Evolutionary Dynamics of rDNAs and U2 Small Nuclear DNAs in *Triportheus* (Characiformes, Triportheidae): High Variability and Particular Syntenic Organization

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Abstract

Multigene families correspond to a group of genes tandemly repeated, showing enormous diversity in both number of units and genomic organization. In fishes, unlike rDNAs that have been well explored in cytogenetic studies, U2 small nuclear RNA (snRNA) genes are poorly investigated concerning their chromosomal localization. All *Triportheus* species (Characiformes, Triportheidae) studied so far carry a ZZ/ZW sex chromosomes system, where the W chromosome contains a huge 18S rDNA cistron. In some species the syntenic organization of rDNAs on autosomes was also verified. To explore this particular organization, we performed three-color-fluorescence *in situ* hybridization using 5S, 18S rDNA, and U2 snRNA genes as probes in eight *Triportheus* species. This work represents the first one analyzing the chromosomal distribution of U2 snRNA genes in genomes of Triportheidae. The variability in number of rDNA clusters, and the divergent syntenies for these three multigene families, put in evidence their evolutionary dynamism, revealing a much more complex organization of these genes than previously supposed for closely related species. Our study also provides additional data on the accumulation of repetitive sequences in the sex-specific chromosome. Besides, the chromosomal organization of U2 snDNAs among fish species is also reviewed.

Keywords: multigene families, sex chromosomes, repetitive DNAs

Introduction

The repetitive DNA content of eukaryotic genomes has received particular attention for being composed by plastic and dynamic elements, providing thus relevant information about the chromosomal structure in genomes of various taxa. Multigene families comprise a group of genes repeated in tandem, showing enormous diversity in both number of units and genomic organization.[1] Among them, genes encoding ribosomal RNAs (rRNA) and U2 small nuclear RNAs (snRNAs) have been used as suited markers for chromosomal investigations.[2–5] rDNAs play an important role in protein synthesis, in which 45S rDNA encodes for 18S, 5.8S and 28S rRNAs, and the 5S rDNA encodes for 5S rRNA.[6] All rDNAs are processed to form ribosome subunits in which 45S rDNAs are generated in the nucleolus (nucleolus organizer region), while 5S rRNAs are first synthesized in nucleoplasm and latter enter the nucleolus to form a functional component of the large ribosomal subunit.[7]

In fishes, rDNAs have been deeply explored in cytogenetic and genomic studies, providing interesting data to elucidate chromosomal evolutionary process in many groups.[8] Concerning the number and position of 45S rDNAs in fish chromosomes, compiled data about 330 species from 22 orders demonstrated that karyotypes of *72% of species carry single 45S rDNA sites, which are located in a terminal position in 87% of cases.[9] On the other hand, the mapping of 5S rDNA in most studied species has showed a conservative interstitial position.[7] Additionally, the most common situation is the localization of both ribosomal genes on different chromosomal pairs.[10] Despite these common patterns, a growing number of studies have pointed to variable numbers and locations of rDNAs loci, which are eventually present in syntenic configuration in many species.[11–16]

Unlike rDNAs, U2 snRNA genes have been poorly investigated in fish chromosomes.[17] U2, U1, U4, U5, and U6 snRNAs are components of a complex of small nuclear ribonucleoproteins that are associated to the splicing process of

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mRNA precursors.[18] Despite the fact that U2 snDNAs appear to be highly conserved in eukaryotes, the number and organization of this multigene family can be highly variable among species.[19,20] The chromosomal mapping of U2 snRNA genes show a broad scenario, with these sequences accumulating in one or more chromosome pairs, and dispersing through the chromosomes.[21–24] By now, only two studies showed a syntenic organization of U2 snDNA and rDNAs among fishes,[18,21] since in most cases these genes are found on separate chromosomes.

The genus *Triportheus* (Characiformes, Triportheidae) is a freshwater fish genus, widely distributed in most of the major river drainages of South America.[25,26] These fishes are commonly known as ''freshwater sardines'' and stands out for their economic importance, constituting an important food resource for traditional communities.[25] Several species have been extensively investigated in cytogenetic studies, in which all of them carry a ZZ/ZW sex chromosomes system.[27,28] The W chromosome is highly differentiated from the Z, being rich in heterochromatin and in repetitive DNAs, including a huge 18S rDNA cistron on its long arms.[27–30] Besides the unusual presence of 18S rDNAs on the sex-specific chromosome, a variable hybridization pattern of rDNAs was also verified on some autosomal pairs, including a syntenic organization of these genes in some species.[11,31,32]

Here, we analyzed the chromosomal organization of three multigene families (18S and 5S rDNAs and U2snDNA) in the genome of eight *Triportheus* species. The results highlighted a hypervariability concerning the number of loci and their presence in the W chromosome, revealing the evolutionary dynamism of the multigene families and their more complex organization than previously supposed for closely related species.

