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Repetitive DNA mapping on *Oligosarcus acutirostris* (Teleostei, Characidae) from the Paraíba do Sul River Basin in southeastern Brazil

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Abstract. Within the Neotropical region, the genus *Oligosarcus* represents an interesting assembly of small-sized freshwater predators. The goal of this study was to cytogenetically analyze *Oligosarcus acutirostris* from the Espírito Santo Stream, Paraíba do Sul River Basin. The following cytogenetic techniques were performed: Giemsa staining, Ag-NOR and C- bandings, Fluorescence *in situ* Hybridization (FISH) using 18S and 5S rDNA probes, and (CA)₁₅ and (GA)₁₅ microsatellite probes. Diploid number was $2n=50$ and the karyotypic formula $4m+14sm+18st+14a$. Ag-NOR sites were present on the subtelocentric chromosome pair number 10. C-banding showed a few pericentromeric and conspicuous terminal heterochromatic blocks. The 18S and 5S rDNA probes marked chromosome pairs number 10 and number 19, respectively. FISH patterns obtained with (CA)₁₅ and (GA)₁₅ probes hybridized pericentromeric and terminal regions in almost all chromosomes, and interstitial regions of some chromosomes. Interestingly, microsatellite (CA)₁₅ showed a conspicuous centromeric mark on chromosome pair number 14, which could be an autapomorphy of this species, or it might characterize some species of this genus. The *Oligosarcus* cytogenetic patterns suggest that this genus is prone to fixation of chromosomal rearrangements and may be useful to detect biogeographical subunits within the coastal Brazilian basins.

Keywords: characiformes, cytotaxonomy, coastal river basins, fluorescence *in situ* hybridization (FISH), freshwater fishes.

INTRODUCTION

The genus *Oligosarcus* Günther, 1864 currently encompasses 22 species adapted to inhabit shallow places with dense vegetation in small tributary-

ies, river channels, although they are also collected in large rivers (Araújo *et al.* 2005; Ribeiro and Menezes 2015; Fricke *et al.* 2021). They are distributed throughout most of South America (Menezes 1988), and its endemism patterns and biogeographic relevance have been addressed (Menezes 1987, 1988; Ribeiro and Menezes 2015; Wendt *et al.* 2019).

Eight *Oligosarcus* species have been studied with cytogenetic techniques, showing a conserved diploid number of $2n = 50$ (Martinez *et al.* 2004; Centofante *et al.* 2006; Rubert and Margarido 2007; Barros *et al.* 2015). Some species have shown high levels of population chromosome variation (Table 1), including 18S rDNA amplification (up to 10 chromosomes) (Barros *et al.* 2015) and the presence of odd numbers (*i.e.* 3, 7, 9) of ribosomal clusters (Hattori *et al.* 2007; Usso *et al.* 2018).

The cytogenetic tools have been instrumental on systematic studies for understanding phylogenetic relationships in several animal groups. Over the recent years, the increasing use of the molecular cytogenetic techniques have added important insights in studies of cryptic and closely related species (Supiwong *et al.* 2013; Yano *et al.* 2016; Utsunomia *et al.* 2018; Conde-Saldana *et al.* 2019; Ibagón *et al.* 2020; Salgado *et al.* 2021), and have been a valuable tool to evidence possible hybridization cases (Peres *et al.* 2012; Gavazzoni *et al.* 2020).

Within the *Oligosarcus* genus, *Oligosarcus acutirostris* Menezes, 1987 is broadly distributed among the rivers belonging to the coastal eastern basins of Brazil (between Espírito Santo and Bahia states) (Menezes 1987; Fricke *et al.* 2021). The aim of this study was to cytogenetically analyze *O. acutirostris* from the Espírito Santo Stream, Paraíba do Sul River Basin, with an additional cytogenetic review of the genus *Oligosarcus*.

MATERIAL AND METHODS

Oligosarcus acutirostris specimens (four males, two females, and one juvenile) were collected in the Espírito Santo Stream, Paraibuna River, Paraíba do Sul River Basin (21°41'27" S 43°28'25" W), with collection license SISBIO 14975-1 issued to Jorge Abdala Dergam. The specimens were identified (Menezes, 1987; Ribeiro and Menezes, 2015) and deposited in the ichthyological collection of the Museu de Zoologia João Moojen in the Universidade Federal de Viçosa, Minas Gerais, Brazil (lot number MZUFV 4104).

