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## Cytogenetic analyses in three species of *Moenkhausia* Eigenmann, 1903 (Characiformes, Characidae) from Upper Paraná River (Misiones, Argentina)

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**Abstract.** *Moenkhausia* Eigenmann, 1903 is one of the most diverse genera within Characidae, being an important component of the Neotropical fish fauna. Three members of this genus were cytogenetically analyzed: *M. dichrourea* Kner, 1858, *M. intermedia* Eigenmann, 1908 and *M. sanctaefilomenae* Steindachner, 1907. The three species showed  $2n = 50$  bi-armed chromosomes (NF = 100) and different karyotype formulas:  $22m + 22sm + 6st$  in *M. dichrourea*,  $16m + 28sm + 6st$  in *M. intermedia*, and  $12m + 32sm + 6st$  in *M. sanctaefilomenae*. In addition, supernumerary chromosomes (or B-chromosomes) were detected in *M. intermedia* and *M. sanctaefilomenae*. C-positive bands were restricted to pericentromeric regions, secondary constrictions and supernumerary chromosomes. Active nucleolus organizer regions (Ag-NORs) and positive CMA<sub>3</sub> bands were observed in a single pair of sm chromosomes. Pericentromeric DAPI positive signals were evidenced on chromosomes of *M. sanctaefilomenae* only. Overall, the three species showed a conservative karyotype macrostructure (diploid number, number of chromosome arms) and variations in microstructure (karyotype formulas, presence/absence of supernumerary chromosomes). We discuss how the observed differences could have been shaped.

**Keywords:** neotropical fishes, *Moenkhausia*, supernumerary chromosomes, heterochromatin, Ag-NORs.

### 1. INTRODUCTION

The genus *Moenkhausia* comprises 109 species (Fricke *et al.* 2021) and is considered *insertae sedis* within the family Characidae (Lima *et al.* 2003). This genus shows a wide distribution along cis-Andean Neotropical rivers (Lima *et al.* 2003). Its members are characterized by a wide variation in morphological attributes and coloration patterns (Carvalho *et al.* 2014), being

frequently used as ornamental fishes. Phylogenetic relationships within *Moenkhausia* based on morphological (Mirande 2010, 2018) and molecular (Mariguela *et al.* 2013) data evidenced their polyphyletic nature, suggesting that this genus could be an artificial grouping.

Several studies addressed the cytogenetic characterization of members of this genus (Portela *et al.* 1988; Foresti *et al.* 1989; Arefjev 1990; Alberdi and Fenocchio 1997; Santos 1999; Portela-Castro *et al.* 2001; Dantas *et al.* 2007; Hashimoto *et al.* 2012; Scudeler *et al.* 2015; Utsunomia *et al.* 2016; Fernandes and Alves 2017; Nascimento *et al.* 2020). However, 10 species have been analyzed so far, mainly from Brazilian populations. Almost all these populations showed a diploid chromosome number of 50 bi-armed chromosomes (Dantas *et al.* 2007; Utsunomia *et al.* 2016; Nascimento *et al.* 2020). Some variations characterized as “cytotypes” were reported in *M. gracilima* Eigenmann 1908 ( $2n = 48$ ) and *M. pittieri* Eigenmann 1920 ( $2n = 49$ ) (Arefjev 1990; Santos 1999), although these observations were not corroborated in subsequent studies. Heterochromatic blocks were mainly reported in centromeric and pericentromeric regions, and Nucleolus Organizer Regions (NORs) were generally observed on a single chromosome pair (Portela *et al.* 1988; Foresti *et al.* 1989; Portela-Castro *et al.* 2001; Portela-Castro and Júlio Júnior 2002; Dantas *et al.* 2007; Hashimoto *et al.* 2012; Utsunomia *et al.* 2016; Fernandes and Alves 2017; Nascimento *et al.* 2020). In addition, supernumerary chromosomes were detected in populations of *M. sanctaefilomenae*, *M. intermedia*, *M. forestii*, and *M. oligolepis* (Foresti *et al.* 1989; Dantas *et al.* 2007; Hashimoto *et al.* 2012; Scudeler *et al.* 2015; Utsunomia *et al.* 2016; Fernandes and Alves 2017; Nascimento *et al.* 2020). More recent studies inquired about the molecular composition of this supernumerary chromosomes by means of chromosomal mapping (Dantas *et al.* 2007; Scudeler *et al.* 2015; Utsunomia *et al.* 2016; Fernandes and Alves 2017; Nascimento *et al.* 2020).

In spite of the taxonomic and cytogenetic diversity observed in *Moenkhausia*, the number of analyzed

species remains scarce. Based on this, the aim of this work was to describe for the first time the karyotypic constitution of Argentinean populations *M. dichrourea*, and new populations of *M. intermedia* and *M. sanctaefilomenae*. Aspects of the chromosomal differentiation between them will also be discussed in an evolutionary context.