Materials and Methods

Materials and chromosomal preparation

Eight *Triportheus* species from different Brazilian river basins were collected and analyzed. The individuals investigated, the collection locations and sexes are listed in Table 1. The sampling permit was obtained from the Brazilian environmental agency ICMBIO/SISBIO (License number 48628-2). All individuals were properly identified and deposited in the fish collection of the Laboratory of Biology and Genetics of Fishes of the Universidade Estadual Paulista (UNESP, Botucatu, SP) (Table 1). The experiments followed ethical conduct, and anesthesia was administered before sacrificing the animals to minimize suffering in accordance with the Ethics Committee on Animal Experimentation of the Universidade Federal de São Carlos (UFSCar, São Carlos, SP—CEUA 1853260315). Mitotic chromosomes were directly prepared from cell suspensions of the anterior kidney according to Bertollo *et al.*[33]

Probe preparation

Three multigene families were directly isolated from the genome of *T. nematurus*, cloned into plasmid vectors and propagated in DH5 *Escherichia coli*-competent cells (Invitrogen). The 18S rDNA probe was obtained according to Cioffi *et al.*[34] and corresponded to a 1400-bp segment of the 18S rRNA gene. The 5S rDNA probe was obtained according to Martins *et al.*[35] and included a 120 bp of 5S rRNA encoding gene and 200 bp of the nontranscribed spacer. The U2 snDNA probe was isolated using the primers described by Cross *et al.*[36]

Both 18S and 5S rDNAs probes were directly labeled using Nick-Translation Mix (Roche), in which 18S rDNA was labeled with Cy5-dUTP and 5S rDNA with Spectrum Green-dUTP (Vysis). The U2 snRNA probe was labeled via PCR using the U2 primers and the cycle program described in Cross *et al.*[36] with Spectrum Orange-dUTP (Vysis).

Fluorescence in situ *hybridization (three-color-fluorescence* in situ *hybridization)*

Chromosomal preparations of males and females of the mentioned *Triportheus* species were used for a three-color fluorescence *in situ* hybridization (FISH) experiment. The slides were first incubated at 37°C for 1 h and subsequently, they were treated with RNAse (10 mg/mL) for 1 h at 37°C in a moist chamber. Next, a 5-min wash using 1· phosphate-buffered saline (PBS) was performed and the slides were treated with 0.005% pepsin solution in 10 mM HCl for 3 min at 37°C. The slides were washed again with 1· PBS for 5 min and the material was fixed with 1% formaldehyde at room temperature (RT) for 10 min. After further washing, the slides were dehydrated with 70%, 85%, and 100% ethanol series, 3 min each. Afterward, chromosomal DNA was denatured in 70% formamide/2· SSC for 3 min at 72°C. The slides were dehydrated in a cold ethanol 70% series and 85% and 100% at RT, 3 min each. The hybridization mixture, containing 100 ng of each denatured probe, 10 mg/mL dextran sulfate, 2· SSC, and 50% formamide in a final volume of 25 1L, was heated to 86°C for 10 min and then applied to the slides. Hybridization was performed during 16–18 h at 37°C in a moist chamber. After hybridization, the slides were washed in 1· SSC for 5 min at 42°C, and in 4· SSCT using a shaker at RT and then quickly rinsed in 1· PBS. Subsequently, the slides were dehydrated again in an ethanol series (70%, 85%,

