Isolation and characterization of 5S rDNA sequences in catfishes genome (Heptapteridae and Pseudopimelodidae): perspectives for rDNA studies in fish by C_0t method

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Abstract Sequences of 5S ribosomal RNA (rRNA) are extensively used in fish cytogenomic studies, once they have a flexible organization at the chromosomal level, showing inter- and intra-specific variation in number and position in karyotypes. Sequences from the genome of *Imparfinis schubarti* (Heptapteridae) were isolated, aiming to understand the organization of 5S rDNA families in the fish genome. The isolation of 5S rDNA from the genome of *I. schubarti* was carried out by reassociation kinetics (C_0t) and PCR amplification. The obtained sequences were cloned for the construction of a micro-library. The obtained clones were sequenced and hybridized in *I. schubarti* and *Microglanis cottoides* (Pseudopimelodidae) for chromosome mapping. An analysis of the sequence alignments with other fish groups was accomplished.

Both methods were effective when using 5S rDNA for hybridization in *I. schubarti* genome. However, the C_0t method enabled the use of a complete 5S rRNA gene, which was also successful in the hybridization of *M. cottoides*. Nevertheless, this gene was obtained only partially by PCR. The hybridization results and sequence analyses showed that intact 5S regions are more appropriate for the probe operation, due to conserved structure and motifs. This study contributes to a better understanding of the organization of multigene families in catfish's genomes.

Keywords C_0t · Genomics micro-library · Hybridization · Pisces

Introduction

The rRNA genes comprise two distinct repeat classes, 45S ribosomal DNA (rDNA) and 5S rDNA. The 45S rDNA contains genes that code 18S, 5.8S and 26S–28S rRNAs, and the second ribosomal class that codifies the 5S rRNA (Long and David 1980). Characterization and isolation of 5S rDNA sequences have been widely used for species identification and phylogenetic analysis of fish species (Alves-Costa et al. 2006; Ardura et al. 2010; Úbeda-Manzanaro et al. 2010). Moreover, chromosomal location of these sites contributes to microstructural analysis, as well to studies in different fish groups.

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The 5S rDNA sequences consist of highly conserved multiple copies of 120 bp of 5S rRNA genes separated by a variable non transcribed region (NTS) (Eickbush and Eickbush 2007). Those sequences have a flexible organization to chromosome level. They vary in number and position in karyotypes, even within the same species, and normally occur in interstitial region of the fish chromosomes (Martins and Wasko 2004).

These rDNA sites may be associated with other multigene families, such as histones, small nuclear RNA (snRNA genes) (Vierna et al. 2012), transposable elements (TEs) or even with extra chromosomal circular DNA (eccDNA) (Raskina et al. 2004; Cohen and Segal 2009). Furthermore, the 5S rDNA could take place in syntenic form with 45S rDNA on the same chromosome in different fish species (Mantovani et al. 2005), although mostly observed in different chromosomes (Martins and Galetti 1999).

5S rDNA studies in Heptapteridae family are scarce, as well as in Siluriformes, and generally reported only in some species or populations (Garcia and Almeida-Toledo 2010a, b; Martinez et al. 2011). Studies performed by Kanteck et al. (2009) show the occurrence of 5S rDNA in *Imparfinis schubarti* (Gomes 1956) syntenic with 18S rDNA sites in the interstitial region of the largest chromosome pair. *Imparfinis mirini* Haseman 1911 and *Imparfinis minutus* (Lutken 1874) analyzed by Ferreira et al. (2014) also showed the same synteny, besides presenting another small metacentric pair, bearer of a single 5S rDNA site in the region near the centromere. In the Pseudopimelodidae family, which has a close phylogenetic relationship with Heptapteridae, hybridization data are even scarcer (Gouveia et al. 2015). The location of 5S rDNA sites are known only in *Lophiosilurus alexandri* Steindachner, 1877, evidenced in the terminal region of a pair of subtelocentric chromosome (Marques et al. 2008).

