Neotropical Ichthyology, 16(1): e170066, 2018
DOI: 10.1590/1982-0224-20170066

Journal homepage: www.scielo.br/ni
Published online: 26 March 2018 (ISSN 1982-0224)
Printed: 31 March 2018 (ISSN 1679-6225)

Original article

Conserved number of U2 snDNA sites in *Piabina argentea*, *Piabarchus stramineus* and two *Bryconamericus* species (Characidae, Stevardiinae)

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The chromosomal location of 5S rRNA and U2 snRNA genes of *Piabina argentea*, *Piabarchus stramineus* and two *Bryconamericus* species from two different Brazilian river basins were investigated, in order to contribute to the understanding of evolutionary characteristics of these repetitive DNAs in the subfamily Stevardiinae. The diploid chromosome number was 2n = 52 for *Bryconamericus* cf. *iheringii*, *Bryconamericus turiuba*, *Piabarchus stramineus* and *Piabina argentea*. The 5S rDNA clusters were located on one chromosome pair in *P. stramineus* and *B.* cf. *iheringii*, and on two pairs in *B. turiuba* and *P. argentea*. The U2 snDNA clusters were located on the one pair in all species. Two-color FISH experiments showed that the co-localization between 5S rDNA and U2 snDNA in *P. stramineus* can represent a marker for this species. Thus, the present study demonstrated that the number of U2 snDNA clusters observed for the four species was conserved, but particular characteristics can be found in the genome of each species.

Keywords: Repetitive DNA, Splicing, 5S rDNA, Chromosome, Diploid number.

A localização cromossômica dos genes de RNAr 5S e RNAsn U2 de *Piabina argentea*, *Piabarchus stramineus* e duas espécies de *Bryconamericus* provenientes de duas bacias hidrográficas foi investigada, com a intenção de contribuir com o entendimento de características evolutivas destes DNAs repetitivos na subfamília Stevardiinae. O número cromossômico diploide foi 2n = 52 para *Bryconamericus* cf. *iheringii*, *Bryconamericus turiuba*, *Piabarchus stramineus* e *Piabina argentea*. Os sítios de DNAr 5S foram localizados em um par cromossômico em *P. stramineus* e *B.* cf. *iheringii*, e em dois pares em *B. turiuba* e *P. argentea*. Os sítios de DNAsn U2 foram localizados em um par em todas as espécies. Experimentos de FISH com duas sondas mostraram que a co-localização entre os DNAr 5S e DNAsn U2 em *P. stramineus* pode representar um marcador para esta espécie. Portanto, o presente estudo demonstrou que o número de sítios de DNAsn U2 observado para as quatro espécies foi conservado, porém características particulares podem ser encontradas no genoma de cada espécie.

Palavras-chave: DNA repetitivo, DNAr 5S, Cromossomo, Número diploide. Splicing.

Introduction

Numerous modifications have been made regarding the phylogenetic relationships of the genera *Bryconamericus* Eigenmann, 1907, *Piabarchus* Myers, 1928 and *Piabina* Reinhardt, 1867, which have already belonged to the group *incertae sedis* in Characidae by Lima *et al.* (2003), as well as many other genera. Nevertheless, studies based on analyses of molecular characters have indicated that *Bryconamericus*, *Piabarchus* and *Piabina* belong to the subfamily Stervadiinae (see, for example, Oliveira *et al.*, 2011; Thomaz *et al.*, 2015).

The karyotype and chromosomal characteristics of *Bryconamericus*, *Piabarchus* and *Piabina* have been described in the literature by some authors utilizing conventional (Giemsa staining, silver staining, C-banding) and molecular (Fluorescence *in situ* hybridization - FISH with rDNA and snDNA probes) cytogenetic techniques (data summarized in Tab. 1). In these studies, the most frequently reported diploid number was 2n = 52 chromosomes and variations involving the number of clusters of 18S and 5S rDNA were also registered.