TABLE 1. BRAZILIAN COLLECTION SITES OF THE *TRIPORTHEUS* SPECIES AND NUMBER OF INDIVIDUALS IN THIS STUDY

Species	*Site*	*Basin*	N	*Deposit number*
Triportheus albus	Araguaia river	Araguaia-Tocantins	(04 \; 04 _)	LBP18620
Triportheus auritus	Araguaia river	Araguaia-Tocantins	(05 \; 04 _)	LBP18622
Triportheus guentheri	Inhuma lake	São Francisco	(12 \; 06 _)	LBP18628
Triportheus nematurus	Paraguai river	Paraguai	(09 \; 07 _)	LBP18624
Triportheus pantanensis	Paraguai river	Paraguai	(01 \; 01 _)	LBP18623
Triportheus aff. *rotundatus*	Paraguai river	Paraguai	(19 \; 21 _)	LBP18625
Triportheus signatus	Piracicaba river	Tietê	(13 \; 24 _)	LBP18619
Triportheus trifurcatus	Araguaia river	Araguaia-Tocantins	(04 \; 11 _)	LBP18621

and 100%), 3 min each. After the complete drying of the slides, the chromosomes were counterstained with 4¢, 6-diamidino-2-phenylindole/antifade (1.2 mg/mL; Vector Laboratories).

Microscope analyses and image processing

At least 30 metaphase spreads were analyzed per individual to confirm the diploid chromosome numbers, karyotype structure, and FISH results. All images were captured by the Zeiss Axioplan microscope using the ISIS digital FISH imaging system (MetaSystems). The longitudinal distribution of signals along the chromosome-pair No. 3 was also analyzed using the ISIS digital FISH imaging software. The chromosomes were classified metacentric (m), submetacentric (sm), or subtelocentric (st) according to their arm ratios.[37]

Results

All *Triportheus* species analyzed have diploid chromosome number (2n) = 52 and karyotype composed by m/sm and some st chromosomes, with a heteromorphic ZZ/ZW sex chromosome systems (Fig. 1), as verified before.[28,31,38,39] Besides, the Z chromosome is metacentric and the largest one within the karyotype, while the W is always smaller than Z, but with variable shapes and sizes among species (Fig. 1).

The W chromosome of all species shows an 18S rDNA cluster on the long (q) arms, however, in *T. albus*, this chromosome also possesses U2 snDNA cluster in the same chromosomal site. Concerning autosomes, the three multigene families depict a variable distribution pattern depending on the species, being located in separate chromosomes or in a synteny (Figs. 1 and 2). The chromosome-pair No. 3 of all species bears an 18S rDNA site on the short (p) arms. Additionally, these sequences are also present on the p arms of the chromosome-pair No. 8 in *T. albus* and pair No. 7 in *T. trifurcatus*. The 5S rDNA sequences are located on the p arms of the chromosome-pair No. 9 in all species. In addition to this site, *T. auritus*, *T. nematurus*, *T. signatus*, and *T. trifurcatus* display 5S rDNA cluster in the chromosome-pair No. 3, and *T. auritus* also in chromosome-pairs Nos. 4, 5, and 6. The U2 snDNA in each species is always clustered on the p arms of four chromosomes: the chromosome-pair No. 7 in all species; the pair No. 3 in *T. auritus*, *T. albus*, *T. nematurus*, *T. signatus*, and *T. trifurcatus* and the pair No. 4 in *T. guentheri*, *T. pantanensis*, and *T.* aff. *rotundatus* (Figs. 1 and 2). A review providing the chromosomal characteristics of U2 snDNAs among fish species, that is, number of sites, location, and syntenic organization with rDNAs sites, is given in Table 2.

In summary, each of the three multigene families is located in separate chromosomes in *T. guentheri*, *T. pantanensis*, and *T.* aff. *rotundatus*. The short arms of the pair No. 3 holds syntenic sequences of 5S and 18S rDNAs and U2 snDNAs in *T. auritus*, *T. nematurus*, *T. signatus*, and *T. trifurcatus*, but only 18S rDNA and U2 snDNA in *T. albus*. *T. trifurcatus* also presents an additional syntenic location of 18S rDNA and U2 snDNA on the p arms of the chromosome-pair 7 (Figs. 1 and 2).