Given the importance of understanding the structure, location and distribution of such sequences in these families, the present study aimed to obtain a 5S rDNA probe from *Imparfinis schubarti* genome, by means of two different methodologies. Our purpose was to compare the efficiency of hybridization in *I. schubarti* and another species, *Microglanis cottoides* (Pseudopimelodidae) (Boulenger 1891), and also, to contribute with new data to characterization studies of 5S rDNA sequences in Siluriformes.

Materials and methods

Biological samples and chromosome preparation

We analyzed five specimens of *Imparfinis schubarti* (2 males and 3 females) from Três Bocas Stream/Londrina/PR (23°23'06.6"S and 51°04'35.8"W) and five specimens (3 males and 2 females) from Laranjinha River/Ribeirão do Pinhal/PR (23°24'9.11"S and 50°27'16.4"W). We also assessed four samples of *Microglanis cottoides* (2 males and 2 females) Pseudopimelodidae family, from Forquetinha River, located in Forquetinha city/RS/Brazil (29°24'21.8"S and 52°03'18.3"W) in Patos Lagoon Hydrographic System/RS. The specimens were deposited in Museum of Zoology of the Universidade Estadual de Londrina (MZUEL) voucher: MZUEL5762 (*I. schubarti* Três Bocas stream); MZUEL5767 (*I. schubarti* Laranjinha River) and MZUEL6033 (*M. cottoides*). The samples were collected with the permission of Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), under the protocol number 11399-1. Mitotic chromosomes were obtained by direct preparation removing the posterior kidney, according to Bertollo et al. (1978).

Obtainment of C₀t fraction and construction of 5S rDNA probe microlibrary

Genomic DNA from *I. schubarti* was extracted from the muscles using phenol/chloroform procedure described by Sambrook and Russel (2001). The 5S rDNA probe was obtained by the C₀t method, which consists of randomly isolating repetitive DNA, using renaturation kinetics (Zwick et al. 1997). This procedure underwent adaptations for acquisition of repetitive DNA in fish (Ferreira and Martins 2008; Vicari et al. 2010) using genomic DNA from *I. schubarti*. Approximately 10 µg of DNA dissolved in 0.3 M NaCl were autoclaved at 1.4 atm (120 °C) for 15 min. The size of the fragments was checked by electrophoresis on 1 % agarose. The sample was denatured at 95 °C for 10 min and transferred to a water bath at 65 °C for 5 min. After that, the sample was incubated at 37 °C for 8 min with S1 nuclease, and the reaction was interrupted by the addition of liquid nitrogen. DNA was purified using phenol: chloroform (1:1, v:v) and subsequently cloned using the Blunt-Ended PCR Cloning Kit (GE Healthcare Life

Sciences, Sao Paulo, Brazil), with *Escherichia coli* TOP 10 competent cells. Inserts were sequenced on a 3500 × L automatic sequencer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's procedures. The target clones were identified through amplification reactions using specific primers for the plasmid. The final reaction had a total volume of 25 µl [50 pmol of each primer: M13 TOPO F (5'GTAAACGACGGCCAG) and R (5'CAGGAAA CAGCTATGAC), 1× buffer (50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris–HCl), 2 mM of each dNTP, and 0.5 U Taq DNA polymerase (Amersham BiosciencesTM, Sao Paulo, Brazil).

PCR amplification and construction of 5S rDNA probe

The 5S rDNA probe was obtained through polymerase chain reaction (PCR) using the primers A (5'TACGCCCGATCTCGTCCGATC) and B (5'CAGGCTGGT ATGGCCGTAAGC), as described by Martins and Galetti (1999). PCR reactions were performed using 20 ng of genomic DNA, 10 pmol of each primer, 2.5 mM of each dNTP, 50 mM MgCl₂, 1U of Taq polymerase for a reaction with a final volume of 25 µl. The cycling time was 2 min at 94 °C (denaturation) followed by 35 cycles of 1 min at 94 °C, 1 min at 62 °C, 1 min at 72 °C, and finally 5 min at 72 °C. Amplifications were visualized on 1 % agarose gel. The 5S rDNA fragments generated were eluted from the gel using the kit PurelinkTM Quick Gel Extraction (Invitrogen, Carlsbad, CA, USA) kit and cloned using the TOPO TA Cloning[®] For Sequencing (Invitrogen) with *Escherichia coli* TOP10 competent cells to obtain sequences larger than 367 bp. Inserts were sequenced on a 3500 × L automatic sequencer (Applied Biosystem), according to the manufacturer's procedures.

