Karyotype variability in six Amazonian species of the family Curimatidae (Characiformes) revealed by repetitive sequence mapping

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Abstract

Fishes of the Curimatidae family represent one of the most important freshwater ichthyofauna groups of Central and South America, with 117 recognized species distributed in eight genera. In this study, six species – Curimata inornata, Curimatella dorsalis, and Psectrogaster falcata collected from the Lower Araguaia River, Pará, Brazil; Curimata vittata, Curimatella meyeri, and Psectrogaster rutiloides collected from the Catalão Lake, Amazonas, Brazil – were cytogenetically analyzed, investigating the occurrence and distribution of repetitive DNA classes in the karyotypes. All species had 2n=54 metacentric/submetacentric chromosomes. Despite the conservative diploid number, we observed variations in the karyotypic structure among species. Ribosomal DNA (rDNA) 18S and 5S were found in single or multiple sites, with the first report of synteny in Curimatella dorsalis, and the occurrence of several interstitial telomeric sequences (ITSs) in species of the genera Curimatella and Psectrogaster. Interspecific karyotypic diversity both concerning structure and location/position of the nucleolar organizer regions (NORs) and ribosomal DNA, suggesting the occurrence of several non-Robertsonian rearrangements driving the evolution of this family.

Keywords: Cytogenetics, rDNA, ITS, chromosomal rearrangements.

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The Curimatidae family currently encompasses 117 fish species, allocated in eight genera: Curimata, Curimatella, Curimatopsis, Cyphocharax, Potamorhina, Psectrogaster, Pseudocurimata, and Steindachnerina (Fricke et al., 2021). The species are widely distributed throughout Central and South America River basins, inhabiting different aquatic environments. Ecologically, these fishes have an important role as food resources for larger predatory fish and act in recycling organic material due to detritivores’ eating habits, being easily distinguished from the other taxa of the Characiformes order by their complete absence of teeth (Vari, 1989, 2003).

Cytogenetically, this family shows 2n=54 with biarmed chromosomes as the most frequent in the analyzed species (Table 1). However, despite this apparent conservative karyotype and chromosome morphology, variations in diploid number have been reported in at least six species, in addition to the occurrence of B chromosomes, as well as interspecific variation in the location/position of the nucleolar organizer regions (NORs) (Venere and Galetti, 1989; Feldberg et al., 1992; Navarrete and Júlio-Júnior, 1997; Brassesco et al., 2004; Venere et al., 2008) (Table 1).

The chromosomal mapping of repetitive sequences, such as 5S and 18S ribosomal DNAs (rDNA) and telomeric DNA (TTAGGG)n, has proven to be an excellent tool for the chromosomal characterization in different groups of Neotropical fishes (Cioffi and Bertollo, 2012; Viana et al., 2017; Ferreira et al., 2020), providing a set of relevant information that can contribute to cytotaxonomy, elucidate geographic distribution patterns and evidence sex chromosomes. In Curimatidae, even with scarce data on mapping these sequences, evident interspecific differences were already observed (De Rosa et al., 2006, 2007; Teribele et al., 2008; Oliveira, 2010; Pinheiro et al., 2016; Sampaio et al., 2016) (Table 1).

The present study aims to investigate the chromosomal composition and structure of the karyotypes of six Amazonian Curimatidae species. The results were compared with the data available in the literature to infer the hypothetical chromosomal rearrangements involved in the chromosomal evolution process.

A total of 52 individuals from six species of the Curimatidae family were cytogenetically analyzed (Table 2). The fishes were collected under authorization from the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio, SISBIO - 28095-1). All procedures followed the guidelines of the Ethics Committee for Experimental Use of Animals of the National Institute of Amazonian Research (004/2018-CEUA/INPA), and the specimens were deposited in the INPA Ichthyology Collection (INPA-ICT 059622 - INPA-ICT 059627).