The animals were anesthetized and euthanized using 300 mg.L⁻¹ clove oil aqueous solution (Lucena *et al.* 2013) following the Universidade Federal de Viçosa Animal Welfare Committee protocols (authorization 68/2014).

Mitotic metaphase chromosomes were obtained through air-drying technique (Bertollo *et al.* 1978). Chromosomes were stained with Giemsa to characterize the diploid number, karyotypic formula and the number of chromosome arms (Fundamental Number - FN). The chromosomes were measured with Image-Pro Plus® software and classified according to the arm ratios proposed by Levan *et al.* (1964) in metacentric, submetacentric, subtelocentric, and acrocentric. The nucleolar organizing regions were detected using silver nitrate impregnation technique (Ag-NOR) (Howell and Black 1980), and the heterochromatic regions were evidenced using C-banding (Sumner 1972) and dyed with DAPI.

The fluorescence *in situ* hybridization (FISH) was used to characterize the chromosomal distribution patterns of 18S and 5S ribosomal sites (double-FISH), and (CA)₁₅ and (GA)₁₅ microsatellites (single-FISH). FISH protocols were carried out according to Pinkel *et al.* (1986). The 18S probe was labeled with biotin using the BIO-Nick Translation Mix kit (Roche Applied Science) and the signal was detected with Avidin-FITC (Sigma), whereas the 5S rDNA probe was labeled with digoxigenin using the DIG-Nick Translation Mix kit (Roche Applied Science) and the signal was detected with Anti-Digoxigenin-Rhodamine (Roche Applied Science). The microsatellite repetitive probes (CA)₁₅ and (GA)₁₅ were synthesized and labeled with fluorochrome Cy3 on the 5' end (Sigma). Digital images were obtained in BX53F Olympus microscopes with Olympus DP73 and XM10 cameras, for Giemsa and fluorescent techniques respectively, both using CellSens imaging software (Olympus).

RESULTS

The diploid number of *O. acutirostris* was $2n = 50$, karyotypic formula of $4m + 14sm + 18st + 14a$, FN = 86, with no differences between males and females (Fig. 1). The Ag-NOR was located on the short arm of the largest subtelocentric chromosome pair number 10 (box on Fig. 1). C-banding evidenced heterochromatic blocks mainly on pericentromeric and terminal regions of the chromosomes, although not all chromosomes showed heterochromatic positive markings (Fig. 2). The 18S rDNA FISH probe marked subtelocentric pair number 10, whereas the 5S rDNA probe marked the acrocentric pair number 19 (Fig. 2).

The microsatellite (CA)₁₅ probe hybridized in pericentromeric and terminal regions of most chromosomes, and in interstitial regions of a few chromosomes, with a conspicuous centromeric mark on pair number 14, observed in both sexes. The (GA)₁₅ probe hybridized in