Analysis of 5S rRNA genes in the *Imparfinis schubarti*

Both strands of 5S rDNA obtained by the above methods were sequenced using specific primers for the plasmid. The assembly of contigs was made using the CAP3 program (Huang 1999). The detection and cleaning of contaminants were performed running Vecscreen with NCBI BLAST network service (Altschul et al. 1997). Clean sequences were deposited in the NCBI database

under accession numbers KJ755999, KJ769232 and KJ769233. The search for repetitive elements was made by the Repeat Masker web interface (Tarailo-Graovac and Chen 2009) and CENSOR software, both with Genetic Information Research Institute data base, Repbase (Kohany et al. 2006), and Zebrafish (Danio rerio) as model. For comparison, the 5S rDNA sequences obtained from the NCBI database and the multiple alignment of the sequences were made using the software MAFFT (Katoh et al. 2002). The UGENE software (Okonechnikov et al. 2012) was used to assemble the contigs and obtain multiple alignments. All sequences obtained were analyzed regarding the presence of probabilistic profiles of sequences and secondary structures of RNA, called covariance models (CMs), for the presence of 5S rDNA using the Infernal software (Nawrocki and Eddy 2013) against the database Rfam v11.0 (Burge et al. 2013). The “boxes” inmates were determined by comparison with previous data observed in the alignments (Rodrigues et al. 2012). The secondary structure was predicted using the RNA fold web server (Gruber et al. 2008).

Fluorescence in situ hybridization (FISH)

Fluorescence in situ hybridization was performed according to Pinkel et al. (1986), with modifications. The 18S and 5S rDNA probe was labeled with Dig-nick translation Kit (Roche[®], Mannheim, Germany) or BioNick TM Labeling System Kit (Invitrogen[®]). The slides were treated with 50 µL of hybridization mixture containing 100 ng of labeled probe (7.5 µL), 50 % formamide (30 µL), dextran sulfate 50 % (12 µL), 20 SSC (10.5 µL). The material was denatured at 80 °C for 10 min, and hybridization was performed overnight at 37 °C in a humidified chamber. Post-hybridization washes were carried out in 2 SSC for 5 min, in 1 × PBS and 1 × PBD (20 × SSC, Triton 100, non-fat milk and distilled water qsp 100, pH 7), all at 45 °C. The probe was detected with 5 µL of Avidin conjugate with FITC (1:100) and Anti-digoxigenin with rodamine conjugate + 45 µL of BSA (5 %). To amplify the signal, 40 µL of amplification solution (1 mL anti-avidin–biotin conjugate and 39 mL of 1 × BPD) were used. The slides were mounted with 25 µL of a medium composed of 23 L of DABCO solution (1,4-diaza-bicyclo (2.2.2)-octane (2, 3 %), 20 mM TrisHCl, pH 8.0, and glycerol (100 %), in distilled water), 1 L of MgCl₂ 50 mM and

1 μ L of 4,6 diamidino-2-phenylindole, dihydrochloride (20 μ g/mL). All images were acquired with a Leica DM 4500 B microscope equipped with a DFC 300F $\times$ camera and Leica IM50 4.0 software.

Results

C_0t , PCR and FISH with rDNA 5S probe

A total of 24 clones were obtained using the C_0t analysis. Out of those, 12 clones were sequenced, as they presented approximately 300 bp. The pIs-1 clone exhibited a 5S rDNA sequence consisting of 311 bp and a complete sequence of 5S rRNA copy with a conserved 118 bp confirmed by the CM obtained through the Infernal software at the position 66–184 bp (Table 1), parts of the NTS could be observed upstream and downstream this region.