We followed the protocol described by Gold et al. (1990) to obtain the mitotic chromosomal preparations. Constitutive heterochromatin (CH) was detected according to Sumner (1972), with modifications where the staining was performed with a solution containing 0.5 µL of propidium iodide in 20 µL of Vectashield®, according to Lui et al. (2012). Active nucleolar organizer regions (NORs) were identified using the silver staining method, according to Howell and Black (1980). For molecular cytogenetic analyses, genomic DNA was extracted from muscle, according to Sambrook et al. (1989).
| Species            | Locality                 | 2n | FN  | Karyotype formula | Ag-NOR pair / position | C Banding | rDNA 18S Pair / position | rDNA 5S Pair / position | Telomere | Reference |
|--------------------|--------------------------|----|-----|-------------------|-------------------------|-----------|--------------------------|--------------------------|-----------|-----------|
| Curimatata         |                          |    |     |                   |                         |           |                          |                          |           |           |
| C. cyprinoides     | Negro and Solimões River/AM | 54 | 108 | 44m+10sm          | 3m/qt                   | –         | –                        | –                        | –         | 3         |
|                    | Araguaia River/MT        | 54 | 108 | 44m+10sm          | 7m/qt                   | –         | –                        | –                        | –         | 16        |
| C. inornata        | Negro and Solimões River/AM | 54 | 108 | 40m+14sm          | 21sm/pi                 | –         | –                        | –                        | –         | 3         |
|                    | Araguaia River/MT        | 54 | 108 | 40m+14sm          | 3m22sm/qt               | pc/t      | 20sm/qt                  | 9m/pi                    | t         | 22        |
| C. knerií          | Negro and Solimões River/AM | 54 | 108 | 40m+12am+2st      | 27st/qt                 | –         | –                        | –                        | –         | 3         |
|                    | Araguaia River/PA        | 54 | 108 | 38m+16sm          | 20sm/qt                 | pc/t      | 2m22sm/qt                | 25sm21sm/qt              | t         | 22        |
| C. ocellata        | Uatumã River/AM          | 54 | 112 | 40m+16sm          | 26sm/pi                 | –         | –                        | –                        | –         | 3         |
| C. vittata         | Negro and Solimões River/AM | 54 | 108 | 42m+12sm          | sm/qt                   | –         | –                        | –                        | –         | 3         |
|                    | Catalão Lake/AM          | 54 | 108 | 38m+16sm          | 20m21sm/qt              | pc/t      | 20m21am/qt               | 25m/pi                   | t         | 22        |
| Curimatella        |                          |    |     |                   |                         |           |                          |                          |           |           |
| C. albina          | Negro and Solimões River/AM | 54 | 108 | 46m+8sm           | 14m/qt                  | –         | –                        | –                        | –         | 3         |
| C. dorsalis        | Miranda River/MS         | 54 | 108 | 46m+8sm           | 13m/qt                  | pc        | –                        | –                        | –         | 7         |
|                    | Paraná River/AR          | 54 | 108 | 54m2sm           | 2m/qt                   | c/t       | –                        | –                        | –         | 11        |
| Araguaia River/PA  | 54 | 108 | 44m+10sm          | 2m/qt                   | pc/t      | 2m/qt                   | 2m/qi                    | t         | 14 pairs  |
| C. immaculata      | Araguaia River/GO        | 54 | 108 | 46m+8sm           | 24sm/qt                 | –         | –                        | –                        | –         | 16        |
| C. leptidura       | São Francisco River/SP   | 54 | 108 | 54m2sm           | 9m/qt                   | –         | –                        | –                        | –         | 2         |