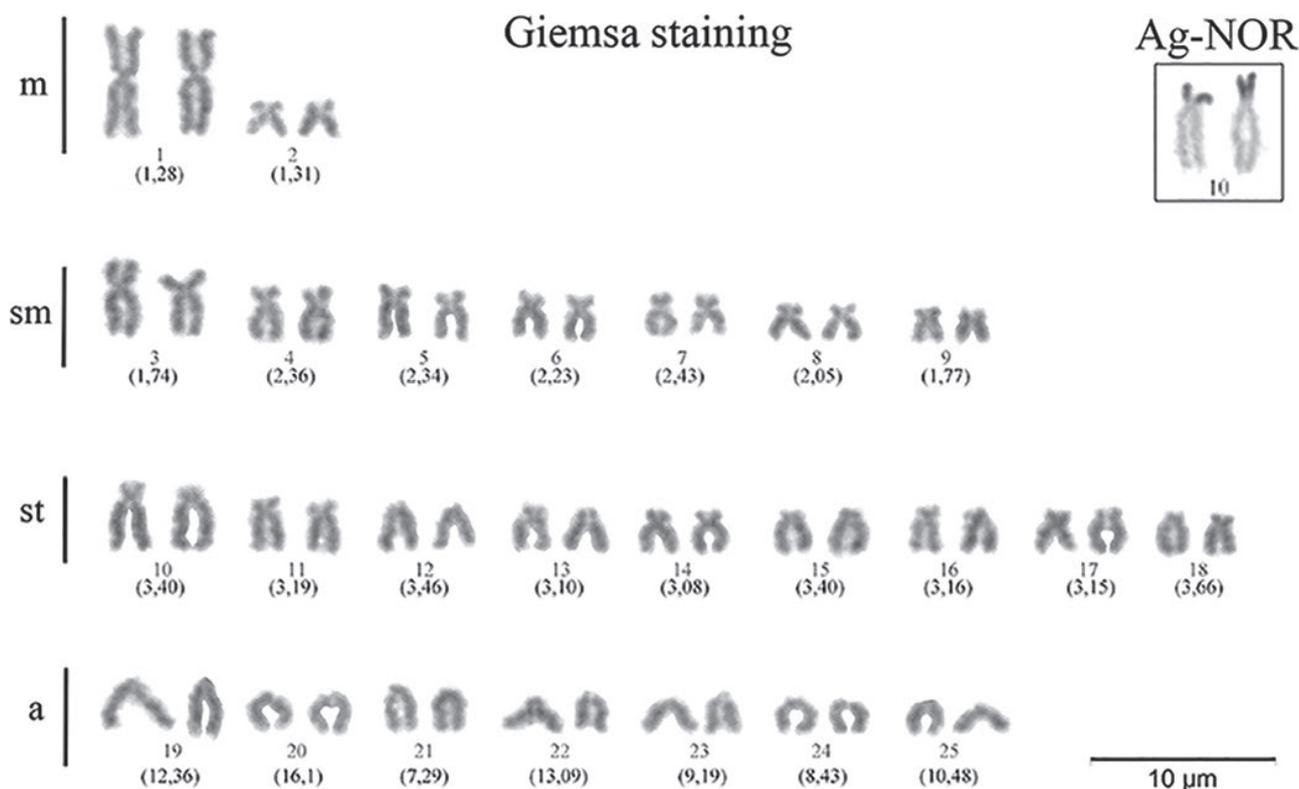


Figure 1. Giemsa-stained karyotype of *Oligosarcus acutirostris* ($2n = 4m + 14sm + 18st + 14a$, $NF = 86$). Mean values of chromosome arm ratios are in parentheses. The Ag-NOR on chromosome pair number 10 is shown in the box.

terminal regions of almost all chromosomes, with a few pericentromeric and interstitial blocks (Fig. 2).

DISCUSSION

All *Oligosarcus* species are characterized by a diploid number of 50 chromosomes, which is considered a plesiomorphic trait within the family Characidae (Kavalco *et al.* 2005). However, the karyotypic formulae and cytogenetic banding patterns are highly variable (Table 1), underlining the relevance of chromosomal inversions and/or translocations in the karyotypic evolution of this group (Centofante *et al.* 2006; Rubert and Margarido 2007; Barros *et al.* 2015). This condition is a stark contrast with the conserved chromosomal macrostructure observed in other families, such as Anostomidae (Salgado *et al.* 2021), and Prochilodontidae (Voltolin *et al.* 2013; Melo *et al.* 2017).

Small amounts of heterochromatin, with few pericentromeric and conspicuous terminal blocks, can be considered a widespread trait of the genus *Oligosarcus* (reviewed in Usso *et al.*, 2018). Within Characidae, closely related genera typically show high levels of inter-

specific karyotypic variation, such as large amounts of heterochromatin found in *Deuterodon taeniatus* (Jenyns, 1842) (Cunha *et al.* 2016), contrasting with the low amounts in *Deuterodon pedri* Eigenmann, 1907 (Coutinho-Sanches and Dergam 2015). Also, there are cases of intraspecific heterochromatin variation, such as in *Astyanax lacustris* (Lütken 1875) (Cunha *et al.* 2019) and *Astyanax scabripinnis* (Jenyns, 1842) (Santos *et al.* 2012).

Among *Oligosarcus* species, Ag-NOR cistrons have been observed on metacentric, submetacentric, subtelocentric, and acrocentric chromosomes (Martinez *et al.* 2004; Rubert and Margarido 2007; Barros *et al.* 2015). Although the occurrence of a single pair of Ag-NORs is common in this genus, up to eight sites have been observed (Table 1). In *O. acutirostris*, coincidental markings of Ag-NOR and 18S rDNA FISH probe demonstrates that the nucleolar organizing region is restricted to one chromosome pair. In some other *Oligosarcus* species, discrepancy between these cytogenetic markers indicate that not all ribosomal sites highlighted by the 18S probe are active (Table 1).

The presence of only one pair of 5S rDNA is the most widespread trait observed in *Oligosarcus* spp., showing less variability than the 18S rDNA clusters (Table 1).