All the multigene families are in adjacent syntenic location in chromosome-pair No. 3, not showing an intercalary

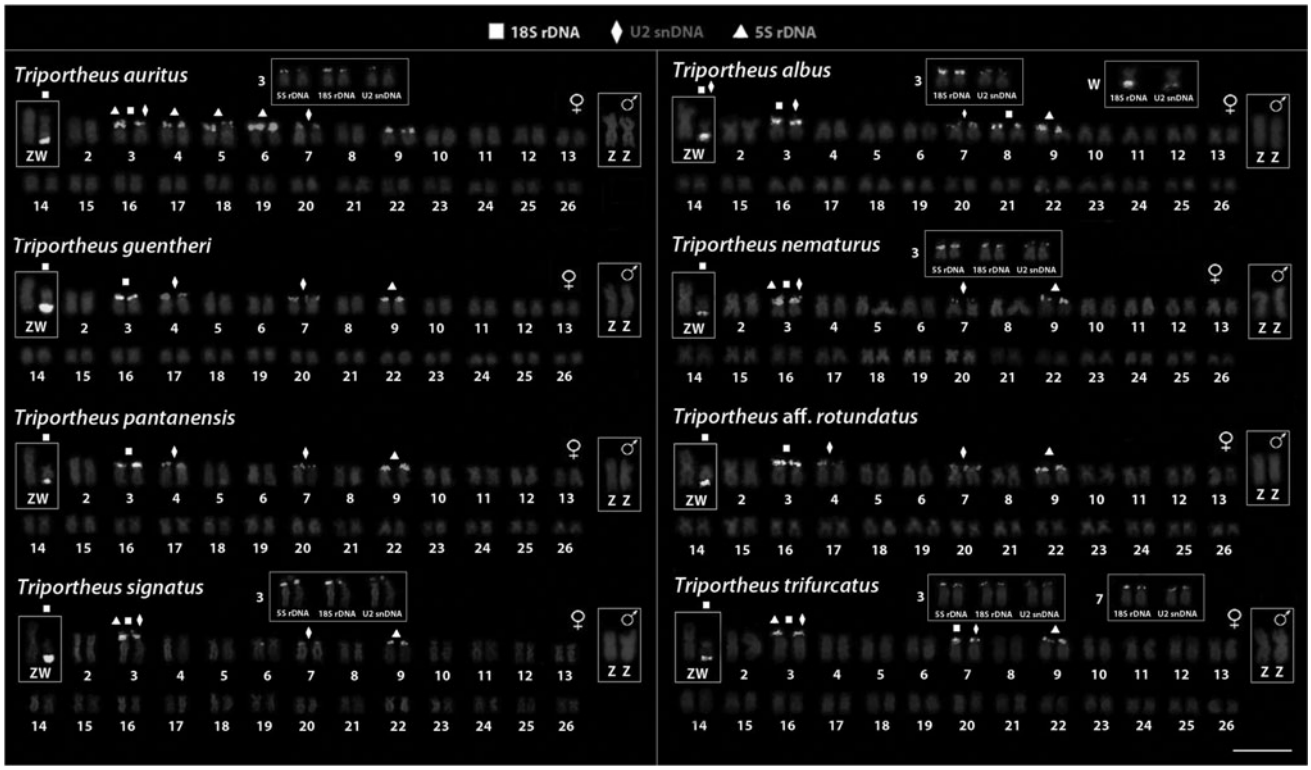

FIG. 1. Karyotypes of eight *Triportheus* species arranged from chromosomes after three color FISH experiment FISH showing the distribution of 18S rDNA (*square*), 5S rDNA (*triangle*), and U2 snDNA (*losenge*) sequences on the chromosomes. The chromosomes bearing syntenic sites are *boxed* and each probe is displayed separately. The Z and W chromosomes are highlighted. Bar = 5 1m. FISH, fluorescence *in situ* hybridization; snDNA, small nuclear DNA.