| C. meyeri          | Negro and Solimões River/AM | 54 | 108 | 46m+8sm           | 9m/qt                   | –         | –                        | –                        | –         | 3         |
|                    | Catalão Lake/AM          | 54 | 108 | 46m+8sm           | 9m/qt                   | pc/t/i    | 7m/qt                   | 9m/qi                    | 26sm/pi   | 14 pairs  |
| Curimatopsis       |                          |    |     |                   |                         |           |                          |                          |           |           |
| C. myersi          | Miranda River/MS         | 46 | 92  | 42m+4sm           | –                      | –         | –                        | –                        | –         | 7         |
| Cyphocharax        |                          |    |     |                   |                         |           |                          |                          |           |           |
| C. gilbert         | Paraibuna River/SP       | 54 | 108 | 44m+10sm          | 2m/qt                   | pc/t      | –                        | –                        | –         | 16        |
| C. cf. gilli       | Bento Gomes River/MT     | 54 | 108 | 54m2sm           | 1m/qi                   | –         | –                        | –                        | –         | 2         |
| C. gouldangi       | Araguaia River/GO        | 54 | 108 | 54m+1B           | 2m/qt                   | –         | –                        | –                        | –         | 16        |
| C. modestus        | Águas de São Pedro/SP    | 54 | 108 | 54m2sm           | 2m/qt                   | –         | –                        | –                        | –         | 2         |
| Três Bocas Stream/PR | 54 | 108 | 54m+sm+B         | 2m/qt                   | pc/t      | 2/qt                    | –                        | –         | 14 pairs  |
| Mogi-Guaçu River/SP | 54 | 108 | 54m+sm+B       | –                      | pc        | –                        | –                        | –         | 8         |
| Taquari River/PR   | 54 | 108 | 54m+sm+B         | 2m/qt                   | pc/t      | 2/qt                    | –                        | –         | 13,15     |
| Tihagi River/PR    | 54 | 108 | 54m2sm           | 2m/qt                   | –         | 2/qt                    | –                        | –         | 15        |
| Água da Floresta River/PR | 54 | 108 | 54m2sm       | 2m/qt                   | –         | 2/qt                    | –                        | –         | 15        |
| Species | Locality | 2n | FN | Karyotype formula | Ag-NOR pair / position | C Banding | rDNA 18S Pair / position | rDNA 5S Pair / position | Telomere | Reference |
|---------|----------|----|----|------------------|------------------------|-----------|------------------------|------------------------|----------|-----------|
| Paranapanema River/SP | 54 | 108 | 54m/sm+B | 2m/qt | pc/t | 2/qt | 3,20/pi | – | 12,14,17 |
| Tietê River/SP | 54 | 108 | 54m/sm+B | 2m/qt | pc/t | 2/qt | 3,20/pi | – | 11,12,14,17 |
| C. naegeli | Mogi-Guçu River/SP | 54 | 108 | 54m/sm | 25/qt | – | – | – | – | 2 |
| | Mogi-Guçu River/SP | 54 | 108 | 46m+8 sm | 1,2,11/1q6/pt | 21/pt | pc/t | – | – | 16 |
| Ribeirão Minhoca/MG | 54 | 108 | 54m/sm+B | 6/qt | pc/t | 6/qt | 3,20/pi | t/ITS 2 pairs | 18 |
| C. platanus | Paraná River/AR | 58 | 116 | 52m/sm+6st | 5m/qt | – | – | – | – | 11 |
| Pirá-Pytá Stream/AR | 58 | 116 | 48m+4sm+6st | 6m/qt | pc/t | – | – | – | 16 |
| C. cf. epiphys | Madeira River/RO | 54 | 108 | 54m/sm | 10m/qt | – | – | – | – | 2 |
| C. stipolus | Paraná River/AR | 54 | 108 | 54m/sm+B | 1/qi | pc/t | – | – | – | 10,11 |
| | Capivara Stream/RS | 54 | 108 | 54m/sm+B | 2/qt | pc/t | 2/qt | – | – | 19,20 |
| | Gasômetro/RS | 54 | 108 | 54m/sm+B | 2/qt | pc/t | 2/qt | 3crom/pi | – | 19,20 |
| C. vanderi | Preto River/SP | 54 | 108 | 54m/sm | 6/qt | – | – | – | – | 2 |
| C. vogae | Bolacha Stream/RS | 54 | 108 | 54m/sm | 6/qt | – | – | – | – | 2 |
| Paraná River/AR | 54 | 108 | 54m/sm | qt | pc/i | – | – | – | 11 |
| Saco da Alemoa River/RS | 54 | 108 | 54m/sm+B | 5/qt | pc/t | 5/qt | – | – | 19,20 |
