidae.[2] However, other diploid chromosome numbers are observed in the *Astyanax* genus, such as the species with $2n = 36$, 46, and 48 examined in this study. Furthermore, species complexes with variable diploid numbers are found in *Astyanax*, such as the ''*scabripinnis* complex'' and the ''*fasciatus* complex'' (see, e.g.,[23,24]). Here, *A. fasciatus* karyomorphs from the same river system were examined that had two different diploid numbers ($2n = 46$ and 50). *Astyanax*

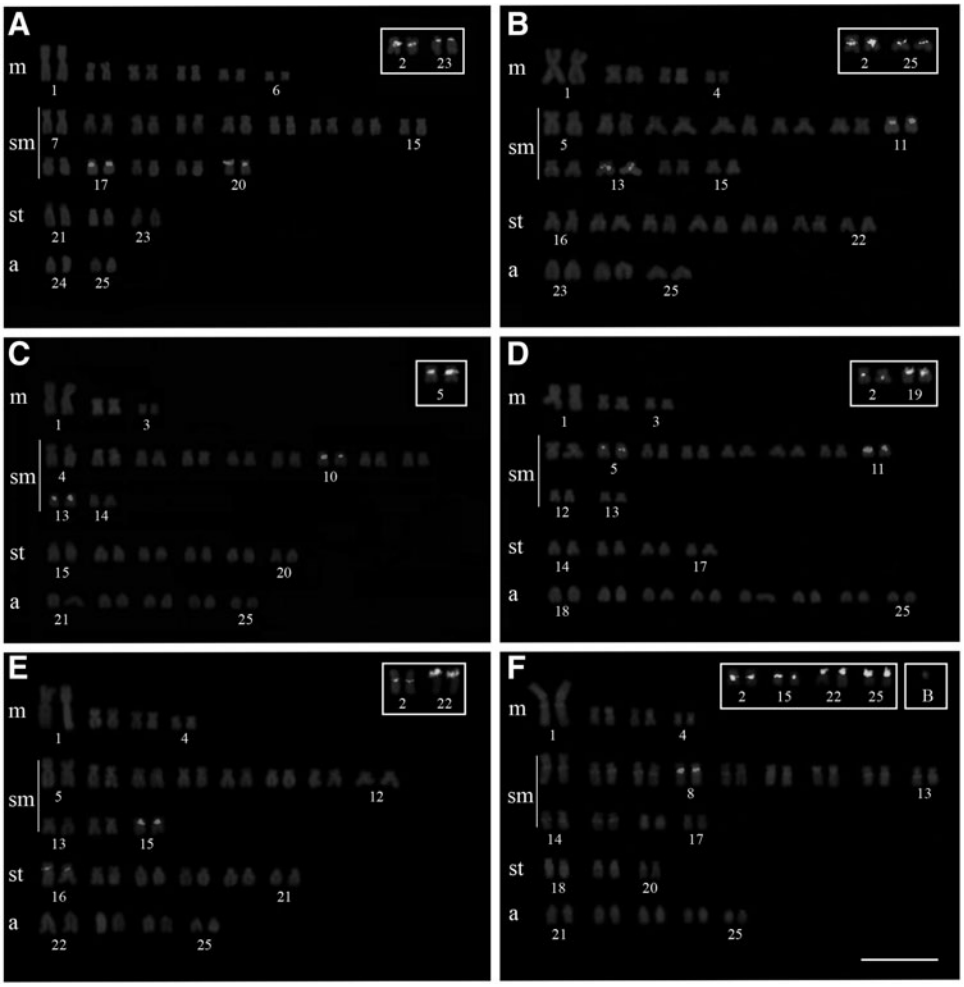

FIG. 1. Locations of U2 snDNA and 5S rDNA clusters on chromosomes of *Astyanax* species with 2n = 50 chromosomes. **(A)** *Astyanax abramis*, **(B)** *Astyanax asuncionensis*, **(C)** *Astyanax altiparanae*, **(D)** *Astyanax bockmanni*, **(E)** *Astyanax eigenmanniorum*, and **(F)** *Astyanax mexicanus*. Karyotypes indicate the chromosome pairs with U2 snDNA clusters, and *boxes* indicate pairs with 5S rDNA clusters. Scale bar = 10 μm.