Unlike rDNAs, U2 snDNA clusters have been poorly investigated in chromosomes of *Bryconamericus*, *Piabarchus* and *Piabina* genera. To date, only studies in *B. ecai* da Silva, 2004 and *Bryconamericus* sp. showed chromosomal mapping of U2 snDNA in this fish group (Santos *et al.*, 2017). The chromosomal mapping of U2 snDNA clusters

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showed a broad scenario in fish chromosomes, with these sequences accumulating in one or more chromosome pairs. In *B. ecai*, *Bryconamericus* sp. (Santos *et al*., 2017), *Astyanax mexicanus* (De Filippi, 1853) (Piscor *et al*., 2016) and *A. jordani* (Hubbs & Innes, 1936) (Silva *et al*., 2015) signals in one chromosome pair were detected. On the other hand, eleven *Astyanax* Baird & Girard, 1854 species showed two chromosomes pairs bearing U2 snDNA clusters (Silva *et al*., 2015; Piscor *et al.*, 2016). The authors showed that, in comparison to other repetitive sequences studied in chromosomes of *Bryconamericus* and *Astyanax*, the U2 snDNA is the most conserved.

Although scarce, the mapping of U2 snDNA sequences in different individuals has demonstrated that these sequences may be linked with other multigene families. According to Yano *et al.* (2017), in four *Triportheus* Cope, 1872 species, the U2 snRNA genes are syntenic with both rDNAs (18S and 5S), while in *Triportheus albus* Cope, 1872, the U2 snRNA genes are syntenic with 18S rDNA and in other three *Triportheus* species, the U2 snRNA genes are not syntenic with rDNAs. The last described pattern is common in fish (Pelliccia *et al.*, 2001; Manchado *et al.*, 2006; Úbeda-Manzanaro *et al.*, 2010; Utsunomia *et al.*, 2014; Scacchetti *et al*., 2015; Silva *et al*., 2015).

Tab. 1. Literature review on the number of chromosomes bearing repetitive sequences in *Piabina*, *Piabarchus* and *Bryconamericus* genera from Brazilian rivers. [a]Cytotypes; [b]Diploid numbers; [c]Extra chromosome; [d]18S rDNA cluster numbers; [e]5S rDNA cluster numbers [f]U2 snDNA cluster numbers; MG = State of Minas Gerais; MS = State of Mato Grosso do Sul; PR = State of Paraná; RS = State of Rio Grande do Sul; SP = State of São Paulo.