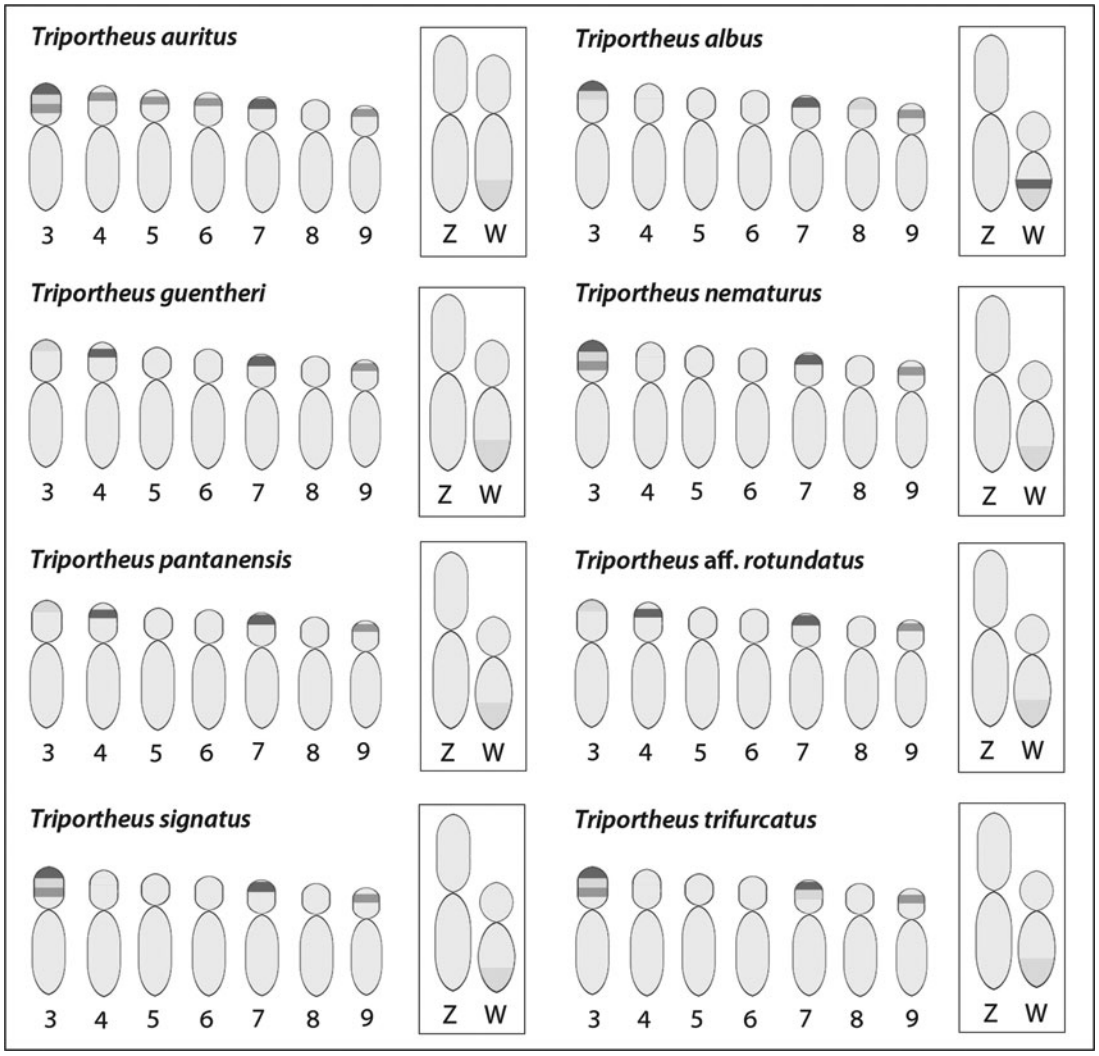

FIG. 2. Partial schematic idiograms showing the chromosomal pairs bearing the multigene families analyzed (18S rDNA - *light gray*, 5S rDNA - *intermediate gray*, and U2 snDNA - *dark gray*) in eight *Triportheus* species analyzed. Note the number of loci and the variable syntenic organization among them. The sex chromosomes are *boxed*.

arrangement. The results obtained by the ISIS digital FISH imaging software, confirmed their flanking configuration (Fig. 3).

Discussion

Repetitive DNA sequences turned out to be excellent markers in cytogenetic studies, mainly considering their inherent dynamism, contributing to identify chromosome rearrangements and other evolutionary process.[40,41] Despite advances in sequencing technologies, more improvements are needed to properly sequence and characterize the repetitive regions.[42] In this sense, cytogenetic techniques provide excellent opportunity to investigate the repetitive fraction of the genome, revealing how the repetitive DNA sequences are structurally organized on the chromosomes.[43] The hypervariability in numbers of rDNA loci highlighted the intense evolutionary dynamics related to these genes, which can generate different chromosomal patterns even in closely related species.[44] Previous studies conducted in *Triportheus* have pointed to distinctly different organization of rDNAs among species.[11,31,32] However, this study aimed to clarify the evolutionary trends behind the particular chromosomal organization of multigene families (including U2 snDNAs). This was realized based on the extensive variation in numbers of loci, the syntenic configuration of these sequences, and their distribution pattern on the W chromosome of these *Triportheus* species.