| | Capivara Stream/RS | 54 | 108 | 54m/sm+B | 5/qt | pc/t | 5/qt | – | – | 19,20 |
| | Gasômetro/RS | 54 | 108 | 54m/sm+B | 5/qt | pc/t | 5/qt | – | – | 19,20 |
| | Barros Lagoon/RS | 54 | 108 | 54m/sm+B | 5/qt | pc/t | 5/qt | 2crom/pi | – | 19,20 |
| | Quadros Lagoon/RS | 54 | 108 | 54m/sm+B | 5/qt | pc/t | 5/qt | – | – | 19,20 |
| C. saladensis | A.E.S. UFRGS Dam/RS | 54 | 108 | 54m/sm+B | 8/qt | pc/t | 8m/qt | 2crom/pi | – | 19,20 |
| Potamorhina | P. altamazonica | Negro and Solimões River/AM | 102 | 106 | 2m+2sm+98a | 5a/qt | pc/i | 5a/qt | 41a/qi | t | 4,21 |
| | P. latior | Negro and Solimões River/AM | 56 | 112 | 52m+2sm+2st | 25m/qt | pc/i | 25m/qt | 4m/qt | t/ITS 18 pairs | 4,21 |
| | P. pristigaster | Negro and Solimões River/AM | 54 | 108 | 42m+12sm | 25m/qt | pc | – | – | – | 4 |
| | Negro and Solimões River/AM | 54 | 108 | 44m+10sm | 5m/qt | pc/t | 5m/qt | 4m/qt | t/ITS 1 pair | 21 |
| | P. squamoralevis | Paraná River/AR | 102 | 116 | 14m+88a | q/i | pc | – | – | – | 11 |
| Psectrogaster | P. amazonica | Araguaia River/MT | 54 | 108 | 44m+10sm | 17m/qt | – | – | – | – | 16 |
| | P. cuiviventricis | Miranda River/MS | 54 | 108 | 42m+12sm | 20m/qt | pc | – | – | – | 7 |
| | Paraná River/AR | 54 | 108 | 54m/sm | q/i | pc/t | – | – | – | 11 |
| | P. falcata | Araguaia River/PA | 54 | 108 | 40m+14sm | 13m/qt | pc/t | 13m/qt | 24sm/pi | t/ITS 15 pairs | 22 |
| | P. rutiloides | Negro and Solimões River/AM | 54 | 108 | 42m+12sm | 9m/qt | – | – | – | 3 |
| | Catalão Lake/AM | 54 | 108 | 46m+8sm | 16m/qt | pc/i | 16m/qt | 5m/qt 22am/qi | t/ITS 18 pairs | 22 |
| Species             | Locality                  | 2n | FN | Karyotype formula | Ag-NOR pair / position | C Banding | rDNA 18S Pair / position | rDNA 5S Pair / position | Telomere | Reference |
|---------------------|---------------------------|----|----|-------------------|------------------------|-----------|-------------------------|-------------------------|-----------|-----------|
| Steinichthysina     |                           |    |    |                    |                        |           |                         |                         |           |           |
| *S. amazonica*      | Araguaia River/GO         | 54 | 108| 42m+12sm          | 2m23sm/qt             | pc/t      | –                       | –                       | –         | 16        |
| *S. biornata*       | Forquetinha River/RS      | 54 | 108| 54m/sm+B           | 3m/qt                  | pc/t      | 4crom/qt                | –                       | –         | 19, 20    |
| *S. brevipinna*     | Miranda River/MS          | 54 | 108| 46m+6sm           | 17m/qt                 | c/t       | –                       | –                       | –         | 7         |
| S. conspersa        | Paraná River/AR           | 54 | 108| 54m/sm            | 15m/qt                 | pc/t      | –                       | –                       | –         | 11        |
| S. elegans          | Paraguai River/MS         | 54 | 108| 54m/sm            | 2m/qi                  | –         | –                       | –                       | –         | 2         |
| S. gracilis         | Paraná River/AR           | 54 | 108| 54m/sm            | 2m/qt                  | pc/t      | –                       | –                       | –         | 11        |
| S. cf guentheri     | São Francisco River/AC    | 54 | 108| 54m/sm            | 24pt                   | pc/t      | –                       | –                       | –         | 9         |
| S. insculpta        | Mogi-Guaçu River/SP       | 54 | 108| 54m/sm            | 25/pt                  | –         | –                       | –                       | –         | 2         |
| S. leucisca         | Mogi-Guaçu River/SP       | 54 | 108| 54m/sm            | 25/pt                  | –         | –                       | –                       | –         | 2         |
| S. lenicua          | Mogi-Guaçu River/SP       | 54 | 108| 54m/sm            | 25/pt                  | –         | –                       | –                       | –         | 2         |
| S. leucisca         | Mogi-Guaçu River/SP       | 54 | 108| 54m/sm            | 25/pt                  | –         | –                       | –                       | –         | 2         |