Genera/Species	Localities/States	2n[b]	18S[d]	5S[e]	U2[f]	References
		Bryconamericus				
B. aff. *exodon*	Três Bocas Stream (PR)	52	8	–	–	Paintner-Marques *et al.* (2002)
B. aff. *iheringii*	Água Floresta River (PR)	52	2	–	–	Paintner-Marques *et al.* (2003)
B. aff. *iheringii* cyt-I[a]	Maringá Stream (PR)	52	6	–	–	Capistano *et al*. (2008)
B. aff. *iheringii* cyt-II[a]	Keller River (PR)	52	10	–	–	
B. aff. *iheringii* cyt-III[a]	Tatupeba Stream (PR)	52	2	–	–	
B. ecai cyt-I[a]	Forquetinha River (RS)	52	4	–	–	Santos *et al.* (2012)
B. ecai cyt-II[a]	Forquetinha River (RS)	52	2	–	–	
B. ecai cyt-III[a]	Forquetinha River (RS)	52 + B[c]	6	–	–	
B. ecai cyt-IV[a]	Forquetinha River (RS)	52	2	–	–	
B. turiuba	Tributary of Passa-Cinco River (SP)	52	4	4	–	Piscor *et al.* (2013)
B. cf. *iheringii*	Tributary of Corumbataí River (SP)	52	2	2	–	
B. aff. *iheringii* cyt-I[a]	Três Bocas Stream (PR)	52	2	–	–	Silva *et al.* (2014)
B. aff. *iheringii* cyt-II[a]	Três Bocas Stream (PR)	52	8	–	–	
B. aff. *iheringii* cyt-III[a]	Três Bocas Stream (PR)	52	6	–	–	
B. aff. *iheringii* cyt-IV[a]	Três Bocas Stream (PR)	52	6	–	–	
B. aff. *iheringii* cyt-V[a]	Três Bocas Stream (PR)	52	8	–	–	
B. aff. *iheringii* cyt-VI[a]	Três Bocas Stream (PR)	52	8	–	–	
B. aff. *iheringii*	Ocoí River (PR)	52	2	–	–	Nishiyama *et al.* (2015)
B. ecai cyt-V[a]	Forquetinha River (RS)	52	4	6	2	Santos *et al.* (2017)
B. ecai cyt-VI[a]	Forquetinha River (RS)	52	13	8	2	
B. ecai cyt-VII[a]	Forquetinha River (RS)	52	10	7	2	
Bryconamericus sp. (Group 1)	Vermelho River (PR)	52	4	6	2	
Bryconamericus sp. (Group 2)	Vermelho River (PR)	52	16	8	2	
Bryconamericus sp. (Cambuta)	Cambuta River (PR)	52	6	2	2	
B. turiuba	Tributary of Passa-Cinco River (SP)	52	–	4	2	Present study
B. cf. *iheringii*	Tributary of Corumbataí River (SP)	52	–	2	2	
		Piabarchus stramineus				
P. stramineus	Guaçu Stream (MS)	52	2	2	–	Piscor *et al.* (2013)
P. stramineus	Guaçu Stream (MS)	52	–	2	2	
		Piabina				
P. argentea	São Francisco River (MG)	52	6	4	–	Peres *et al*. (2008)
P. argentea	Municipality of Itatinga (SP)	52	2	4	–	Pazian *et al.* (2012)
P. argentea	Municipality of Botucatu (SP)	52	4	4	–	
P. argentea	Municipality of Bauru (SP)	52	6	6	–	
P. anhembi	Municipality of Salesópolis (SP)	52	2	2	–	
P. argentea	Tributary of Passa-Cinco River (SP)	52	–	4	2	Present study

The aim of the present study was to analyze the chromosomal location of two multigene families (5S rDNA and U2 snDNA) in the genome of *Piabina argentea* Reinhardt, 1867, *Piabarchus stramineus* (Eigenmann, 1908) and two *Bryconamericus* species, in order to obtain a better knowledge about the relationship among U2 snRNA and 5S rRNA genes of species of the subfamily Stevardiinae.

Material and methods

All institutional guidelines for the care and use of laboratory animals were followed. Animals were captured with the permission of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio; number 23434-1).

Two *Bryconamericus* species, *Piabarchus stramineus* and *Piabina argentea* were obtained from locations in Brazil as follows: seven individuals of *B. turiuba* Langeani, Lucena, Pedrini & Tarelho-Pereira, 2005 (five males and two females) and five *B.* cf. *iheringii* (Boulenger, 1887) (all males) from a tributary of the Passa-Cinco River and a tributary of the Corumbataí River (Corumbataí River basin, State of São Paulo), respectively; twenty-one individuals of *P. stramineus* (12 males and nine females) from Guaçu Stream (Iguatemi River basin, State of Mato Grosso do Sul); and eleven individuals of *P. argentea* (five males and six females) from a tributary of the Passa-Cinco River (Corumbataí River basin, State of São Paulo). Voucher specimens were deposited in the fish collection of the Laboratório de Citogenética (LC), Universidade Estadual Paulista, SP, Brazil, as *B. turiuba* (LC 1421), *B.* cf. *iheringii* (LC 1424), *P. stramineus* (LC 1502), and *P. argentea* (LC 1074). Chromosomes were obtained as described by Foresti *et al.* (1981) and chromosome morphologies were determined according to the arm ratios (Levan *et al*., 1964).