## 2. MATERIALS AND METHODS

We collected 24 individuals of *Moenkhausia dichrourea* Kner 1858, 12 individuals of *M. intermedia* Eigenmann 1908, and 12 individuals of *M. sanctaefilomenae* Steindachner 1907 from tributaries of the Upper Paraná river (Misiones province, Argentina) (Table 1). The specimens were deposited in the collection of Grupo de Investigación en Citogenética Animal y Monitoreo Ambiental (IBS-UNaM-CONICET).

Mitotic preparations were obtained from kidney cells following the protocol described in Moreira-Filho and Bertollo (1991). C-banding followed Sumner (1972), and NORs were evidenced by silver nitrate impregnation (Ag-NOR; Howell and Black 1980). AT and GC-rich regions were detected with fluorochromes DAPI (4',6-diamidin-2-phenylindol) and CMA<sub>3</sub> (chromomycin A<sub>3</sub>), respectively (Schweizer 1980).

At least 30 metaphases were analyzed per specimen, and those exhibiting optimal chromosomal morphologies were used in karyotype analysis. Chromosomes were classified as metacentrics (m), submetacentrics (sm), subtelocentric (st) and acrocentrics (a) according to their arm ratios (Levan *et al.* 1964). Metacentric, submetacentric and subtelocentric chromosomes were considered as bi-armed, in order to determine the number of chromosome arms (NF). Chromosome measures were obtained in KaryoType v2 (Altinordu *et al.* 2016) and karyograms were assembled in Adobe Photoshop®CS6 (San Jose, California, USA).

**Table 1.** Specimens of *Moenkhausia* collected. F: females, M: males, ?: undetermined sex.

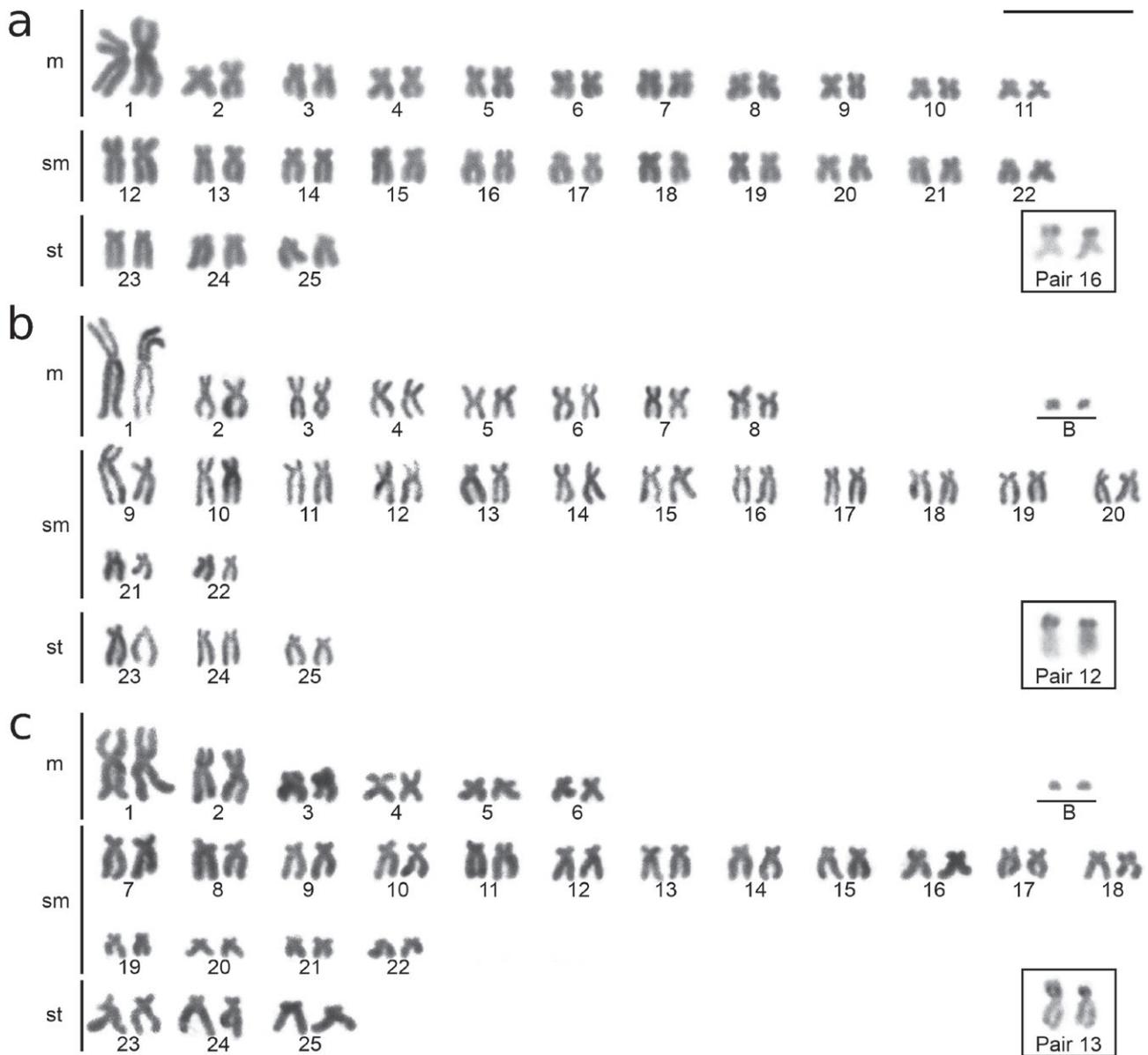
Voucher	Species	Stream/locality	Coordinates	Sex
2733-47, 2764-68 2758-61 2751-57	<i>Moenkhausia dichrourea</i>	A° Pindapoy Grande/Garupá/MN/Arg. A° Mártires/Posadas/MN/Arg.	27°28'58"S, 55°49'10"W 27°22'50"S, 55°57'14"W	10F, 7M, 3? 1F, 1M, 2?
2770, 2773, 2775, 2777, 2779	<i>Moenkhausia intermedia</i>	A° Pindapoy Grande/Garupá/MN/Arg. A° Yabebiry/Santa Ana/MN/Arg.	27°29'41"S, 55°49'13"W 27°17'40"S, 55°33'40"W	? 1F, 1M 3?
2771-72, 2774, 2776, 2778, 2780-86	<i>Moenkhausia sanctaefilomenae</i>	A° Yabebiry/Santa Ana/MN/Arg.	27°17'40"S, 55°33'40"W	8F, 4M