TABLE 1. CHROMOSOMAL DATA IN SPECIES OF *ASTYANAX* WITH U2 snDNA CLUSTERS

Species	*2n*	*Karyotype formulae*	*FN*[a]	*5S*[b]	*U2*[c]	*References*
Astyanax abramis	50	12m + 28sm + 6st + 4a	96	4	4	Present study
Astyanax asuncionensis	50	8m + 22sm + 14st + 6a	94	4	4	Present study
Astyanax altiparanae	50	6m + 22sm + 12st + 10a	90	2	4	Present study
A. altiparanae	50	10m + 16sm + 16st + 8a	–	2	4	Silva *et al.*[13]
Astyanax bockmanni	50	6m + 20sm + 8st + 16a	84	4	4	Present study
A. bockmanni	50	8m + 14sm + 12st + 16a	–	4	4	Silva *et al.*[13]
Astyanax eigenmanniorum	50	8m + 22sm + 12st + 8a	92	4	4	Present study
Astyanax aff. *fasciatus* (karyotype I)	50	8m + 20sm + 12st + 10a	90	4	4	Present study
A. fasciatus (karyotype II)	46	8m + 18sm + 16st + 4a	88	4	4	Present study
A. fasciatus	46	10m + 14sm + 14st + 8a	–	4	4	Silva *et al.*[13]
Astyanax jordani	50 + B[d]	8m + 18sm + 12st + 12a	–	10	2	Silva *et al.*[13]
Astyanax marionae	48	8m + 24sm + 10st + 6a	90	2	4	Present study
Astyanax mexicanus	50 + B[d]	8m + 26sm + 6st + 10a	90	8	2	Present study
Astyanax paranae Eigenmann, 1914	50 + B[d]	8m + 22sm + 10st + 10a	–	4	4	Silva *et al.*[13]
Astyanax schubarti	36	12m + 16sm + 4st + 4a	68	4	4	Present study

[a]Fundamental numbers.
[b]Numbers of clusters (5S rDNA).
[c]Numbers of clusters (U2 snDNA).
[d]B chromosomes.
FN, fundamental number.