Table 1 - Cont.

1- Venere and Galetti (1985); 2- Venere and Galetti (1989); 3- Feldberg et al. (1992); 4- Feldberg et al. (1993); 5- Oliveira and Foresti (1993); 6- Martins et al. (1996); 7- Navarrete and Júlio Jr (1997); 8- Venere et al. (1999); 9- Carvalho et al. (2001); 10- Fenocchio et al. (2003); 11- Brassesco et al. (2004); 12- De Rosa et al. (2006); 13- Gravena et al. (2007); 14- De Rosa et al. (2007); 15- Teribele et al. (2008); 16- Venere et al. (2008); 17- De Rosa et al. (2008); 18- Oliveira (2010); 19- Sampaio et al. (2011); 20- Sampaio et al. (2016); 21- Pinheiro et al. (2016); 22- Present study.
Table 2 - Cytogenetic data of fish species from Curimatidae family analyzed in this study. M= male; F= female; ?= Unknown sex; 2n= diploid number; FN= fundamental number; Ag-NOR= nucleolar organizer regions; rDNA= ribosomal DNA; m= metacentric; sm= submetacentric; p= short arm; q= long arm; t= terminal; i= interstitial.

| Species                     | Sex | Locality / Coordinates                  | 2n | FN   | 2n  | 18S rDNA Pair / Position | 5S rDNA Pair / Position | Ag-NOR Pair / Position |
|-----------------------------|-----|------------------------------------------|----|------|-----|--------------------------|-------------------------|------------------------|
| Curimatella meyeri          | 4   | Catalão lake, AM                         | 54 | 108  | 108 | 9m/pt                    | 16m/pt                  | 10m/pt                 |
| Curimatella inornata        | 2   | Araguaia river, PA                       | 25 | 54   | 54  | 6m/pt                    | 26m/pt                  | 9m/pt                  |
| Curimatella dorsalis        | 4   | Catalão lake, AM                         | 54 | 108  | 108 | 9m/pt                    | 16m/pt                  | 10m/pt                 |
| Psectrogaster rutiloides    | 10  | Catalão lake, AM                         | 54 | 108  | 108 | 9m/pt                    | 16m/pt                  | 10m/pt                 |

Ribosomal DNA (rDNA) 18S, 5S, and telomeric probes were amplified by Polymerase Chain Reaction (PCR) using the following primers: 18Sf (5’-CCGCTTTTGTTGACTCTTGAT-3’) and 18Sr (5’-CCGAGGACCTACTAAACCA-3’) (Gross et al., 2010), 5Sf (5’-TAC GCC CGA TCT CGT CGG ATC) and 5Sr (5’-CAGGCT GTG ATG GCC GTA AGC-3’) (Martins and Galetti Jr., 1999), (TTAGGG) 5 and (CCCTAA) 5 (Ijdo et al., 1991). Probes were labeled using nick-translation with biotin-14-dATP (Biotin Nick Translation Mix; Invitrogen) for SS rDNA and digoxigenin-11-dUTP (Dig-Nick Translation Mix; Roche) for 18S rDNA and telomere, following the recommendations of the manufacturer.

FISH followed Pinkel et al. (1986), with modifications. The slides with chromosome preparations were denatured in 70% formamide/2x SSC at 70 °C, pH 7, and dehydrated in 100% ethanol. Then, 20 μL of hybridization mix (100 ng of each probe, 100% formamide, 20x SSC buffer, and 10% dextran sulfate) were placed on each slide, being hybridized at 37 °C for 24 h in a humid chamber, containing distilled water. Chromosomes were counterstained with DAPI (1.2 μg/mL) in an antifade solution (Vector, Burlingame, CA, EUA).