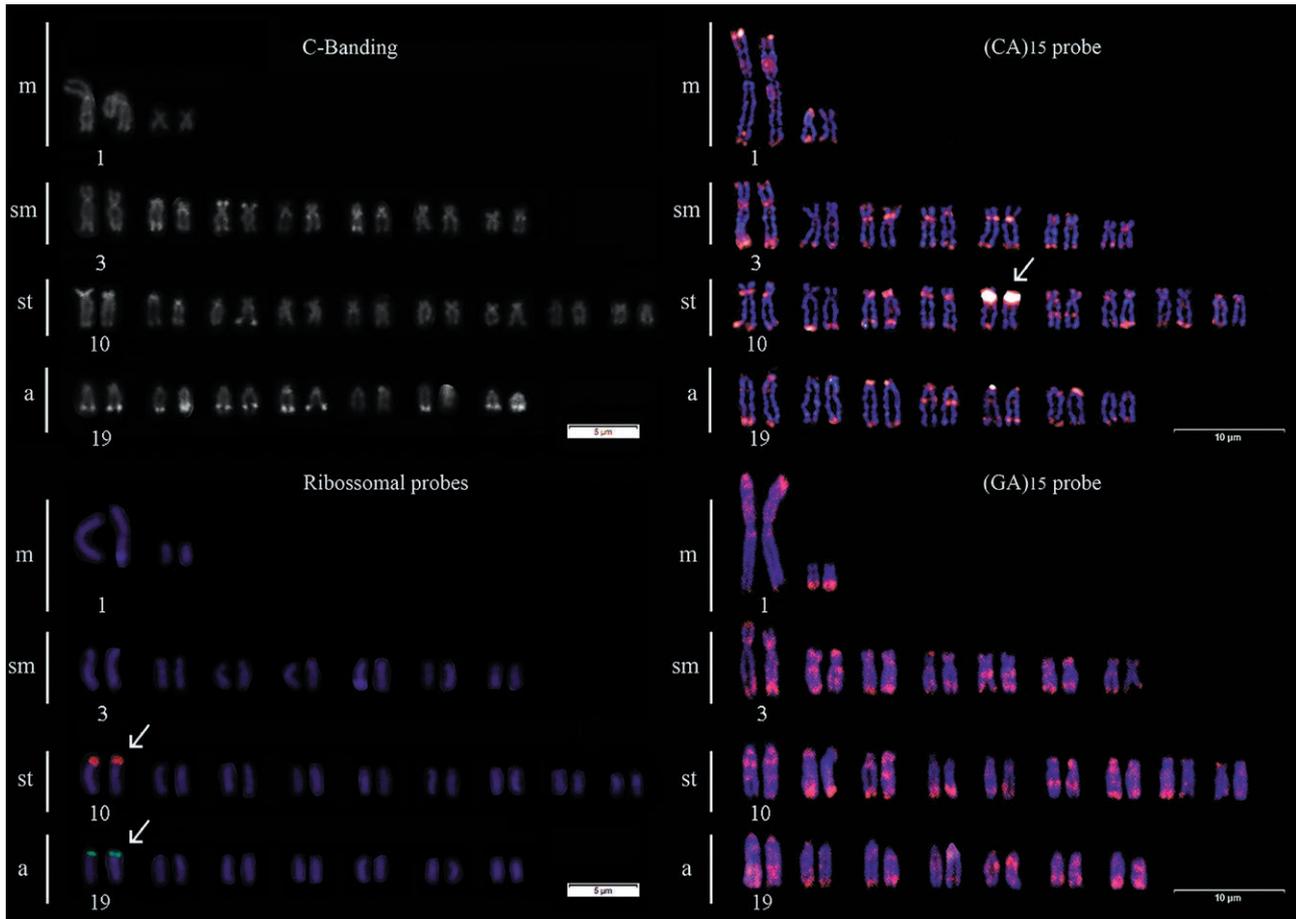


Figure 2. DAPI-stained C-banding and fluorescence *in situ* hybridization (FISH) patterns of *Oligosarcus acutirostris*. Double-FISH was performed with the probes 18S (pair number 10) and 5S rDNA (pair number 19), and single-FISH with the repetitive microsatellite probes (CA)₁₅ and (GA)₁₅. A conspicuous centromeric mark on pair number 14 was observed with the (CA)₁₅ probe (indicated by the arrow).