3. RESULTS

All three *Moenkhausia* species showed  $2n = 50$  bi-armed chromosomes (NF = 100). Sexual differences were not observed. The analysis of karyotype formula revealed subtle differences distinctive of each species (Fig. 1): *M. dichroura* ( $22m + 22sm + 6st$ ), *M. intermedia* ( $16m + 28sm + 6st$ ), and *M. sanctaefilomenae* ( $12m + 32sm + 6st$ ). We have not observed differences in karyotype formula among different populations of the same species. In addition to the basic karyotype, *Moenkhausia*

*intermedia* and *M. sanctaefilomenae* showed a variation from one to three supernumerary microchromosomes (mean = 2 on both species), both in males and females (Fig. 1; Table 2).

Silver nitrate staining allowed the identification of one pair of NOR-bearing chromosomes in the three species, which showed size heteromorphism. This chromosomes corresponded to pair 16 in *M. dichroura*, pair 12 in *M. intermedia*, and pair 13 in *M. sanctaefilomenae* (Fig. 1). Heterochromatic C-bands were allocated in centromeric and pericentromeric regions, in the short arms



**Figure 1.** Giemsa stained karyotypes of *Moenkhausia* species: (a) *M. dichroura*, (b) *M. intermedia* and (c) *M. sanctaefilomenae*. NOR-bearing chromosomes of each species are depicted in the boxes.

**Table 2.** B chromosome counts in metaphase cells of *Moenkhausia intermedia* and *M. sanctaefilomenae*. F: females, M: males, ?: undetermined sex

Species	Voucher	Sex	Number of Bs			N cells with Bs
			1B	2Bs	3Bs	
<i>Moenkhausia intermedia</i>	2752	?	-	2	-	2
	2753	?	2	6	13	21
	2754	?	-	-	6	6
	2755	M	3	6	-	9
	2757	?	3	8	1	12
	2773	?	5	16	-	21
	2775	?	10	-	-	10
	2779	F	4	3	7	14
	N cells	27	41	27	95	
	Proportion		0.28	0.43	0.28	1
<i>Moenkhausia sanctaefilomenae</i>	2771	F	-	4	-	4
	2776	F	1	6	1	8
	2781	F	24	2	1	27
	2782	M	2	13	1	16
	2783	F	-	4	-	4
	2785	M	15	-	-	15
	2786	F	4	14	-	18
		N cells	46	43	3	92
	Proportion		0.5	0.47	0.03	1

of NOR-bearing chromosomes, and the supernumerary chromosomes (Fig. 2). The Ag-NOR bands showed correspondence with bright positive signals when stained with CMA<sub>3</sub>, and dark negative bands when stained with DAPI (Fig. 3). Besides, the staining with CMA<sub>3</sub> made more evident the size heteromorphism evidenced by silver nitrate. Bright DAPI bands were observed in the pericentromeric region of several chromosomes in *M. sanctaefilomenae*, matching positive C-bands (Fig. 3).