U2 snDNA genes were mapped for the first time in *Triportheus*. This represents the first available data for Triportheidae family, contributing to characterize these sequences in Characiformes, since only some *Astyanax* (Characidae) and *Characidium* (Crenuchidae) species were analyzed until now[45] (Table 2). The U2 snDNAs are quite conserved in all eukaryotes, but their genomic and chromosomal organization can be very distinct among species.[18,46,47] Although dispersed U2 snRNA signals have been found in species from the Batrachoididae family,[21] most studies have identified these sequences clustered in one chromosome pair[4,5,48] (Table 2). In *Triportheus*, two autosomal pairs carried U2 snRNA sites in the terminal region of the p arms in all species (Figs. 1 and 2), which appears to hint at a conserved number of sites in this genus. However, the location of one cluster is variable, being

Table 2. Cytogenetic Data of U2 Small Nuclear RNA Genes in Fish Chromosomes

Chromosomal characterization of U2 snRNA genes in fishes						
Orders and species	*Number of chromosomes*	*Position*	*Syntenic 18S/28S rDNA*	*Syntenic 5S rDNA*	*With both rDNAs*	*References*
Batrachoidiformes						
Amphichthys cryptocentrus	Dispersed	Dispersed	Negative	—	Negative	21
Batrachoides manglae	Dispersed[a]	Dispersed[a]	Negative	Negative	Negative	21
Halobatrachus didactylus	2	Interstitial/q arms	Negative	Negative	Negative	17
Porichthys plectrodon	Dispersed	Dispersed	Negative	—	Negative	21
Thalassophryne maculosa	2/dispersed	Interstitial/q arms	Positive	—	—	21
Characiformes						
Astyanax altiparanae	4	Proximal	—	Negative	Negative	45
Astyanax bockmanni	4	Proximal	—	Negative	Negative	45
Astyanax fasciatus	4	Proximal	—	Negative	Negative	45
Astyanax jordani	2	Proximal	—	Negative	Negative	45
Astyanax paranae	4	Proximal	—	Negative	Negative	45
Characidium cf. *zebra*	2	Subcentromeric	Negative	Positive	Negative	60
Characidium tenue	2	Subcentromeric	Negative	Positive	Negative	60
Characidium xavante	2	Subcentromeric	Negative	Positive	Negative	60
Characidium stigmosum	2	Subcentromeric	Negative	Positive	Negative	60
Characidium sp1	2	Subcentromeric	Negative	Positive	Negative	60
Characidium sp2	2	Subcentromeric	Negative	Negative	Negative	60
Characidium sp3	2	Subcentromeric	Negative	Positive	Negative	60
Characidium sp4	2	Subcentromeric	Negative	Negative	Negative	60
Characidium sp5	2	Subcentromeric	Negative	Positive	Negative	60
Characidium vestigipinne	2	Subcentromeric	Negative	Positive	Negative	60
Characidium rachovii	2	Subcentromeric	Negative	Positive	Negative	60
Characidium orientale	2	Subcentromeric	Negative	Positive	Negative	60
Characidium aff. *C. vidali*	2	Subcentromeric	Negative	Negative	Negative	60
Triportheus albus	4 + W	Terminal	Positive 1 pair	Negative	Negative	Present study
Triportheus auritus	4	Terminal	Positive 1 pair	Positive 1 pair	Positive	Present study
Triportheus guentheri	4	Terminal	Negative	Negative	Negative	Present study
Triportheus nematurus	4	Terminal	Positive 1 pair	Positive 1 pair	Positive	Present study
Triportheus pantanensis	4	Terminal	Negative	Negative	Negative	Present study
Triportheus aff. *rotundatus*	4	Terminal	Negative	Negative	Negative	Present study
Triportheus signatus	4	Terminal	Positive 1 pair	Positive 1 pair	Positive	Present study
Triportheus trifurcatus	4	Terminal	Positive 2 pairs	Positive 1 pair	Positive	Present study
Gadiformes						
Merluccius merluccius	2	Subcentromeric	Negative	Negative	Negative	5
Gymnotiformes						
Gymnotus carapo	2	Terminal	Negative	Negative	Negative	24
Gymnotus inaequilabiatus	2	Terminal	Negative	Negative	Negative	24
Gymnotus javari	2	Terminal	Negative	Negative	Negative	24
Gymnotus pantanal	12 \/10 _ + X1	Terminal	Negative	Negative	Negative	24
Gymnotus pantherinus	2	Terminal	Negative	Negative	Negative	24
Gymnotus sylvius	2	Terminal	Negative	Negative	Negative	24
Perciformes						
Argyrosomus regius	2	Subcentromeric[b]	Negative	Negative	Negative	23
Dicentrarchus labrax	2	Telomeric/q arms	Negative	Negative	Negative	4
Dicentrarchus punctatus	2	Telomeric/q arms	Negative	Negative	Negative	4
Diplodus sargus	2	Subcentromeric	Negative	Negative	Negative	48
Pagrus auriga	2	Subcentromeric	Negative	Negative	Negative	48
Pagrus pagrus	2	Subcentromeric	Negative	Negative	Negative	48
Plectorhinchus mediterraneus	2	Subcentromeric	Negative	Negative	Negative	22
Pleuronectiformes						
Solea senegalensis	4	Subcentromeric	Positive[c]	Positive	Positive	18
Siluriformes						
Mystus bocourti	2	Telomeric	Negative	Negative	Negative	47