The two 5S rDNA sequences obtained by PCR amplification are composed of 367 bp and have an rRNA partial copy that covers positions from 1 to 95 bp in the first partial sequence, and from 1 to 96 bp in the second partial sequence (Table 1). The PCR amplification of the gel shows no additional bands, indicating the absence of two size classes NTS regions (Supplementary material S1.pdf.). The covariance model detected that these sequences were truncated at the 5' end (Table 1).

The NTS regions extend over the 251 bp exhibiting a TATA-like region at its end, as well as the first 21 bps of the following 5S region as summarized in a diagram (Fig. 1), which shows the characteristics of 5S rDNA and NTS regions of *Imparfinis schubarti*. The Repeat Masker software identified repetitions from 5S rRNA class in all sequences in regions corresponding to covariance analysis. Moreover, Repeat Masker also detected (TTAT) n simple repeat class in the NTS region of the two partial sequences (Fig. 1). Search for repetitive elements by CENSOR software shows an excerpt from 107 bp, in the full sequence, and 65 bp in the sequences with incomplete 5S, similar to the SINE3-1 element (Supplementary material S2.pdf.).

The chromosome structure of *Imparfinis schubarti* and *Microglanis cottoides* were analyzed by Gouveia et al. (2013, 2015), respectively. *Imparfinis schubarti* has 58 chromosomes distributed in 30 m + 28sm and features a secondary constriction in the first metacentric pair coincident with the Ag-NOR and 18S rDNA. Diversely,

Microglanis cottoides showed $2n = 54$, with 30 m + 14sm + 6st + 4a. The two 5S rDNA probes obtained with the two different methods were hybridized to the chromosomes of *I. schubarti* from the two populations. They were located in the interstitial region of a small metacentric chromosome pair (pair 10) close to the centromere. The double FISH experiments did not show the occurrence of synteny with the 18S rDNA site located in pair 1 (Fig. 2a). On the other hand, hybridizations in *Microglanis cottoides* chromosomes were performed with both probes, only the probe obtained by the C_0t method with complete sequence of rRNA gene was hybridized in this species. Such probe was observed in the interstitial region of the short arm of a subtelocentric chromosome pair (pair 23) close to the centromere, not showing synteny with the 18S rDNA site (Fig. 2b).

5S rDNA alignment analysis

The CMs detected by the Infernal software for sequences obtained from NCBI nucleotide database are shown in Table 1. The species *Leporinus elongatus* Valenciennes, 1850, *Pimelodus blochii* Valenciennes, 1840, *Pseudoplatystoma fasciatum* (Linnaeus 1766), *Phractocephalus hemiliopterus* (Bloch and Schneider 1801), *Ageneiosus inermis* (Linnaeus 1766) and *Brachyplatystoma rousseauxii* (Castelnau 1855) presented incomplete fragments of 5S rDNA, truncated at the 5' end. The species *Leporinus obtusidens* (Valenciennes 1836) and *Bryconcephalus* (Gunther 1869) had a truncated fragment at the 5' end and an intact fragment downstream of the NTS. *Oreochromis niloticus* (Linnaeus 1758) was the only species having three 5S rDNA fragments.

In addition, *Leporinus elongatus*, *Pimelodus blochii*, *Phractocephalus hemiliopterus* and *Ageneiosus inermis* showed a small stretch of the following 5S region at the end of their sequences. The alignment with the MAFFT software best arranged the intact blocks of the 5S rDNA regions which show sequences with conserved 5S rDNA region in most species, as well as extremely variable NTS regions and elements typical of 5S rDNA, such as, A box, Intermediate Element (IE), C box and a thymine rich terminal region (Fig. 3a, b). Figure 4a, b shows only the 5' region, characterized and aligned according to the rDNA region and to the 3' region 55 bp downstream of rDNA. Inverted TATA-like motifs and putative poly-T terminator regions can be observed.