At least 30 metaphase spreads of each individual were analyzed to confirm the diploid number and karyotype structure. The chromosomes were classified as metacentric (m) and submetacentric (sm) (Levan et al., 1964).

The six species analyzed presented 2n=54 and FN=108 (Fundamental number) (Figure 1, Table 2), it is highlighted that the karyotype of Psectrogaster falcata is presented here for the first time. CH was observed in pericentromeric blocks in all chromosomes of the six species, except in pairs 5 and 18 of P. falcata. Furthermore, additional blocks located in the terminal portions of several chromosomes of the six species were also observed. C. meyeri showed interstitial blocks in the long arms of pair 5; and pairs 2, 19, and 21 in P. rutiloides (Figure 1).

Five species presented NOR in only one chromosome pair in the terminal portion of the short arms: C. inornata, P. falcata, and P. rutiloides (pairs 20, 13, and 16, respectively), and in the end of the long arms in C. dorsalis and C. meyeri (pairs 2 and 9, respectively). C. vittata exhibited NORs in two chromosome pairs (multiple NORs) in the terminal portion of the long arms (pairs 20 and 21). The six species showed the NORs colocated with heterochromatic blocks (Figure 1, box Ag-NOR).

The rDNA mapping corroborates the NORs in all the species studied, including an additional site observed in the end of the short arm of pair 7 in C. meyeri, which is also colocalized to the constitutive heterochromatin (Figure 2, 18S). The SS rDNA sequences mapping revealed a species-specific pair with interstitial signals: pair 9 in C. inornata, pair 25 in C. vittata, pair 2 in C. dorsalis, pair 26 in C. meyeri, and pair 24 in P. falcata. P. rutiloides presented SS sites in two pairs: pair 5 in the terminal portion of the short arm and interstitial in pair 22. C. dorsalis showed synteny of 5S and 18S (Figure 2). Telomeric sequences (TTAGGG), were located in the terminal region of all chromosomes of the six species. Additionally, interstitial telomeric sequences (ITSs) were observed in several chromosomes of Curimatella and Psectrogaster species, with some conspicuous blocks (Figure 2).
Chromosomal evolution of the family Curimatidae was defined as being highly conservative chromosome morphology and diploid number: $2n=54$ m-sm, $FN=108$ for the majority of the species (Table 1). These traits, considered plesiomorphic for the family, were also evidenced in the species analyzed here in. According to Oliveira et al. (1988) and De Oliveira et al. (2009), this conservative chromosomal structure may be related to the ecological characteristics of these fishes, that is, high vagility and large shoal formation, allowing high rates of gene flow and genetic diversity (Landínez-García and Marquez, 2018). However, this apparent conservation is revealed when other cytogenetic markers, such as repetitive DNA sequences (e.g., ribosomal and telomeric) are applied.

Curimatids, in general, have a large amount of HC, and in *Psectrogaster* species for example, pericentromeric and terminal blocks were observed in several chromosome pairs (Figure 1). Beyond that, large heterochromatic blocks are often coincident or adjacent to the NORs, with interspecific

**Figure 1.** Karyotypes of the species of the Curimatidae family analyzed in conventional Giemsa stain (left), C banding (right) and nucleolar organizer regions (NOR, box). Scale bar=10μm.
and interpopulation differences, both in the number of loci (single or multiple NORs) and in the chromosomal location/position in the karyotype (Table 1), as seen in the present study as well as in previous studies (Feldberg et al., 1992; Navarrete and Júlio-Júnior, 1997; Brassesco et al., 2004; Venere et al., 2008). These differences may be related to the
repetitive and highly transcribed structure of rDNA, where the number of copies might vary owing to rearrangements of the chromosomal microstructure, such as duplications, translocations and/or inversions (Symonová et al., 2013; Goffová and Fajkus, 2021).

The mapping of the 18S rDNA sequence confirmed Ag-NOR in all species with an additional site in C. meyeri, similar situation also reported by Sampaio et al. (2016) in Steindachnerina biornata. This additional site might be related to the lack of transcriptional activity, which depends on cell activity (Rosa et al., 2012), or simply associated with the presence of pseudogenic rDNA variants (Gong et al., 2021).