Light gray represents the *Triportheus* species of the present study. The *dark gray* area represents the cases in which the U2 snRNA genes are syntenic with both rDNAs (18S and 5S). Positive synteny with both rDNAs.

[a]A degree of clustering in a specific chromosome pair in *B. manglae*.

[b]In addition, the U2 snRNA gene showed minor spots scattered throughout the genome of *A. regius*.

[c]See the chromosomal location of 18S rDNAs in *Solea senegalensis*, data are available in Cross *et al.*[62]

snRNA, small nuclear RNA.

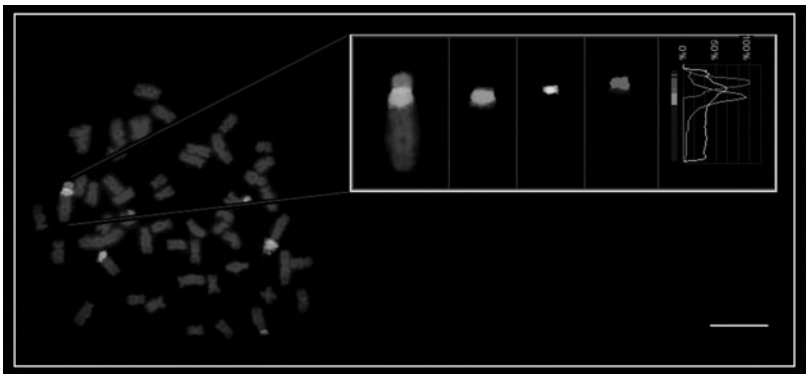

FIG. 3. Analysis of the syntenic configuration of the multigene families on the chromosome-pair No. 3 of *Triportheus signatus.* Metaphase plate of *T. signatus* and the pair No. 3 in detail; note the longitudinal distribution of the 5S (*first site*), 18S (*second site*), and U2 snDNA (*third site*) along this chromosome pair. Bar = 5 1m.

located in chromosome-pair No. 4 or in syntenic organization with rDNAs in chromosome-pair No. 3 depending on the species (Figs. 1 and 2). Regarding the W chromosome, only *T. albus* showed U2 snRNA sequences, syntenic with the 18S rDNA site. The presence of U2 snRNA genes in sex chromosomes was only verified on the X_1 chromosome of *Gymnotus pantanal*.[24] However, one can rule out their ubiquitous presence on the sex chromosomes since only few data are currently available (Table 2).

Contrary to the conserved number of U2 snDNAs sites, the number of rDNAs is quite variable among the different *Triportheus* species. It has been assumed that a single pair of 45S rDNA site is the most common scenario found among teleosts, including the ancestral groups.[9] In all *Triportheus* species, the 18S rDNA sequences are clustered in the chromosome-pair No. 3 and in W chromosome, with an additional cluster present in *T. albus* and *T. trifurcatus* (Fig. 2). According to Martins and Wasko,[7] the number of 45S rDNA loci is more variable in fishes compared to the conserved number of 5S rDNAs. However, the scenario shown in *Triportheus* is quite the opposite, since 5S rDNAs are even more variable then the 18S rDNA loci. Indeed, despite 5S rDNA clusters being always found in the pair No. 9 in all species, their variability is demonstrated by additional sites being found in *T. nematurus*, *T. signatus*, and *T. trifurcatus* (where they occur in a syntenic organization with 18S rDNAs and U2 snDNAs in pair 3), and by the presence of eight additional sites in *T. auritus* (Fig. 2).