Table 1 5S rRNA regions detected by Infernal software in the 5S rRNA sequences available in a public database

Species	Accession	CM	From	To	Strand	Trunc	E value
<i>Imparfinis schubarti</i> 5S complete (Heptapteridae)	KJ755999	5S_rRNA	66	184	+	No	1.6e−27
<i>Imparfinis schubarti</i> 5S partial 01 (Heptapteridae)	KJ769232	5S_rRNA	1	95	+	5′	3.9e−18
<i>Imparfinis schubarti</i> 5S partial 02 (Heptapteridae)	KJ769233	5S_rRNA	1	96	+	5′	3.9e−18
<i>Leporinus obtusidens</i> (Anostomidae)	AF284747	5S_rRNA	180	298	+	No	4.2e−22
<i>Leporinus obtusidens</i> (Anostomidae)	AF284747	5S_rRNA	1	95	+	5′	2.6e−16
<i>Leporinus elongatus</i> (Anostomidae)	AF284728	5S_rRNA	1	96	+	5′	1.6e−18
<i>Brycon cephalus</i> (Characidae)	AF250529	5S_rRNA	1	95	+	5′	2.4e−14
<i>Brycon cephalus</i> (Characidae)	AF250529	5S_rRNA	329	447	+	No	1.4e−24
<i>Salmo gairdnerii</i> (Salmonidae)	J01861	5S_rRNA	1	119	+	No	2.6e−28
<i>Misgurnus fossilis</i> (Cobitidae)	V00647	5S_rRNA	1	119	+	No	2.2e−27
<i>Misgurnus fossilis</i> (Cobitidae)	V00648	5S_rRNA	1	119	+	No	3.3e−23
<i>Hypophthalmichthys molitrix</i> (Cyprinidae)	X63146	5S_rRNA	67	185	+	No	8.2e−28
<i>Hypophthalmichthys nobilis</i> (Cyprinidae)	X63147	5S_rRNA	67	185	+	No	2.9e−27
<i>Cyprinus carpio</i> (Cyprinidae)	AB015590	5S_rRNA	1	119	+	No	2.9e−28
<i>Oreochromis niloticus</i> (Ciclidae)	AF478461	5S_rRNA	1	119	+	No	9.4e−27
<i>Oreochromis niloticus</i> (Ciclidae)	AF478461	5S_rRNA	1078	960	−	No	7.6e−23
<i>Oreochromis niloticus</i> (Ciclidae)	AF478461	5S_rRNA	577	481	−	No	0.00081
<i>Oreochromis niloticus</i> (Ciclidae)	AF478462	5S_rRNA	1	119	+	No	9.1e − 26
<i>Pangasianodon hypophthalmus</i> (Pangasiidae) ^a	HQ681111	5S_rRNA	1	119	+	No	6.2e − 28
<i>Ictalurus punctatus</i> (Pimelodidae) ^a	L49398	5S_rRNA	1	118	+	No	1.1e − 23
<i>Porichthys plectrodon</i> (Brachyodidae) ^a	GU645672	5S_rRNA	1	119	+	No	8.2e − 26
<i>Pimelodus blochii</i> (Pimelodidae) ^a	GQ403958	5S_rRNA	1	58	+	5′	9.7e − 10
<i>Pseudoplatystoma fasciatum</i> (Pimelodidae) ^a	GQ403963	5S_rRNA	1	48	+	5′	8.7e − 08
<i>Phractocephalus hemiliopterus</i> (Pimelodidae) ^a	GQ403962	5S_rRNA	1	48	+	5′	5.4e − 08
<i>Ageneiosus inermis</i> (Aucheniteridae) ^a	GQ403954	5S_rRNA	1	41	+	5′	6.1e − 06
<i>Brachyplatystoma rousseauxi</i> (Pimelodidae) ^a	GQ403956	5S_rRNA	1	29	+	5′	0.0069