Based on non-simultaneous FISH patterns, Hattori *et al.* (2007) suggested the existence of synteny between the 18S and 5S rDNA cistrons in *O. hepsetus*, *O. pintoii*, and *O. jenynsii*. However, this putative syntenic pattern has not been observed in other studies that applied double-FISH (Barros *et al.* 2015; Usso *et al.* 2018; present study). The ribosomal 18S and 5S probes constitute potential phylogenetic markers for populations or species groups in the family Characidae (Kavalco *et al.* 2004; Coutinho-Sanches and Dergam 2015; Piscor *et al.* 2019).

The family Characidae has a complex evolutionary history, and the phylogenetic relationship of its members have been assessed using morphological and molecular data (Mirande 2010; Oliveira *et al.* 2011; Silva *et al.* 2017; Wendt *et al.* 2019). Most small-sized fish of this family, which includes the genus *Oligosarcus*, have complex taxonomic issues. Although this is the first study using microsatellite DNA probes to characterize an *Oli-*

gosarcus species, the conspicuous mark on chromosome pair number 14 with the (CA)₁₅ probe in *O. acutirostris* could be an autapomorphy of this species, or it might be a cytotaxonomic marker for some species within this genus. In other fish groups, these probes are distributed mainly in terminal chromosome regions, but additional interstitial markings have been useful as cytotaxonomic markers (Supiwong *et al.* 2013; Cunha *et al.* 2016; Salgado *et al.* 2021), as well as in the identification of sex chromosome systems (Cioffi *et al.* 2011; Poltronieri *et al.* 2014; Yano *et al.* 2016).

Most of the *Oligosarcus* species are allopatric, just a few are sympatric but not syntopic (Ribeiro and Menezes 2015). This habitat partitioning together with competitive exclusion may act as geographical or ecological barriers isolating populations, favoring the diversification and speciation of this taxon. Classical chromosomal evolutionary models suggest that high rates of chromo-

Table 1. Cytogenetic variation in the *Oligosarcus* species regarding the karyotypic formulae and the number of chromosomes marked by the Ag-NOR, 18S and 5S rDNA markers.

Species	Locality	Karyotype	Ag-NOR	18S rDNA	5S rDNA	References
<i>O. acutirostris</i>	Espírito Santo Stream, Paraíba do Sul Basin	4m+14sm+18st+14a	2	2	2	Present study
<i>O. argenteus</i>	Doce River Basin	6m+12-14sm+16-20st+12-14a	4	8 [#] -10 [#]	2	Barros <i>et al.</i> 2015
<i>O. hepsetus</i>	Grande Stream, Paraíba do Sul Basin	6m+12sm+14st+18a	3	4	-	Centofante <i>et al.</i> 2006
<i>O. hepsetus</i>	Santo Antônio Stream, Paraíba do Sul Basin	4m+12sm+16st+18a	3	6	-	Centofante <i>et al.</i> 2006
<i>O. hepsetus</i>	Ipiranga and Juquia rivers, Paraíba do Sul Basin	2m+26sm+4st+18a	-	-	-	Falcão and Bertollo 1985
<i>O. hepsetus</i>	Paraíba do Sul River, Paraíba do Sul Basin	2m+16sm+16st+16a	2	2-3	2	Hattori <i>et al.</i> 2007
<i>O. hepsetus</i>	Paraitinga River and Jacui Stream, Paraíba do Sul Basin	6m+10sm+16st+18a	2	4	4	Kavalco <i>et al.</i> 2005
<i>O. jenynsii</i>	Ipiranga Rivers, Paraíba do Sul Basin	6m+22sm+6st+16a	-	-	-	Falcão and Bertollo 1985
<i>O. jenynsii</i>	Uruguay River, Santa Catarina State, Brazil	2m+24sm+10st+14a	2	2	2	Hattori <i>et al.</i> 2007
<i>O. longirostris</i>	Iguaçu River, Upper Paraná Basin	4m+10sm+16st+20a	2	-	-	Rubert and Margarido 2007
<i>O. longirostris</i>	Iguaçu River, Upper Paraná Basin	2m+20sm+10st+18a	4	-	-	Martinez <i>et al.</i> 2004
<i>O. macrolepis</i>	Turvo River, Minas Gerais State	8m+20sm+6st+16a	-	-	-	Falcão and Bertollo 1985
<i>O. paranensis</i>	Keller River, Upper Paraná Basin	2m+26sm+8st+14a	2-6	-	-	Martinez <i>et al.</i> 2004
<i>O. paranensis</i>	Tunas River, Upper Paraná Basin	4m+10sm+16st+20a	2-6	-	-	Rubert and Margarido 2007
<i>O. paranensis</i>	Três Bocas Stream, Tibagi Basin	8m+18sm+10st+14a	2-8	7	2	Usso <i>et al.</i> 2018
<i>O. paranensis</i>	Quexada River, Ivaí Basin	6m+10sm+16st+18a	2-6	9	2	Usso <i>et al.</i> 2018
<i>O. pinto</i>	Mogi-Guaçu River, Upper Paraná Basin	4m+20sm+10st+16a	-	-	-	Falcão and Bertollo 1985
<i>O. pinto</i>	Mogi-Guaçu River, Upper Paraná Basin	2m+20sm+12st+16a	2	3	3	Hattori <i>et al.</i> 2007
<i>O. pinto</i>	Tunas River, Upper Paraná Basin	4m+10sm+16st+20a	2-4	-	-	Rubert and Margarido 2007
<i>O. solitarius</i>	Doce River Basin	4m+14-16sm+14-20st+12-18a	2	6 [#]	2	Barros <i>et al.</i> 2015
<i>Oligosarcus</i> sp.	Das Velhas River, São Francisco Basin	6m+14sm+18st+12a	4	10 [#]	2	Barros <i>et al.</i> 2015