The 5S rDNA localization in interstitial region, ranging from two to four chromosomes, is a pattern found in most curimatids corroborated in the present study. However, markings in terminal chromosomal regions have also been reported in this family (Pinheiro et al., 2016; present study), again evidencing the occurrence of non-Robertsonian rearrangements in chromosome microstructure of these species. The location of 18S and 5S rDNA in different chromosome pairs is a trait found in all curimatid species (Table 1). Interestingly, Curimatella dorsalis seems to be the first case to show synteny between these rDNAs in curimatids, which may have arisen independently during non-Robertsonian rearrangements (Symonová et al., 2013), demonstrating the dynamic nature of the 18S and 5S rDNA sites, prone to recombination events. Synteny between 18S and 5S rDNA is an atypical situation, including for the superfamly Anostomoidae (Anostomidae, Chilodontidae, Prochilodontidae and Curimatidae), which has been reported only in lineages derived of the Anostomidae (De Barros et al., 2017; Dulz et al., 2019), Prochilodontidae (Vicari et al., 2006; Terencilio et al., 2012; Voltolin et al., 2013) and Curimatidae families (present study).

Chromosome mapping of telomeric sequences revealed a high degree of chromosome structure variation in Curimatella and Pscectrogaster species, presenting ITSs in several chromosome pairs. ITS has been observed in several vertebrate species and is classified into short ITS (s-ITS) and heterochromatic ITS (Het-ITS) (Bolzán, 2017). In the case of the curimatids here analyzed, we classify the ITSs as Het-ITSs, since the signals are colocalized with heterochromatic blocks. Many authors relate the presence of Het-ITSs to ancestral chromosomal fusion events and are generally associated with a reduction in diploid number (Meyne et al., 1990; Rosa et al., 2012; Schneider et al., 2013; Sember et al., 2015). Similarly, there are reports of Het-ITSs in species that present the conserved karyotype (Metcalfe et al., 2004; Di-Nizio et al., 2020), as observed in the present study, considering that 2n=54 is the ancestral diploid number for the whole superfamly Anostomoidae.

Thus, the appearance of these Het-ITSs may be related to other mechanisms, such as (1) occurrence of pericentric inversions or translocations with the insertion of s-ITSs, followed by amplification of these regions and subsequent heterochromatinization; (2) transpositions, mediated by transposable elements, which are internally reinserted into the chromosomes and undergo an amplification process; and, (3) telomeric sequences (TTAGGG), would constitute the main repetitive motif of centromeric DNA, as observed in amphibians and marsupials (Meyne et al., 1990; Paço et al., 2012; Bolzán, 2017; Clemente et al., 2020).

Regardless of the mechanism that gave rise to Het-ITSs in the curimatids here analyzed, these sequences are an important component of the karyotype diversification. As observed in another genus of Curimatidae, in Potamorhina ITSs are involved in multiple chromosomal fissions in the ancestor of the species P. latior (2n=56, 18 pairs with ITS), P. altamazonica (2n=102), and P. squamoralevis (2n=102) (Pinheiro et al., 2016), as suggested in molecular phylogeny of Dorini et al. (2020). Thus, the Het-ITSs present in Curimatella and Pscectrogaster can signal the presence of “hot spots” for the occurrence of recombination, which according to Bolzán (2017), can lead to new karyotypes and even new species.

Thus, despite the conservative diploid number for most species of the Curimatidae (2n=54), our data highlights a high level of variation in repetitive DNA sequences among species, suggesting that additional integrative analyzes, involving the mapping of other repetitive sequences classes as well as investigation in other species/populations of curimatids, will produce a more complete picture of the chromosomal evolution of this family.

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Conflict of Interest
The authors declare no conflict of interest.

Author Contributions
JNM and EF conceived the study and collected the fish; JNM, VSPF, and EF analyzed the karyotype data; JNM, VSPF, RMF, PFV, and EF conducted the experiments, supervised the study and contributed to the preparation of the manuscript. All the authors revised and approved the final manuscript.

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Karyotype variability in curimatids

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