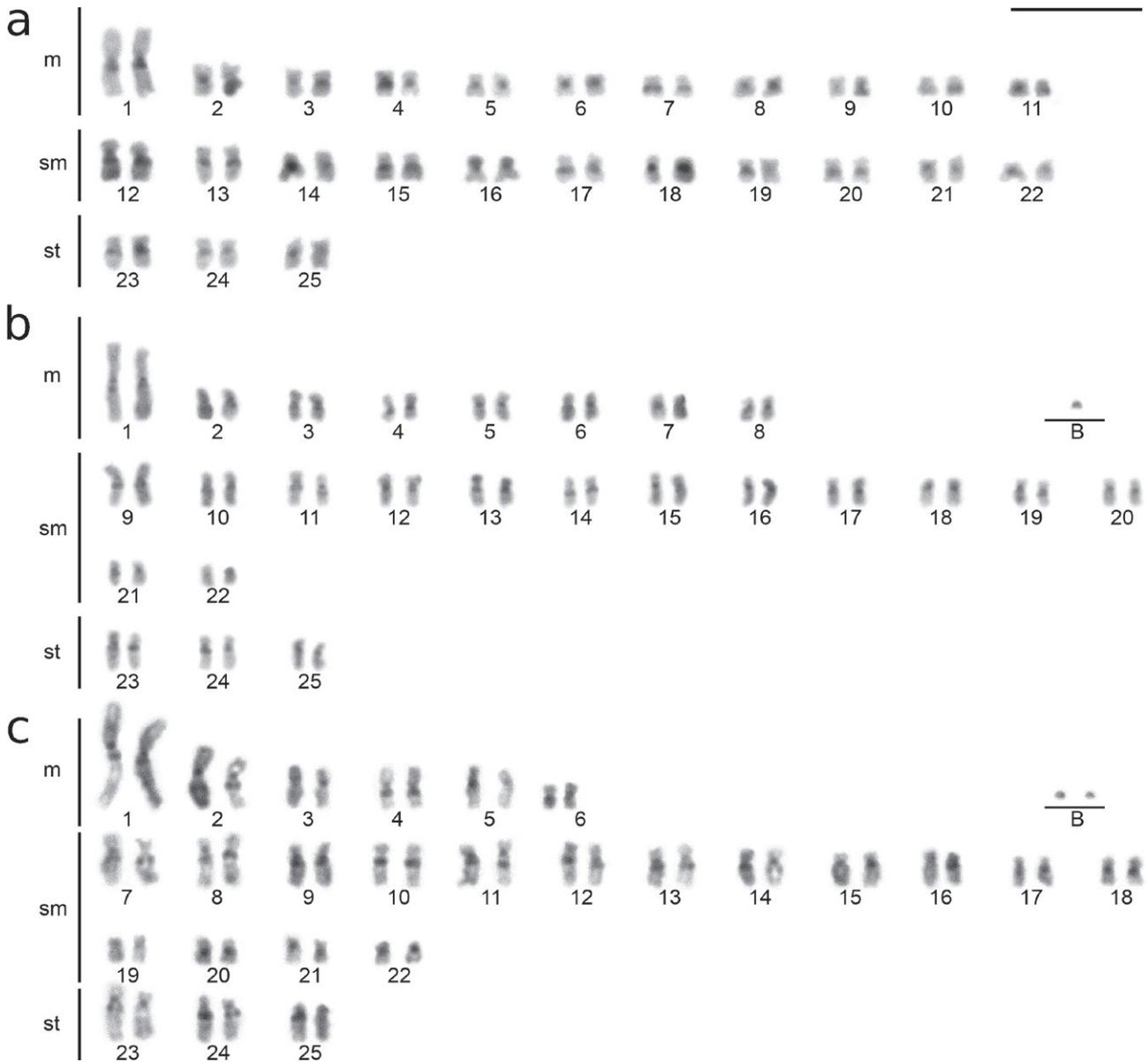
#### 4. DISCUSSION

Diploid number of 50 bi-armed chromosomes are common features of the genus *Moenkhausia*, in agreement with our observations (Portela *et al.* 1988; Arefjev 1990; Foresti *et al.* 1989; Alberdi and Fenocchio 1997; Portela-Castro *et al.* 2001; Portela-Castro and Júlio Júnior 2002; Dantas *et al.* 2007; Hashimoto *et al.* 2012; Scudeler *et al.* 2015; Utsunomia *et al.* 2016; Fernandes and Alves 2017; Nascimento *et al.* 2020). However, cytotypes with  $2n = 48$  and  $2n = 49$  were described in *M. gracilima* and *M. pittieri*, respectively (Arefjev 1990; Santos 1999). Variations reported in karyotype formu-

las suggests that structural rearrangements could be involved in the karyotypic differentiation in *Moenkhausia*, such as non-Robertsonian translocations, inversions and/or translocations (Tenório *et al.* 2013; Nascimento *et al.* 2020). Some authors have also postulated that these chromosomal rearrangements could have an important role in the diversification of certain families and orders of Neotropical fishes (Galetti Jr. *et al.* 2000; Silva *et al.* 2013; Takagui *et al.* 2014; Cioffi *et al.* 2017).