[#] Some chromosomes showed biterminal markings.

some rearrangement fixation are associated with species subdivided in small populations (King 1987; Sites and Moritz 1987), but they may also arise when selection favors reduction of crossing-over rates between chromosome regions, favoring chromosome rearrangement

fixation and speciation (Faria and Navarro 2010). We conclude that *Oligosarcus* species are prone to fixation of chromosomal rearrangements and this characteristic may be useful to detect biogeographical subunits within the coastal Brazilian basins.

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STATEMENT OF ETHICS

The protocols followed the Universidade Federal de Viçosa Animal Welfare Committee authorization 68/2014.

AUTHORS' CONTRIBUTIONS

M.S.C. and S.M. collected the data; M.S.C, S.M., and F.S.S. analyzed the data; all authors contributed to the manuscript writing and approved the final version.

REFERENCES

- Araújo FG, Andrade CC, Santos RN, Santos AFGN, Santos LN. 2005. Spatial and seasonal changes in the diet of *Oligosarcus hepsetus* (Characiformes: Characidae) in a Brazilian reservoir. *Braz J Biol.* 65:1-8.
- Barros LC, Santos U, Cioffi MB, Dergam JA. 2015. Evolutionary divergence among *Oligosarcus* spp. (Ostariophysi, Characidae) from the São Francisco and Doce River basins: *Oligosarcus solitarius* Menezes, 1987 shows the highest rates of chromosomal evolution in the Neotropical region. *Zebrafish.* 12:102-110.
- Bertollo LAC, Takahashi CS, Moreira-Filho O. 1978. Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). *Rev Bras Genet.* 1:103-120.
- Centofante L, Bertollo LAC, Moreira-Filho O. 2006. Chromosomal differentiation between populations of *Oligosarcus hepsetus* (Teleostei, Characidae) from small tributaries at opposite margins of the Paraíba do Sul River (Brazil). *Braz Arch Biol Techn.* 49:981-987.
- Cioffi MB, Kejnovsky E, Bertollo LAC. 2011. The chromosomal distribution of microsatellite repeats in the genome of the wolf fish *Hoplias malabaricus*, focusing on the sex chromosomes. *Cytogenet Genome Res.* 132:289-296.
- Conde-Saldana CC, Cunha MS, Albornoz-Garzón JG, Barreto CAV, Ibagón N, Villa-Navarro FA, Dergam JA. 2019. Karyotypic divergence of two co-occurring species of Andean Climbing catfishes (Siluriformes: Loricarioidei: Astroblepidae). *Zebrafish.* 16:106-114.
- Coutinho-Sanches N, Dergam JA. 2015. Cytogenetic and molecular data suggest *Deuterodon pedri* Eigenmann, 1907 (Teleostei: Characidae) is a member of an ancient coastal group. *Zebrafish.* 12:357-365.
- Cunha MS, Reis VJC, Dergam JA. 2016. Closely related syntopic cytotypes of *Astyanax taeniatus* (Jenyns, 1842) from the Upper Piranga River, Upper Doce Basin in southeastern Brazil. *Zebrafish.* 13:112-117.
- Cunha MS, Fregonezi AR, Fava L, Hilsdorf AW, Campos LAO, Dergam JA. 2019. Phylogeography and historical biogeography of the *Astyanax bimaculatus* species complex (Teleostei: Characidae) in coastal southeastern South America. *Zebrafish.* 16:115-127.
- Falcão JN, Bertollo LAC. 1985. Chromosome characterization in Acestrorhynchinae and Cynopotaminae (Pisces, Characidae). *J Fish Biol.* 27:603-610.
- Faria R, Navarro A. 2010. Chromosomal speciation revisited: rearranging theory with pieces of evidence. *Trends Ecol Evol.* 25:660-669.
- Fricke R, Eschmeyer WN, Van der Laan R. 2021. Eschmeyer's catalog of fishes: genera, species, references. Available online at <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcat-main.asp>. Last accessed 25 February 2021.
- Gavazzoni M, Pavanelli CS, Graça WJ, Melo BF, Gubiani ÉA, Margarido VP. 2020. Detection of natural hybridization and delimitation of two closely related operational taxonomic units of the *Astyanax fasciatus* (Teleostei: Characidae) complex through integrative approaches. *Biol J Linn Soc.* 129:687-700.
- Hattori RS, Daniel-Silva MFZ, Almeida-Toledo LF. 2007. Karyotype characterization and gene mapping of 5S and 18S rDNA in three species of *Oligosarcus* (Teleostei: Characidae). *Caryologia.* 60:372-378.
- Howell WM, Black DA. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia.* 36:1014-1015.
- Ibagón N, Maldonado-Ocampo JA, Cioffi MB, Dergam JA. 2020. Chromosomal diversity of *Hoplias malabaricus* (Characiformes, Erythrinidae) along the Magdalena River (Colombia-northern South America) and its significance for the Neotropical Region. *Zebrafish.* 17:211-219.
- Kavalco KF, Pazza R, Bertollo LAC, Moreira-Filho O. 2004. Gene mapping of 5S rDNA in eight fish species from the Paraíba do Sul River Basin, Brazil. *Cytogenet Genome Res.* 106:107-110.