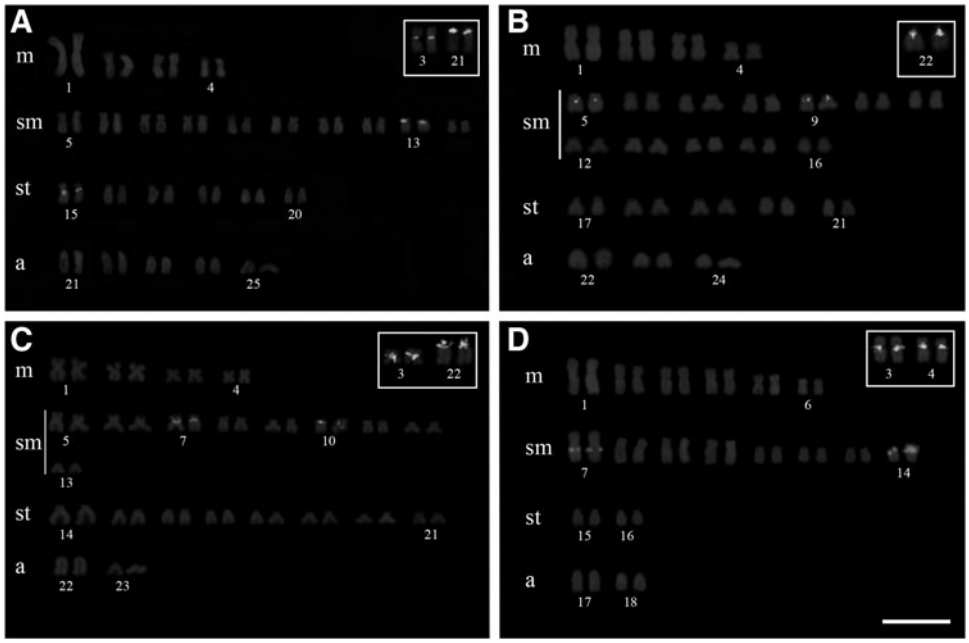

FIG. 2. Locations of U2 snDNA and 5S rDNA clusters on chromosomes of *Astyanax marionae* (2n = 48), *Astyanax schubarti* (2n = 36), and two *Astyanax fasciatus* populations (karyomorph I, 2n = 50, and karyomorph II, 2n = 46). **(A)** *Astyanax* aff. *fasciatus* (karyomorph I), **(B)** *A. marionae*, **(C)** *A. fasciatus* (karyomorph II), and **(D)** *A. schubarti*. Karyotypes indicate the chromosome pairs with U2 snDNA clusters, and *boxes* indicate pairs with 5S rDNA clusters. Scale bar = 10 μm.

fasciatus is known to have several karyomorphs, mostly with $2n = 46$, 48, and 50 chromosomes.[7]

The *Astyanax* genus is distinguished cytogenetically by diploid number variability from $2n = 36$ chromosomes for *A. schubarti*[2] and *Astyanax correntinus* Holmberg, 1891,[25] to $2n = 50$ chromosomes for most species, for example, *A. altiparanae* and *A. bockmanni*.[3,4] Variations in karyotype formula and fundamental number (FN) are also widely observed even in populations of the same species. For example, Fernandes and Martins-Santos[3] reported two different karyotype formulae and FN in two *A. altiparanae* populations from the Índios and Paraná rivers (PR, Brazil), and these values differ from those of the Piracicaba River (SP, Brazil) studied here. In contrast, FISH examination has identified that repetitive sequences showed similar chromosomal locations in *Astyanax* species.

Chromosomal locations of 5S rDNA are conserved in some *Astyanax* and exhibit three forms.[26] The form found in most species, including the *A. fasciatus* karyomorphs analyzed here, exhibits one metacentric pair and one acrocentric or subtelocentric pair with 5S rDNA sites located on the long arm, both near the centromere.[26] According to Vicari *et al.*,[27] *Astyanax* species with two chromosome pairs bearing 5S rDNA sites exhibit probable synapomorphic features.

In fish, 5S rDNA and other repetitive DNA clusters may be located on the same chromosome pair. For example, while 5S rRNA and histone genes can occur on the same chromosome in *Astyanax* species,[10,14] the 5S rDNA is close to 18S rDNA clusters in *Bryconamericus* aff. *iheringii* Boulenger, 1887,[16] another characid fish. The chromosomal locations of the 5S rDNA and U2 snDNA clusters were not consistently linked in the *Astyanax* species examined here because that these represent two distinct classes of repetitive DNA with completely different functions. Supiwong *et al.*[28] found that U2 snDNA and 5S rDNA sequences were also carried on different chromosome pairs in the naked catfish *Mystus bocourti* Bleeker, 1864 (Siluriformes). This spatial separation of 5S rDNA and U2 snDNA appears to be a common feature in fish (see, e.g.,[29,30]).