Genomic DNA was extracted from fin and liver samples of *Bryconamericus* and *Piabina* species according to Sambrook, Russell (2001). The 5S rDNA probe was prepared using polymerase chain reaction (PCR) with primers described by Pendás *et al.* (1994) (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.* (2013) (U2F, 5′-ATC GCT TCT CGG CCT TAT G–3′; and U2R, 5′-TCC CGG CGG TAC TGC AAT A-3′). The 5S rDNA probes were labeled by PCR with biotin-14-dATP (Invitrogen, San Diego, CA, USA), and the U2 snDNA probes were labeled by PCR with digoxigenin-11-dUTP (Roche, Mannheim, Germany). Probes labeled with digoxigenin-11-dUTP were detected using anti-digoxigenin-rhodamine (Roche, Mannheim, Germany), and probes labeled with biotin-14-dATP were detected using Alexa Fluor 488 conjugated streptavidin (Invitrogen, San Diego, CA, USA). Single and two-color FISH experiments were performed using mitotic metaphase chromosomes according to Pinkel *et al.* (1986) with modifications described by Cabral-de-Mello *et al.* (2010). Chromosomes were counterstained with Vectashield Mounting Medium (Vector, Burlingame, CA, USA) 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).

Results

The diploid chromosome number was 2n = 52 for *Piabarchus stramineus* (karyotype formula: 6 m + 10sm + 16st + 20a), *Bryconamericus turiuba* (karyotype formula: 8 m + 10sm + 14st + 20a), *B.* cf. *iheringii* (karyotype formula: 10 m + 14sm + 18st + 10a), and *Piabina argentea* (karyotype formula: 6m, 8sm, 24st and 14a). These data were reported in previous studies by Piscor *et al.* (2013) for *Bryconamericus* species and Piscor *et al.* (2017) for *P. argentea*. The 5S rDNA clusters were observed on the pericentromeric regions of one acrocentric pair in *B.* cf. *iheringii*, one submetacentric pair in *P. stramineus*, two acrocentric pairs in *B. turiuba* and two pairs in *P. argentea* (one acrocentric and one subtelocentric) (Fig. 1).

The U2 snDNA clusters were observed on the pericentromeric regions of the long (q) arm of one chromosome pair in all four species under study: on the submetacentric pair in *Bryconamericus turiuba* and *Piabarchus stramineus* and on the subtelocentric pair in *B.* cf. *iheringii* and *Piabina argentea* (Fig. 1). The *P. stramineus* species showed 5S rDNA and U2 snDNA clusters on the same chromosome in adjacent position, while for *B.* cf. *iheringii*, *B. turiuba*, and *P. argentea* these clusters are found on separate chromosomes (Fig. 1).

The chromosomes bearing U2 snDNA clusters observed in present study are summarized in Fig. 2.

Discussion

In two *Bryconamericus* species, *Piabarchus stramineus* and *Piabina argentea* under study, as well as shown by Santos *et al.* (2017), the U2 snDNA clusters were observed on the interstitial/pericentromeric regions of the long arm of one chromosome pair in all species, except fo *Bryconamericus* sp. (Cambuta River) that showed one pair bearing U2 snDNA clusters in interstitial position on the short arm. These observations make it clear that, regardless of chromosomal positions, the number of U2 snDNA sites is conserved for *Bryconamericus* genus, as well as described for *Astyanax* genus (Silva *et al.*, 2015; Piscor *et al.*, 2016). However, in *Astyanax* genus almost all species showed two pairs bearing U2 snDNA sites. The location of U2 snRNA gene is described here for the first time in *Piabina argentea*. Thus, in the future, extending these observations to other *Piabina* species could help us confirm if in the genus the number of clusters of this gene is also conserved.