- Kavalco KF, Pazza R, Bertollo LAC, Moreira-Filho O. 2005. Molecular cytogenetics of *Oligosarcus hepsetus* (Teleostei, Characiformes) from two Brazilian locations. *Genetica*. 124:85-91.
- King M. 1987. Chromosomal rearrangements, speciation and the theoretical approach. *Heredity*. 59:1-6.
- Levan A, Fredga K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas*. 52:201-220.
- Lucena CAS, Calegari BB, Pereira EHL, Dallegrave E. 2013. O uso de óleo de cravo na eutanásia de peixes. *Bol Soc Bras Ictiol*. 105:20-24.
- Martinez ERM, Oliveira C, Júlio-Júnior HF. 2004. Cytogenetic analysis of species of the genera *Acestrorhynchus*, *Oligosarcus* and *Rhaphiodon* (Teleostei: Characiformes). *Caryologia*. 57:294-299.
- Melo S, Utsunomia R, Penitente M, Sobrinho-Scudeler PE, Porto-Foresti F, Oliveira C, Foresti F, Dergam JA. 2017. B chromosome dynamics in *Prochilodus costatus* (Teleostei, Characiformes) and comparisons with supernumerary chromosome system in other *Prochilodus* species. *Comp Cytogenet*. 11:393-403.
- Menezes NA. 1987. Três espécies novas de *Oligosarcus* Günther, 1864 e redefinição taxonômica das demais espécies do gênero (Osteichthyes, Teleostei, Characidae). *Boletim de Zoologia da Universidade Federal de São Paulo*. 11:1-39.
- Menezes NA. 1988. Implications of the distribution patterns of the species of *Oligosarcus* (Teleostei, Characidae) from Central and Southern South America. In: Vanzolini PE, Heyer WR (eds). *Proceedings of a workshop on Neotropical distribution patterns*. Academia Brasileira de Ciências, Rio de Janeiro; p. 295-304.
- Mirande JM. 2010. Phylogeny of the family Characidae (Teleostei: Characiformes): from characters to taxonomy. *Neotrop Ichthyol*. 8:385-568.
- Oliveira C, Avelino GS, Abe KT, Mariguela TC, Benine RC, Ortí G, Vari RP, Castro RMC. 2011. Phylogenetic relationships within the speciose family Characidae (Teleostei: Ostariophysi: Characiformes) based on multilocus analysis and extensive ingroup sampling. *BMC Evol Biol*. 11:275.
- Peres WAM., Bertollo LAC, Buckup PA, Blanco DR, Kantek DLZ, Moreira-Filho O. 2012. Invasion, dispersion and hybridization of fish associated to river transposition: karyotypic evidence in *Astyanax "bimaculatus" group* (Characiformes: Characidae). *Rev Fish Biol Fish*. 22:519-526.
- Pinkel D, Straume T, Gray JW. 1986. Cytogenetic analysis using quantitative, high sensitivity, fluorescence hybridization. *P Natl Acad Sci USA*. 83:2934-2938.
- Piscor D, Pozzobon APB, Fernandes CA, Centofante L, Parise-Maltempi PP. 2019. Molecular clock as insight to estimate the evolutionary history and times of divergence for 10 nominal *Astyanax* species (Characiformes, Characidae): an evolutionary approach in species with 2n= 36, 46, 48, and 50 chromosomes. *Zebrafish*. 16:98-105.