It was proposed that the relative conserved nature of 5S rDNA loci in fishes might be due to their interstitial chromosome position, which is also found in other animal groups.[7] Therefore, the variable number of loci of these genes can be related with their chromosomal terminal position in *Triportheus*. In fact, this chromosomal location appears to facilitate frequent rearrangements, due to the proximity of the chromosome ends in the interphase nucleus allowing the spreading of sequences through the genome.[49,50] Moreover, some studies have also pointed out the involvement of transposable elements (TEs) in rDNA movement, as verified in *Diplodus sargus* and cichlid fishes, since the variable number of rDNA genes in these species were associated with the presence of TEs in their flanking regions.[44,48] In *Erythrinus erythrinus*, the huge chromosomal dispersion of 5S rDNAs was associated with the activity of the non-long terminal report retrotransposons *Rex 3*, since they were colocalized in the centromeric region of several chromosomes.[3] Regarding *Triportheus* species, this feature appears to be less plausible, at least for *Rex* retrotransposons, since neither they are found in synteny with rDNA genes nor occupy a particular chromosomal region.[29,30] In addition, the movement of ribosomal genes could be also mediated by extrachromosomal circular DNA, as verified in many taxa.[51]

Distinct chromosomal location of 5S and 18S rDNAs is common in fishes and other taxa.[10] The syntenic organization, not only for the rDNAs, but also for the U2 snDNAs, reveals a very particular pattern in *Triportheus* species (Figs. 1 and 2). Indeed, this study shows a very rare situation for fishes, in which U2 snDNA clusters are closely located to both rDNAs (Table 2). However, the divergent distribution of these sequences, even in closely related species, involving the syntenic state and their distribution in distinct chromosome pairs, reflects the instability of the particular region bearing all the three multigene families, that is, the heterochromatic p arms of the chromosome-pair No. 3. Alongside with other reported mechanisms, the constitutive heterochromatin is also associated with chromosomal rearrangements[9,52] and could have facilitated the spreading of those sequences. The fact that these three multigene families are arranged in an adjacent way and not interspersed with each other (Fig. 3), may also explain their turnover organization along the chromosomal evolution of *Triportheus*.

The presence of 18S rDNAs on the W chromosome of *Triportheus* appears to be a conserved feature, since all species studied so far display this distribution. It is common sense that sex chromosomes evolve from an ancestral autosomal pair, in which structural and/or DNA changes occur in the specific chromosome bearing sex determining factors.[53,54] Partial suppression of recombination in the proto-sex chromosome pair is a crucial step in this process.[55] Once recombination stopped, repetitive sequences can invade and be amplified in the sex-specific chromosome, leading its differentiation.[56] Beside 18S rDNAs, the W chromosome of *Triportheus* is rich in other repetitive DNA classes, as evidenced by the variable accumulation of microsatellites.[30] Additionally, the W chromosome of *T. albus* also bears a particular U2 snDNA cluster, reflecting the dynamic mechanisms of transposition and amplification of repetitive DNAs, especially in the sex-specific chromosomes. The size of the 18S rDNA cluster on the Wq is also well differentiated among *Triportheus* species (Fig. 1). These changes in size are probably associated with independent evolutionary process followed by the W chromosome in this fish group, as demonstrated by their differential size, morphology, and repetitive DNA content.[28,30] The presence of 18S rDNA sequences on the sex chromosomes has already been reported in fishes

and in other groups.[57–60] As demonstrated in *Salmonella typhimurium*, unequal crossing over occurs more frequently at the rDNA sites than at other loci.[61] This appears to be a powerful evolutionary force and, among others, an attractive hypothesis to explain the occurrence of these sequences on the sex chromosomes of several species.

Conclusions

In general, our study showed a particular scenario concerning the distribution of three multigene families in *Triportheus* genomes and provided new chromosomal data for U2 snRNA genes in fish species. The variability in numbers of rDNA clusters, and the various syntenic status for these three multigene families, put in evidence the evolutionary dynamism of these genes, even in closely related species. Furthermore, our study provided additional data on the accumulation of repetitive sequences in the sex-specific chromosome and their significance in the differentiation of the pair of sex chromosomes.

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Disclosure Statement

No competing financial interests exist.

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