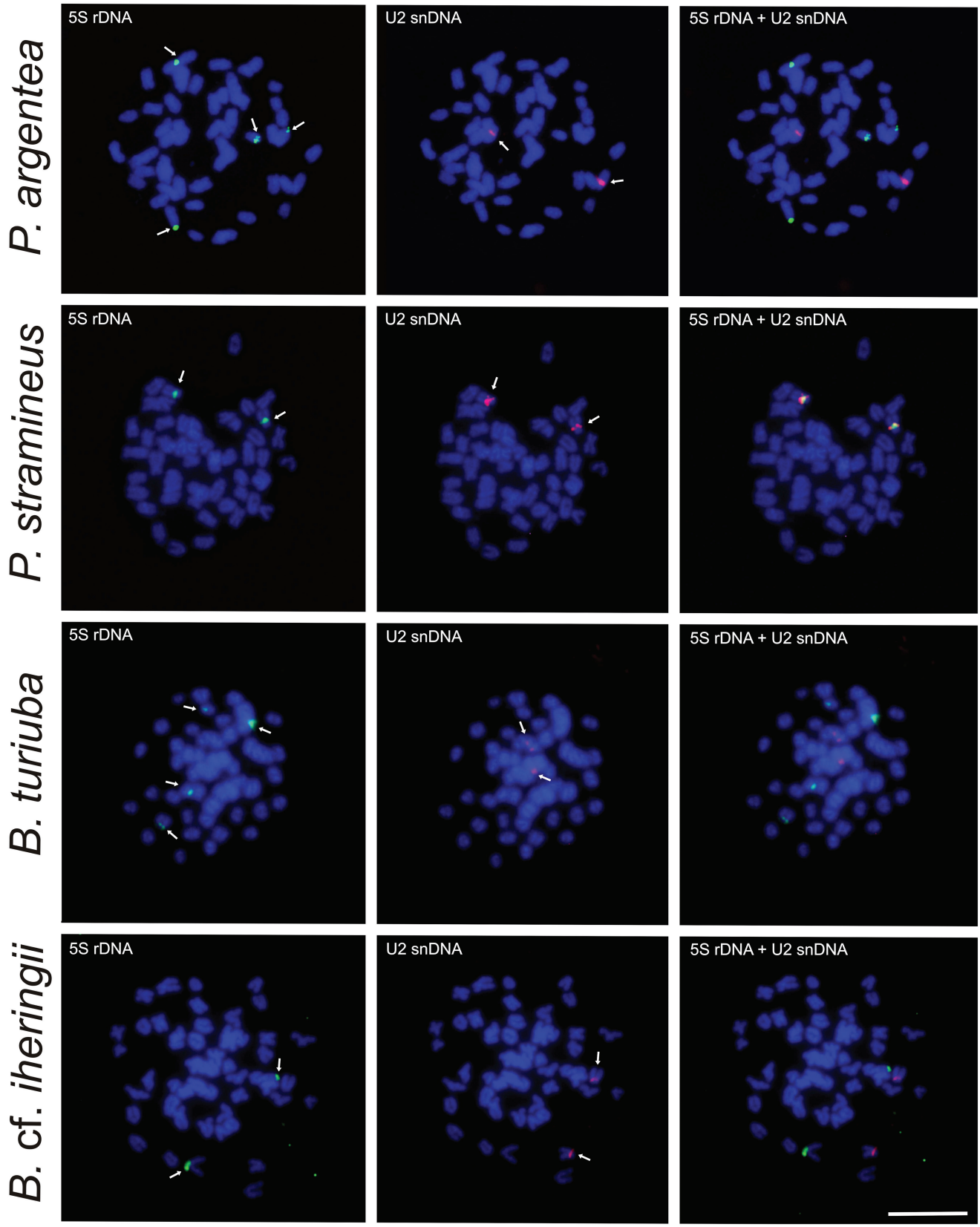

Fig. 1. Sequential metaphases of the chromosomal locations of U2 snDNA and 5S rDNA clusters using two-color FISH in species of the genera *Piabina*, *Piabarchus* and *Bryconamericus*. The arrows indicate the fluorescent signals. Note that, in *P. stramineus*, the two repetitive DNA are located adjacently on the same pair. Scale bar = 10 μm.

