DE GRUYTER

Biologia **72**/6: 680—685, 2017
Section Zoology
DOI: 10.1515/biolog-2017-0071

Chromosomal characteristics of rDNA in a conserved karyotype of two *Sternopygus macrurus* (Gymnotiformes: Sternopygidae) populations from upper Paraná River basin

Carlos Alexandre Fernandes[1], Lucas Baumgärtner[2], Leonardo Marcel Paiz[2,3], Vladimir Pavan Margarido[2,3] & Ana Luiza de Brito Portela-Castro[4]

[1] *Universidade Estadual de Mato Grosso do Sul, Unidade Universitária de Mundo Novo, 79980-000 Mundo Novo, Mato Grosso do Sul, Brazil; e-mail: fxande@gmail.com*
[2] *Universidade Estadual do Oeste do Paraná, Centro de Ciencias Biológicas e da Saúde, 85819-110 Cascavel, Paraná, Brazil*
[3] *Universidade Estadual de Maringá, Centro de Ciencias Biológicas, 87020-900 Maringá, Paraná, Brazil*
[4] *Universidade Estadual de Maringá, Departamento de Biotecnologia, Genética e Biologia Celular, 87020-900 Maringá, Paraná, Brazil*

Abstract: Karyotype and chromosomal characteristics of both minor and major rDNA of *Sternopygus macrurus,* a weakly electric South American fish, from two populations of the upper Paraná River basin, were investigated using conventional (Giemsa staining, silver staining, C-banding and base-specific fluorochromes) and molecular (fluorescent *in situ* hybridization (FISH) with 5S and 18S rDNA probes) cytogenetic techniques. Diploid chromosome number was invariably $2n = 46$ and karyotype composed of 23 pairs of biarmed chromosomes (28m+18sm). The nucleolus organizer regions (NORs) were located in the secondary constriction of the p arm of pair No. 2; this site corresponded with CMA_3 positive as well as with 18S rDNA signals, respectively. This 18S rDNA cluster was not syntenic to the 5S rDNA sites located at pairs Nos. 1, 5 and 15. The karyotypes and other chromosomal characteristics of individuals from the two populations in the upper Paraná River basin were identical. The karyotype differences among individuals identified as *S. macrurus* from Paraná River and the São Francisco and Amazon River basins, respectively, may indicate that these taxa might represent distinct species.

Key words: fish cytogenetics; chromosome banding; NORs; repetitive sequences; Neotropical fishes

Introduction

Sternopygidae, one of the five families of the order Gymnotiformes, contains fishes that are characterized by their electrogenic ability (Mago-Leccia 1978) and comprises five genera: *Archolaemus* Korringa, 1970, *Distocyclus* Mago-Leccia, 1978, *Eigenmannia* Jordan & Evermann, 1896, *Rhabdolichops* Eigenmann & Allen, 1942 and *Sternopygus* Müller & Troschel, 1846 (Albert 2003; Albert & Crampton 2005). The genus *Sternopygus* includes nine recognized species (Froese & Pauly 2017) found throughout Neotropical river drainages from Panama and Colombia to the Paraguay-Paraná River basin in Paraguay (Albert 2003; Albert & Crampton 2005).

Available cytogenetic data for this family are known for representatives of the genera *Eigenmannia* and *Sternopygus*. *Eigenmannia* species display a remarkable karyotype diversification, with diploid chromosome numbers ranging from $2n = 28$ to 38 , as well as different sex chromosome systems (Almeida-Toledo et al. 2001; Henning et al. 2008; Silva et al. 2009; Fernandes et al. 2010; Sene et al. 2014). However, cytogenetic studies in representatives of the genus *Sternopygus* are restricted to the single species *Sternopygus macrurus* (Bloch & Schneider, 1801), which possessed an invariably diploid chromosome number of $2n = 46$ and different karyotypes for individuals from different hydrographic basins (Almeida-Toledo et al. 1993; Silva et al. 2008). Cytogenetic studies on the distribution of ribosomal genes are restricted to karyotypes of six species of the genus *Eigenmannia*, demonstrating that these multigene families may be extremely either variable (5S rDNA) or conserved (18S rDNA) (Silva et al. 2009; Sene et al. 2014).

The aim of the present study was the investigation of the karyotype and other chromosomal characteristics of both minor and major rDNA of *S. macrurus,* a weakly electric South American fish from two populations of the upper Paraná River basin, using conventional (Giemsa staining, silver staining, C-banding and base-specific fluorochromes) and molecular (fluorescent in situ hybridization (FISH) with 5S and 18S rDNA probes) cytogenetic techniques.