The presence of B chromosomes in Neotropical fishes has been reported for the first time in *Prochilodus lineatus* (cited as *P. scrofa* in Pauls and Bertollo 1983), Characiformes being the group with the higher number of species having this special type of chromosomes (Carvalho *et al.* 2008). The presence of supernumerary chromosomes in the genus *Moenkhausia* was reported for the first time by Portela *et al.* (1988), in a population of *M. intermedia* from Mogi-Guaçu river (São Paulo, Brasil). In a later study, a population of this species from Paraná river was analyzed, but the authors could not detect supernumerary chromosomes (Portela-Castro and Júlio Júnior 2002). Thus, our results extends the presence of B-chromosomes in *M. intermedia*. Nearly all analyzed populations of *M. sanctaefilomenae* have shown supernumerary chromosomes, including our results, even as numerical polymorphisms within populations (Foresti *et al.* 1989; Alberdi and Fenocchio 1997; Portela-Castro *et al.* 2001; Dantas *et al.* 2007; Hashimoto *et al.* 2012; Scudeler *et al.* 2015; Utsunomia *et al.* 2016; Fernandes and Alves 2017). Recent molecular cytogenetic approaches have also revealed an autosomic origin of this elements (Scudeler *et al.* 2015; Utsunomia *et al.* 2016). It has been suggested that numerical polymorphisms of B-chromosomes in *M. sanctaefilomenae* could represent a genetic diversification process, related to populations restricted to small rivers and tributaries (Portela-Castro *et al.* 2001; Hashimoto *et al.* 2012). This can also be attributed to somatic non-disjunction, as suggested in Camacho *et al.* (2000). Interestingly, we detected supernumerary chromosomes on specimens of both sexes, contrary to the results of Portela-Castro *et al.* (2001), who found their presence only in males.

C-banding showed several heterochromatic bands at centromeric and pericentromeric regions in the three species, in concordance with previous studies (Foresti *et al.* 1989; Portela-Castro *et al.* 2001; Portela-Castro and Júlio Júnior 2002; Dantas *et al.* 2007; Hashimoto *et al.* 2012; Fernandes and Alves 2017). B-chromosomes detected in *M. intermedia* and *M. sanctaefilomenae* exhibited positive C-bands, agreeing partially with studies that demonstrated the occurrence of euchromatic and heterochromatic supernumerary chromosomes (Foresti



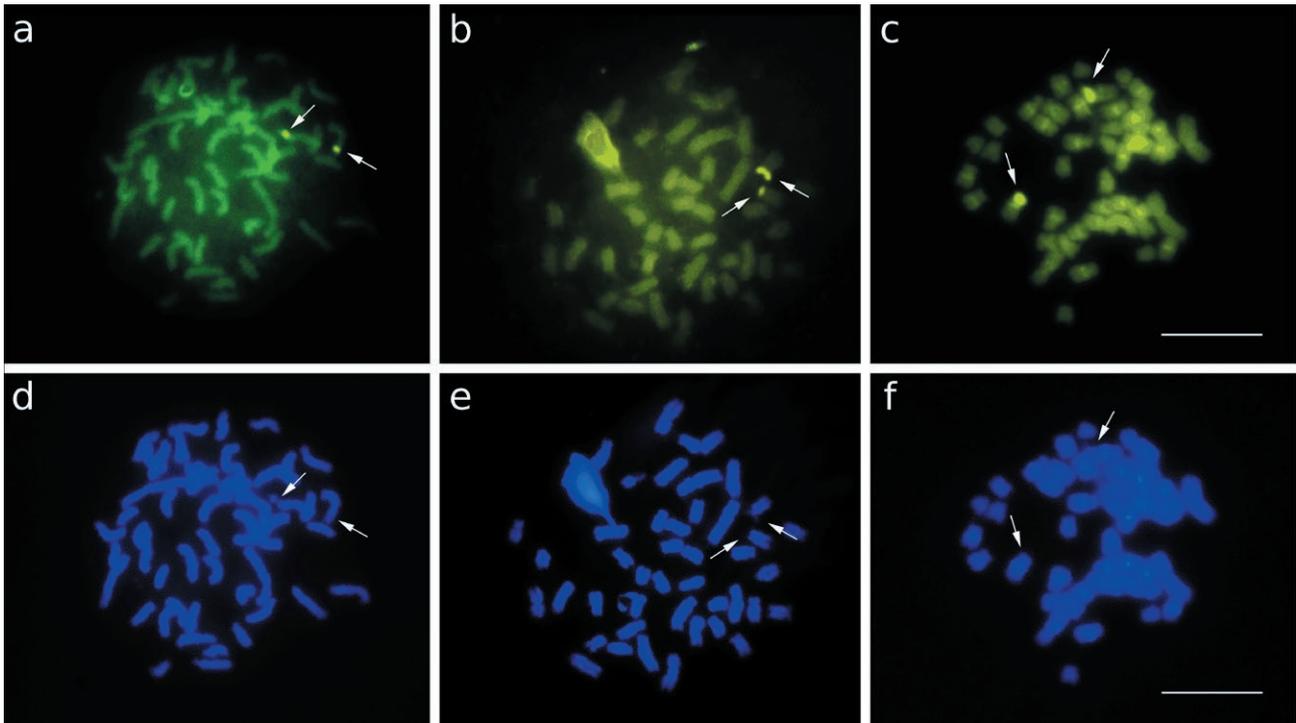
**Figure 2.** C-banded chromosomes of (a) *Moenkhausia dichroua*, (b) *M. intermedia* and (c) *M. sanctaefilomenae*.