- Poltronieri J, Marquioni V, Bertollo LAC, Kejnovsky E, Molina WF, Liehr T, Cioffi MB. 2014. Comparative chromosomal mapping of microsatellites in *Leporinus* species (Characiformes, Anostomidae): Unequal accumulation on the W chromosomes. *Cytogenet Genome Res*. 142:40-45.
- Ribeiro AC, Menezes N. 2015. Phylogenetic relationships of the species and biogeography of the characid genus *Oligosarcus* Günther, 1864 (Ostariophysi, Characiformes, Characidae). *Zootaxa*. 3949:41-81.
- Rubert M, Margarido PV. 2007. Cytogenetic studies in three species of the genus *Oligosarcus*. *Braz Arch Biol Techn*. 50:127-135.
- Salgado FS, Cunha MS, Melo S, Dergam JA. 2021. Cytogenetic analysis of *Hypomasticus copelandii* and *H. steindachneri*: relevance of cytotaxonomic markers in the Anostomidae family (Characiformes). *Comp Cytogenet*. 15:65-76.
- Santos NM, Ferreira-Neto M, Artoni RF, Vicari MR, Bakali M, Oliveira CD, Foresti F. 2012. A comparative structural cytogenetic study in three allopatric populations of *Astyanax scabripinnis* (Teleostei: Characidae). *Zoologia*. 29:159-166.
- Silva PC, Malabarba MC, Malabarba LR. 2017. Using ancient DNA to unravel taxonomic puzzles: the identity of *Deuterodon pedri* (Ostariophysi: Characidae). *Neotrop Ichthyol*. 15:e160141.
- Sites Jr JW, Moritz C. 1987. Chromosomal evolution and speciation revisited. *Syst Zool*. 36:153-174.
- Sumner AT. 1972. A simple technique for demonstrating centromeric heterochromatin. *Exp Cell Res*. 75:304-306.
- Supiwong W, Liehr T, Cioffi MB, Chaveerach A, Kosyakova N, Pinthong K, Tanee T, Tanomtong A. 2013. Karyotype and cytogenetic mapping of 9 classes of repetitive DNAs in the genome of the naked catfish *Mystus bocourti* (Siluriformes, Bagridae). *Mol Cytogenet*. 6:51.
- Uso MC, Mortati AF, Morales-Blanco AG, Giuliano-Caetano L, Dias AL. 2018. Molecular cytogenetics in different populations of *Oligosarcus paranensis* (Characidae): comparative analysis of the genus with 5S and 18S rDNA probes. *Caryologia*. 71:103-108.
- Utsunomia R., Melo S, Scacchetti PC, Oliveira C, Machado MA, Pieczarka JC, Nagamachi CY, Foresti F. 2018.

- Particular chromosomal distribution of microsatellites in five species of the genus *Gymnotus* (Teleostei, Gymnotiformes). *Zebrafish*. 15:398-403.
- Voltolin TA, Penitente M, Mendonça BB, Senhorini JA, Foresti F, Porto-Foresti F. 2013. Karyotypic conservatism in five species of *Prochilodus* (Characiformes, Prochilodontidae) disclosed by cytogenetic markers. *Genet Mol Biol*. 36:347-352.
- Wendt EW, Silva PC, Malabarba LR, Carvalho TP. 2019. Phylogenetic relationships and historical biogeography of *Oligosarcus* (Teleostei: Characidae): Examining riverine landscape evolution in southeastern South America. *Mol Phylogenet Evol*. 140:106604.
- Yano CF, Bertollo LAC, Liehr T, Troy WP, Cioffi MB. 2016. W chromosome dynamics in *Triportheus* species (Characiformes, Triporthidae): an ongoing process narrated by repetitive sequences. *J Hered*. 107:342-348.