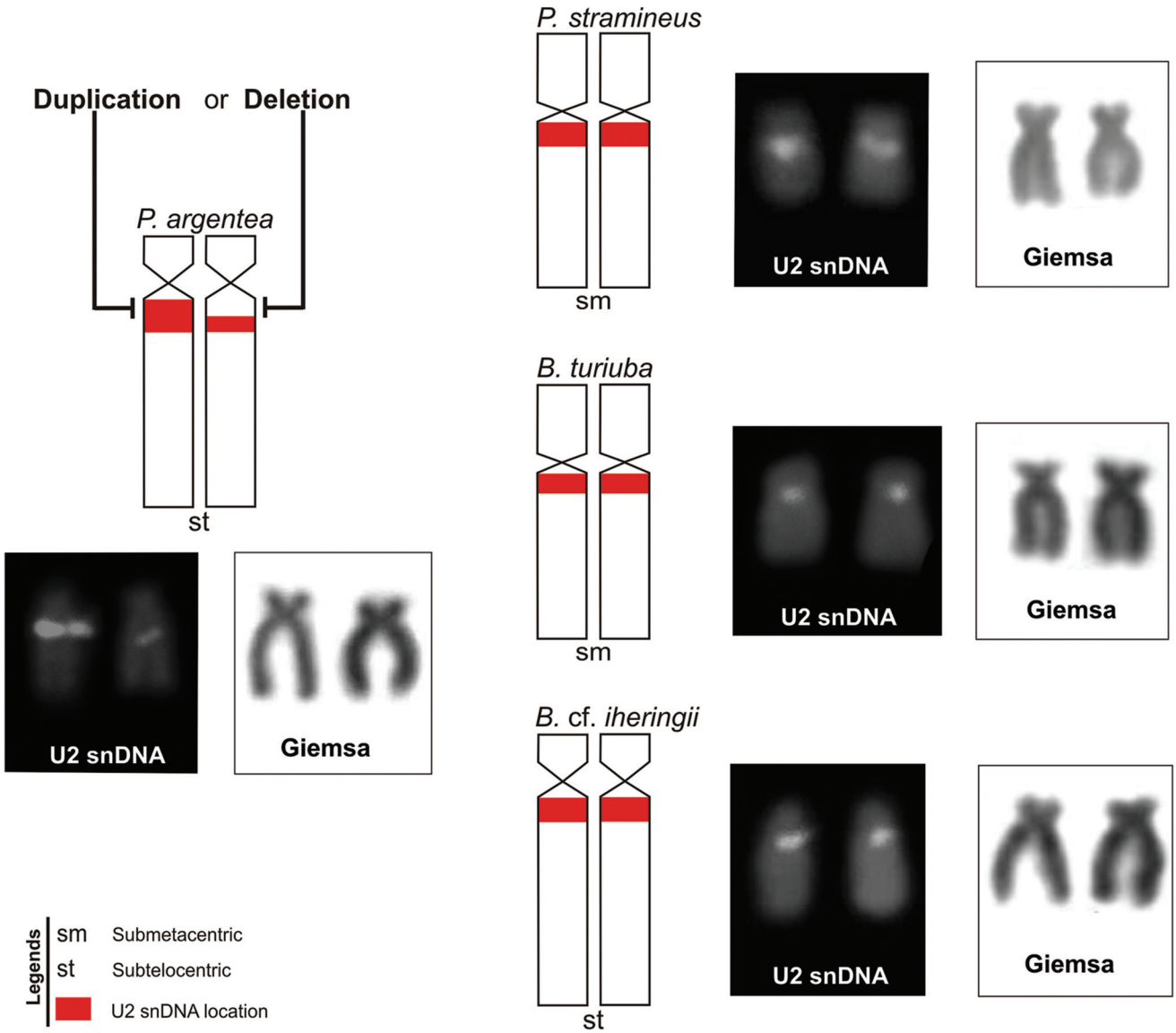

Fig. 2. Scheme showing the number of U2 snDNA sites on the real pairs and ideograms in species of the genera *Piabina*, *Piabarchus* and *Bryconamericus*. Note the size heteromorphism of U2 snDNA clusters between homologous chromosomes in *P. argentea*.