*et al.* 1989; Hashimoto *et al.* 2012; Utsunomia *et al.* 2016; Fernandes and Alves 2017).

*Moenkhausia intermedia* has been characterized by Ag-NORs in a single chromosome pair, in agreement with our results (Portela *et al.* 1988; Portela-Castro and Júlio Júnior 2002; Dantas *et al.* 2007). On the contrary, simple and multiple NORs have been described in *M. sanctaefilomenae* (Foresti *et al.* 1989; Portela-Castro and Júlio Júnior 2002; Dantas *et al.* 2007; Hashimoto *et al.* 2012; Fernandes and Alves 2017). Ag-NORs were not described in *M. dichroua*, this study being the first report. Some populations of *M. sanctaefilomenae* analyzed previously

exhibited active NORs on supernumerary chromosomes (Foresti *et al.* 1989; Hashimoto *et al.* 2012). This has led to the suggestion that these elements are not completely inert, being able to contribute to cellular functions (Hashimoto *et al.* 2012; Utsunomia *et al.* 2016). In addition, it has been hypothesized that B chromosomes had a relevant role in the evolutionary history of this species (Portela-Castro *et al.* 2001). We have not observed Ag-NOR bands in any supernumerary chromosome.

Fluorochromes that stain preferentially GC base repetitions were employed as an additional method to detect nucleolar organizers independently of their activ-



**Figure 3.** Metaphasis after sequential fluorescent staining with CMA<sub>3</sub> (a) and DAPI (b) of *Moenkhausia dichroua*, *M. intermedia* and *M. sanctaefilomenae*. Arrows indicate the NOR-bearing chromosomes.

ity (Amemiya and Gold 1986). Ag-NOR bearing chromosomes of *M. intermedia* and *M. sanctaefilomenae* showed positive CMA<sub>3</sub> signals on secondary constrictions, according to previous observations (Portela-Castro and Júlio Júnior 2002). *Moenkhausia dichroua* exhibited a similar pattern. The observation of pericentromeric DAPI<sup>+</sup> blocks restricted only to *M. sanctaefilomenae* could indicate a prevalence of AT-rich regions in these species. Pericentromeric DAPI<sup>+</sup> heterochromatic blocks were also detected in other Neotropical fish species such as *Astyanax argyrimarginatus* (Tenório *et al.* 2013), *Bryconamericus aff. iheringii* (da Silva *et al.* 2014), and *Hollandichthys multifasciatus* (Balén *et al.* 2013). This fact could be an exception since it has been suggested that bright DAPI<sup>+</sup> regions are not common in fishes, negative bands coincident with CMA<sub>3</sub><sup>+</sup> sites being more frequently observed (Souza *et al.* 2008). Supernumerary chromosomes were not stained by the fluorochromes, neither in *M. intermedia* nor *M. sanctaefilomenae*, preventing us to make inferences about their molecular composition.

The species of *Moenkhausia* analyzed here showed a conservative macrostructure of bi-armed chromosomes, similar C-band patterns and simple NORs systems. However, species-specific differences were evidenced regarding composition of chromosome types (m, sm and st), position of Ag-NORs, and DAPI banding patterns.

Overall, *M. dichroua* and *M. intermedia* showed more similarities between them in comparison to *M. sanctaefilomenae*, supporting phylogenetic hypotheses that grouped *M. dichroua* and *M. intermedia* in a branch separated from *M. sanctaefilomenae* (Mariguela *et al.* 2013; Mirande 2010, 2018).

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#### REFERENCES

- Alberdi AJ, Fenocchio AS. 1997. Karyotypes of five Tetragonopterinae species (Pisces, Characidae) from Argentina. *Cytologia*. 62:171–176.