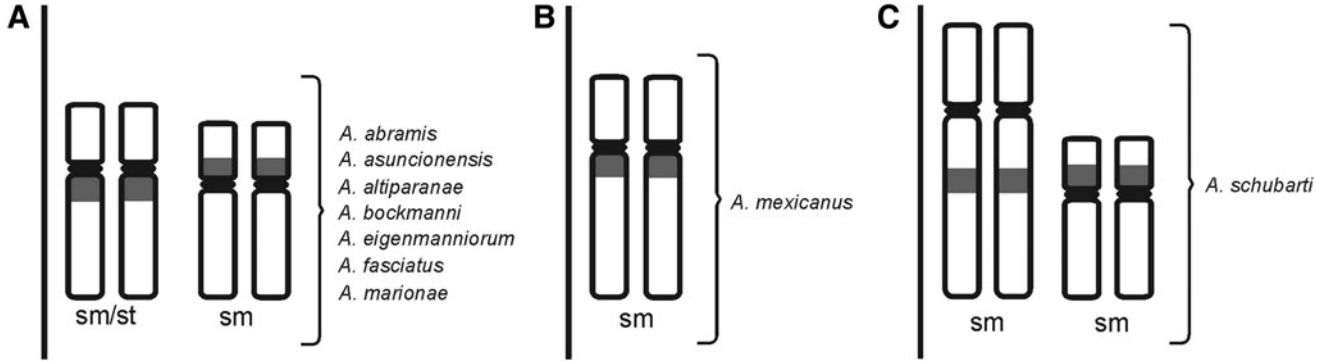

FIG. 3. Diagram indicating the chromosome pairs bearing U2 snDNA clusters in the nine *Astyanax* species. **(A)** Species with very similar chromosome pairs (pericentromeric regions), **(B)** *A. mexicanus*, with only one chromosome pair carrying U2 snDNA (pericentromeric region), and **(C)** *A. schubarti*, with two chromosome pairs carrying U2 snDNA (pair 7, interstitial location, and pair 14, pericentromeric location). U2 snDNA clusters are shown in *gray*.

All the species examined here had clusters of U2 snDNA on two chromosome pairs, with the exception of *A. mexicanus* (one pair; Fig. 3). Therefore, our results suggest that the two pairs with U2 snDNA clusters may represent a similar form shared by species of the first group (Fig. 3A), and the only one chromosome pair of *A. mexicanus* (Fig. 3B) and two pairs of *A. schubarti* (Fig. 3C) represent different forms of genomic organization of U2 snRNA genes. These two different forms may be explained due to probable reduction in the diploid number ($2n = 36$) of *A. schubarti* and ancient separation of *A. mexicanus* from the South America as previously proposed by Piscor and Parise-Maltempi.[14]

Recently, Silva *et al.*[13] found U2 snDNA clusters on two chromosome pairs in different *Astyanax* species, except in *Astyanax jordani* Hubbs and Innes, 1936 (one pair). Silva *et al.*[13] also showed that, while U1 and U2 snRNA genes were located on different chromosome pairs in different species, the numbers of U1 and U2 sites per genome were strongly conserved. Martins and Galetti[19] proposed that 5S rDNA on a single pair of chromosomes probably represented a more ancient genomic condition in *Leporinus* Spix, 1829 (Anostomidae).

The eyed epigean form (surface fish) of *A. mexicanus* is widely distributed in northeastern Mexico and southern Texas, and the eyeless hypogean forms (cavefish) live in some caves inside this extension.[31] Therefore, an ancestral link is possible between the single pair of chromosomes carrying U2 snDNA in *A. mexicanus* from Mexico and single U2 snDNA pair in other species of *Astyanax* from North and Central America, as for example, *A. jordani* studied by Silva *et al.*[13] that also showed one chromosome pair bearing U2 snDNA clusters.

In summary, the variability in diploid chromosome number in the *Astyanax* genus is not reflected in the chromosomal organization of the U2 snRNA genes. However, U2 snDNA sites appear to be located on two chromosome pairs with medium size and similar morphologies in almost all *Astyanax* species. The U2 snDNA cluster stability could be the result of an evolutionary advantage or association with specific DNA segments or particular regions of the genome, which may have facilitated the maintenance of U2 snDNA on two chromosome pairs in South American *Astyanax* species.

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Disclosure Statement

No competing financial interests exist.

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