Species, species from which the sequence was obtained; Accession, NCBI nucleotide access number; CM, Rfam Covariance Model detected by Infernal software; From and To, localization of the model detected; Strand, strand where the model is detected; Trunc, Truncated model at 5′ or 3′ for incomplete sequences or “No” if sequence is considered complete; E value, expected value of confidence, the lower the better

^a Fishes of siluriforme order

Fig. 1 *Imparfinis schubarti* 5S rDNA. Typical elements of 5S rDNA, such as a TATA like region, A box, Intermediate Element (IE) and C box can be observed. Besides, a class of simple repeats (TTATn) could be observed before TATA-Like region

Discussion

The current study showed that isolation of 5S rDNA probes from genomic DNA of *Imparfinis schubarti*, through two different techniques, was able to reveal

unprecedented characteristics of that sequence for that species. Hence, it was possible to observe differences in hybridization efficiency with the different 5S rDNA sequences obtained in this study. The probe characterized with a complete segment of 5S rRNA gene

Fig. 2 Location of 5S rDNA in the karyotype of *Imparfinis schubarti* (Heptapteridae) of the Três Bocas stream and Laranjinha River: (a) probe isolated by the C_0t and PCR method showing the double FISH with 18S rDNA (green signal) in pair 1 and 5S rDNA probes (red signal) in pair 10. In b karyotype of

Microglanis cottoides (Pseudopimelodidae) from the Forquetinha River, with probe insulated by the C_0t method with double FISH with 18S rDNA (green signal) in pair 24 and 5S rDNA probes (red signal) in pair 23. Bar represents 5 μ m. (Color figure online)

hybridized with *I. schubarti* and also with *Microglanis cottoides*, another fish species of Pseudopimelodidae family. On the other hand, the probe with the partial segment showed to be inefficient for the physical mapping of the 5S rDNA region in the latter species.

Comparison of the sequences isolated in this paper with sequences of other fish groups shows that most species of Siluriformes with sequencing data deposited in the database have only partial 5S rDNA sequences at their ends (Table 1^a). C_0t method can isolate repeat sequences randomly, based on denaturation and renaturation kinetics of the fragmented DNA (Zwick et al. 1997). This technique proved to be efficient in searching for the 5S rRNA genes found in

multiple copies in the genome. Moreover, it enabled the identification of a region with the characteristics required for transcription and with a model of secondary structure compatible with that expected for the 5S rRNA family (Supplementary material S3 Figure of model of secondary structure of 5S rRNA. pdf.).

The hybridization results showed that intact 5S rRNA regions are more appropriate for the probe operation. This fact is comparably with due conserved structure and motifs of 5S rRNA in different groups of the fish genome. This may be the reason why the probe isolated by the C_0t method operated in a different family. In contrast other studies have shown that small

Fig. 4 Characterization of the region immediately upstream of the 5S rDNA section in some species of fish. **a** shows a TATA box and the 23 bp of the 5S rDNA region. Note that there are different organizations for each species; some are reversed. In **b**,

characterization of the region immediately downstream of 5S rDNA and highlighting of the GAACAAA sequences identical to the sequence observed in Anurans, which act as Poly-T regions in fish. (**) Siluriforme species of South America

differences in NTS regions, without 5S rDNA complete sequence, are sufficient to stop the operation of the probe in fish (Martins and Galetti 2001). The same seems to be true for other species, as in frogs, where the NTS region shows nonspecific hybridization in the absence of 5S region (Rodrigues et al. 2012).

NTS regions vary extensively, not only in size, but also among the composition of the elements immediately upstream and downstream 5S rDNA region, like specific terminator regions and TATA like motifs (Wang and Stumph 1995). Moreover, probable specific terminator regions can influence this variation. For example, the default GAACAAA observed in species of the *Leporinus* genus was identical to the sequence observed in Anurans (Rodrigues et al. 2012) and similar to GAAACAA, which supposedly acts as a terminator region in fish. Only one variant of the NTS region was detected in the present study for *I. schubarti* (Supplementary material S1). However, other species exhibited from 4 to 14 different NTS variants (Rebordinos et al. 2013), this way a probe lacking the 5S region may be nonspecific if used for different species.