- Altınordu F, Peruzzi L, Yu Y, He X. 2016. A tool for the analysis of chromosomes: KaryoType. *Taxon*. 65(3):586–592. Available from: <http://doi.wiley.com/10.12705/653.9>.
- Amemiya CT, Gold JR. 1986. Chromomycin A<sub>3</sub> stains nucleolus organizer regions of fish chromosomes. *Copeia*. 1986(1):226. Available from: <https://www.jstor.org/stable/1444915?origin=crossref>.
- Arefjev VA. 1990. Problems of karyotypic variability in the family Characidae (Pisces, Characiformes) with the description of somatic karyotypes for six species of tetras. *Caryologia*. 43(3-4):305–319.
- Balen RE, Noletto RB, Vicari MR, Artoni RF, Cestari MM. 2013. Comparative cytogenetics among populations of *Hollandichthys multifasciatus* (Teleostei: Characidae). *Zoolog Sci*. 30(2):105–109.
- Camacho JPM, Sharbel TF, Beukeboom LW. 2000. B-chromosome evolution. *Philos Trans R Soc London Ser B Biol Sci*. 355(1394):163–178. Available from: <http://www.royalsocietypublishing.org/doi/10.1098/rstb.2000.0556><https://royalsocietypublishing.org/doi/10.1098/rstb.2000.0556>.
- Carvalho FR, Sarmiento-Soares LM, Martins-Pinheiro RF, Carvalho FR, Sarmiento-Soares LM, Martins-Pinheiro RF. 2014. Redescription of *Moenkhausia doceana* (Steindachner, 1877) (Ostariophysi: Characiformes): a characid from the Northeastern Mata Atlântica ecoregion, Brazil. *Neotrop Ichthyol*. 12(2):377–388. Available from: [http://www.scielo.br/scielo.php?script=sci\\_arttext&pid=S1679-62252014000200377&lng=en&tlng=en](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1679-62252014000200377&lng=en&tlng=en).
- Carvalho RA, Martins-Santos IC, Dias AL. 2008. B chromosomes: an update about their occurrence in freshwater Neotropical fishes (Teleostei). *J Fish Biol*. 72(8):1907–1932.
- Cioffi MdB, Yano CF, Sember A, Bertollo LAC. 2017. Chromosomal evolution in lower vertebrates: Sex chromosomes in neotropical fishes. *Genes*. 8(10). Available from: <https://www.mdpi.com/2073-4425/8/10/258>.
- da Silva L, Giuliano-Caetano L, Dias A. 2014. Karyotypic diversity in a population of *Bryconamericus aff. iheringii* (Characidae). *Genet Mol Res*. 13(1):2069–2081. Available from: <http://www.funpecrp.com.br/gmr/year2014/vol13-1/pdf/gmr3381.pdf>.
- Dantas ESdO, Vicari MR, Souza IL, Moreira-Filho O, Bertollo LAC, Artoni RF. 2007. Cytotaxonomy and karyotype evolution in *Moenkhausia Eigenmanni*, 1903 (Teleostei, Characidae). *Nucl*. 50(3):505–518.
- Fernandes CA, Alves DS. 2017. Occurrence of multiple euchromatic B microchromosomes in *Moenkhausia sanctaefilomenae* (Pisces, Characidae) from the Upper Paraná River basin, Brazil. *Cytologia*. 82(5):547–550. Available from: [https://www.jstage.jst.go.jp/article/cytologia/82/5/82\\_{\\_}MS1992/{\\_}article](https://www.jstage.jst.go.jp/article/cytologia/82/5/82_{_}MS1992/{_}article).
- Foresti F, Almeida-Tolcdo LF, Toledo SA. 1989. Supernumerary chromosome system, Cbanding pattern characterization and multiple nucleolus organizer regions in *Moenkhausia sanctaefilomenae* (Pisces, Characidae). *Genetica*. 79(2):107–114.
- Fricke R, Eschmeyer WN, van der Laan R. 2021. Eschmeyer's catalog of fishes: genera, species, references. [accessed 2021-03-2]. Available from: <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>.
- Galetti Jr PM, Aguilar CT, Molina WF. 2000. An overview of marine fish cytogenetics. *Hydrobiologia*. 420(1):55–62.
- Hashimoto DT, Voltolin TA, Paes ADNVdA, Foresti F, Bortolozzi J, Porto-Foresti F. 2012. Cytogenetic analysis of B chromosomes in one population of the fish *Moenkhausia sanctaefilomenae* (Steindachner, 1907) (Teleostei, Characiformes). *Comp Cytogenet*. 6(2):141–151.
- 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.
- Levan A, Fredga K, Sandberg AA. 1964. Nomenclature for centromeric positions on chromosomes. *Hereditas*. 52(2):201–220. Available from: <http://doi.wiley.com/10.1111/j.1601-5223.1964.tb01953.x>.
- Lima FCT, Malabarba LR, Buckup PA, da Silva JFP, Vari RP, Harold A, Benine R, Oyakawa OT, Pavanelli CS, Menezes NA, *et al.* 2003. Genera *incertae sedis* in Characidae. In: Reis RE, Kullander SO, Ferraris Jr CJ, editors. Check list of the freshwater fishes of south and central america. Porto Alegre: EDIPUCRS; p. 106–168.
- Mariguela TC, Benine RC, Abe KT, Avelino GS, Oliveira C. 2013. Molecular phylogeny of *Moenkhausia* (Characidae) inferred from mitochondrial and nuclear DNA evidence. *J Zool Syst Evol Res*. 51(4):327–332.
- Mirande JM. 2010. Phylogeny of the family Characidae (Teleostei: Characiformes): from characters to taxonomy. *Neotrop Ichthyol*. 8(3):385–568.
- Mirande JM. 2018. Morphology, molecules and the phylogeny of Characidae (Teleostei, Characiformes). *Cladistics*. 35(3):282–300. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/cla.12345>.
- Moreira-Filho O, Bertollo LAC. 1991. Extraction and use of the cephalic kidney for chromosome studies in small fish. *Brazilian J Genet*. 14(4):1085–1090.