In *Piabina argentea*, a size heteromorphism of U2 snDNA was detected between homologous chromosomes of males and females, indicating that this polymorphism has no association with sex and reflects differences in the number of U2 snDNA copy among one and another homologues chromosome. This attribute suggests that rearrangement processes occurred during meiosis, as *e.g.*, deletion or duplication of these segments. Chromosomal rearrangements tend to be most common in specific regions or "hotspots", and deletions and/or duplications of single-base pairs typically arise during homologous recombination (Clancy, Shaw, 2008). Similar results were reported by Carvalho, Dias (2007), which verified an interindividual size heteromorphism of 18S rDNA clusters in *Iheringichthys labrosus* (Lütken, 1874) (Pimelodidae).

The karyotypes of *Bryconamericus turiuba*, *B.* cf. *iheringii* and *Piabina argentea* shared the non-syntenic sites of 5S rDNA and U2 snDNA in their genomes, a common characteristic of several fish groups (Supiwong *et al.*, 2013; Utsunomia *et al.*, 2014; Piscor *et al.*, 2016). On the other hand, in *Piabarchus stramineus*, the U2 snDNA and 5S rDNA clusters were found in adjacent positions. This syntenic organization of these clusters were not observed in the other species studied here, demonstrating that co-localization between 5S rDNA and U2 snDNA in *P. stramineus* seems to represent a derived condition and could be used as a marker for this species.

A similar example of co-localization between the 5S and 18S rDNA (on the pair 24) was verified for *Bryconamericus* cf. *iheringii* (Piscor *et al.*, 2013), however these clusters presented telomeric location, while co-localization between

5S rDNA and U2 snDNA in *Piabarchus stramineus* under study were observed on the pericentromeric regions. According to Schweizer, Loidl (1987), the proximity of telomeric regions within interphase nuclei would facilitate genetic material transfer, as predicted by Rabl's model. Therefore, the pericentromeric location of the 5S rDNA/U2 snDNA clusters in *P. stramineus* would not facilitate transference events, as suggested by Piscor *et al.* (2013) for *B.* cf. *iheringii*. Thus, probably this co-localization in *P. stramineus* could be explained by association between these multigene families and mobile elements, common association in distinct groups for different repetitive DNAs (Cioffi *et al.*, 2010; Nakajima *et al.*, 2012; Anjos *et al.*, 2015).

Other repetitive sequences were studied near 5S rDNA clusters in other fish groups, *e.g.*, in *Astyanax*, the 5S rDNA was observed co-located to GATA repeats in four species [*Astyanax lacustris* (Lütken 1875) (= *A. altiparanae* Garutti & Britski, 2000), *A. fasciatus* (Cuvier, 1819), *A. marionae* Eigenmann, 1911 and *A. schubarti* Britski, 1964] (Piscor, Parise-Maltempi, 2016). The authors believe that the 5S-GATA co-location can have been maintained in different *Astyanax* species because represents an evolutionary advantage (Piscor, Parise-Maltempi, 2016).

In general, our study showed that one chromosome pair bearing U2 snDNA clusters was conserved for the two genera (*Bryconamericus* and *Piabina*) of the subfamily Stevardiinae, with non-syntenic organization of 5S rDNA and U2 snDNA in their genomes, except for *Piabarchus stramineus* that presented a derived condition (co-localization).

Acknowledgments

The authors are grateful to Dr. Francisco Langeani Neto and Dr. Ricardo Britzke by species identifications. This study was supported by Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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Submitted June 06, 2017
Accepted January 15, 2018 by Claudio Oliveira