The small number of hybridization data with 5S rDNA probe in Heptapteridae family shows that this ribosomal sequence is located in the interstitial region of chromosomes in most species, as well in other fish

groups (Martins and Galetti 2001; Martins and Wasko 2004). However, there is variation regarding the chromosome type and presence of such sequences either on the short or long arm, even within the same species (Kantek et al. 2009; Garcia and Almeida-Toledo 2010a, b; Martinez et al. 2011). The Heptapteridae family also shows variation in chromosome number bearing this site, and only one pair of chromosomes (Kantek et al. 2009; Garcia and Almeida-Toledo 2010a; Martinez et al. 2011) or two pairs of chromosome carriers are found (Garcia and Almeida-Toledo 2010a, b; Ferreira et al. 2014).

In contrast, Kantek et al. (2009) observed this site only in a big submetacentric chromosome pair of the constriction that also showed synteny with the 18S rDNA site in *I. schubarti* from Pihumi River/MG/Brazil. However, the two populations of *I. schubarti*, in this study, showed the 5S rDNA site on a small metacentric chromosome (pair 10), which does not show synteny with the 18S rDNA site, as was observed with the double FISH technique. Ferreira et al. (2014) observed 5SrDNA site in two chromosomes: synteny with the 18S rDNA site in the constriction pair and in another small metacentric chromosome in species of *I. mirini* and *I. minutus*. Considering these data together with those presented in this study, location of 5S

rDNA does not seem to follow a particular pattern within *Imparfinis* genus.

In Pseudopimelodidae family, data about 5S rDNA location are even scarcer, hindering a comparative analysis. This site had only been evidenced in *Lophiosilurus alexandri*, located in the terminal region on a pair of subtelocentric chromosomes (Marques et al. 2008). Conversely, it had been observed in *Microglanis cottoides*, located in interstitial region. Therefore, despite few species analyzed in Pseudopimelodidae family, 5S rDNA sites do not seem to follow a pattern. This can be attributed to the action of other genomic elements, since the variation of rDNA genes has also been described in karyotypes of different species within this family, including species of the same genus (Gouveia et al. 2015).

This extensive variation in rDNA locations in eukaryotes may be mediated by heterologous recombination, transposable elements (TEs) or even extra circular chromosomal DNA (eccDNA), which make transposition of new loci, followed or not by removal of original rDNA loci (Raskina et al. 2004; Cohen and Segal 2009). In fish, such intermediate elements have been identified in various classes of repetitive DNA, including rDNA, and several authors suggest that TEs may be related to the movement of rRNA genes in fish genomes (Nakajima et al. 2012; Sczepanski et al. 2013; Merlo et al. 2013; Barbosa et al. 2015).

Contrastingly a region of SINE3-1 transposable element, showed similarity to a segment in 5S partial region of sequences obtained from *I. schubarti*. This element may have originated accidentally from a 5S rRNA gene and a retrotransposon CR1-like non-LTR (Kapitonov and Jurka 2003), which explain its similarity to 5S incomplete region. Thus, presence of other multigene families (LINES, SINES, non-LTR retrotransposons), especially regarding the NTSs, appears to be suitable among fish. These multigene families seems to collaborate with variability in this region (see review Rebordinos et al. 2013).

This work highlights the characterization of species-specific probes 5S rDNA. Given the broad variability of NTS regions, incomplete sequences can provide false positive results while probes with 5S rDNA intact can hybridize with no major problems in different species. With the characterization it was also possible to understand a little more about the molecular composition of these sequences. Given these data, and in contrast to the literature, the location of these

sequences seems not to follow a pattern within Heptapteridae and Pseudopimelodidae families, suggesting that additional elements may be involved in the evolution of rDNA 5S fish.

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