- Nascimento CND, Troy WP, Alves JACP, Carvalho ML, Oliveira C, Foresti F. 2020. Molecular cytogenetic analyses reveal extensive chromosomal rearrangements and novel B chromosomes in *Moenkhausia* (Teleostei, Characidae). *Genetics and Molecular Biology*. 43. Available from: [http://www.scielo.br/scielo.php?script=sci\\_arttext&pid=S1415-47572020000600205&nrm=iso](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1415-47572020000600205&nrm=iso).
- Pauls E, Bertollo LAC. 1983. Evidence for a system of supernumerary chromosomes in *Prochilodus scrofa* Steindachner, 1881 (Pisces, Prochilodontidae). *Caryologia*. 36(4):307–314. Available from: <http://www.tandfonline.com/doi/abs/10.1080/00087114.1983.10797671>.
- Portela ALBS, Galetti Jr PM, Bertollo LAC. 1988. Considerations on the chromosome evolution of Tetragonopterinae (Pisces, Characidae). *Rev Bras Genética*. 11:307–316.
- Portela-Castro ALdB, Júlio Júnior HF. 2002. Karyotype relationships among species of subfamily Tetragonopterinae (Pisces, Characidae): cytotaxonomy and evolution aspects. *Cytologia*. 67(3):329–336.
- Portela-Castro ALdB, Júlio Júnior HF, Nishiyama PB. 2001. New occurrence of microchromosomes B in *Moenkhausia sanctaefilomenae* (Pisces, Characidae) from the Paraná River of Brazil: analysis of the synaptonemal complex. *Genetica*. 110:277–283.
- Santos AF. 1999. Estudos citogenéticos em peixes dos gêneros *Bryconops* e *Moenkhausia* (Teleostei, Characidae, Tetragonopterinae) [dissertation]. Universidade Federal de São Carlos/Fundação Universidade do Amazonas.
- Schweizer D. 1980. Simultaneous fluorescent staining of R bands and specific heterochromatic regions (DAPI bands) in human chromosomes. *Cytogenet Cell Genet*. 27:190–193.
- Scudeler PES, Diniz D, Wasko AP, Oliveira C, Foresti F. 2015. Whole chromosome painting of B chromosomes of the red-eye tetra *Moenkhausia sanctaefilomenae* (Teleostei, Characidae). *Comp Cytogenet*. 9(4):661–669. Available from: <http://compcytogen.pensoft.net/articles.php?id=5460>.
- Silva DMZA, Pansonato-Alves JC, Utsunomia R, Daniel SN, Hashimoto DT, Oliveira C, Porto-Foresti F, Foresti F. 2013. Chromosomal organization of repetitive DNA sequences in *Astyanax bockmanni* (Teleostei, Characiformes): dispersive location, association and co-localization in the genome. *Genetica*. 141(7-9):329–336. Available from: <http://link.springer.com/10.1007/s10709-013-9732-7>.
- Souza IL, Santos-Silva LK, Venere PC, Moreira-Filho O. 2008. Molecular cytogenetics of *Salminus* fish (Characiformes) based on 5S and 18S rRNA genes hybridization, fluorochrome staining and C-banding. *Micron*. 39(7):1036–1041. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0968432807001424>.
- Sumner AT. 1972. A simple technique for demonstrating centromeric heterochromatin. *Exp Cell Res*. 75:304–306.
- Takagui FH, Venturelli NB, Dias AL, Swarc AC, Vicari MR, Fenocchio AS, Giuliano-Caetano L. 2014. The importance of pericentric inversions in the karyotypic diversification of the species *Loricariichthys anus* and *Loricariichthys platymetopon*. *Zebrafish*. 11(4):300–305. Available from: <http://www.liebertpub.com/doi/10.1089/zeb.2014.0985>.
- Tenório RCCdO, Vitorino CdA, Souza IL, Oliveira C, Venere PC. 2013. Comparative cytogenetics in *Astyanax* (Characiformes: Characidae) with focus on the cytotaxonomy of the group. *Neotrop Ichthyol*. 11(3):553–564. Available from: [http://www.scielo.br/scielo.php?script=sci\\_arttext&pid=S1679-62252013000300553&lng=en&tlng=en](http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1679-62252013000300553&lng=en&tlng=en).
- Utsunomia R, De Andrade Silva DMZ, Ruiz-Ruano FJ, Araya-Jaime C, Pansonato-Alves JC, Scacchetti PC, Hashimoto DT, Oliveira C, Trifonov VA, Porto-Foresti F, et al. 2016. Uncovering the ancestry of B chromosomes in *Moenkhausia sanctaefilomenae* (Teleostei, Characidae). *PLoS One*. 11(3):1–20.