Material and methods

The 16 individuals of *S. macrurus* analysed were collected from populations in the upper Paraná River basin: 7 males and 5 females from Dourado stream (23°51′04.9″

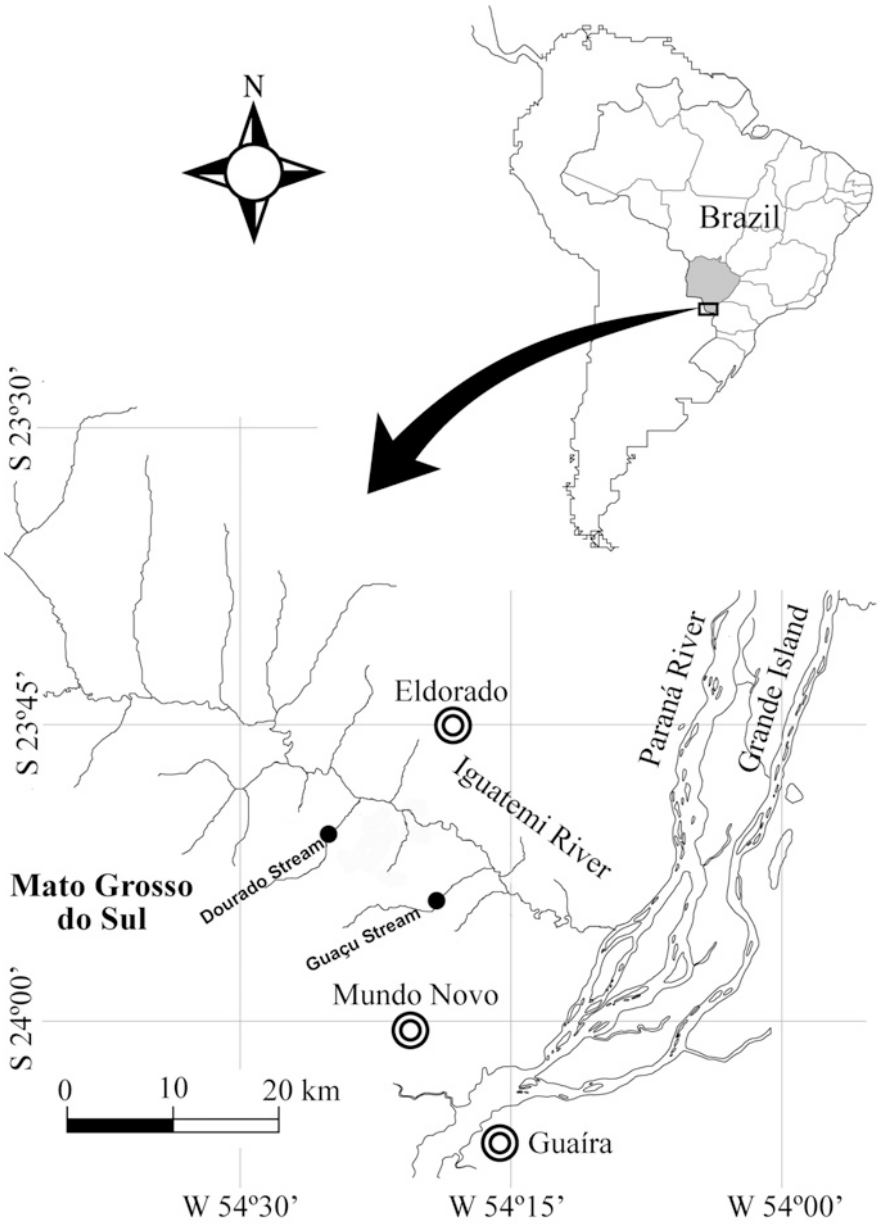

Fig. 1. Location of Dourado and Guaçu streams from the upper Paraná River basin, where *Sternopygus macrurus* individuals were captured. Dark circle indicates the sampling points.

S, 54°25′13.9″ W) and 3 males and 1 female from Guaçu stream (23°54′19.6″ S, 54°21′43.4″ W). The Dourado and Guaçu streams are tributaries of the right margin of the Iguatemi River (Fig. 1).

The individuals were identified and deposited in the Universidade Estadual do Mato Grosso do Sul, Mundo Novo. The experiments followed the ethical conducts, and before euthanasia, the fish were anesthetized by an overdose of clove oil (Griffiths 2000). Metaphase chromosomes were obtained from anterior kidney cells using the air-drying technique (Bertollo et al. 1978). The C-positive heterochromatin (C-bands) visualized by the procedure of Sumner (1972), with some minor adaptations. NORs were detected by means of silver nitrate staining (Ag-NORs), according to Howell & Black (1980). GC- and AT- rich regions were detected by fluorochromes Chromomycin A_3 (CMA_3) and DAPI (4'6-diamidino-2-phenylindole), respectively, according to Schmid (1980).

At least 30 metaphases were analysed for each individual and those with better chromosome morphology were used for the karyotype analysis. The chromosomes were classified as metacentric (m), and submetacentric (sm) according to Levan et al. (1964). For the determination of the number of chromosome arms (NF value), the m and sm chromosomes were scored as bearing two arms.

The location of the 5S and 18S rDNA sites in the chromosomes was performed by fluorescence *in situ* hybridization (FISH) (Pinkel et al. 1986) with modifications (Margarido & Moreira-Filho 2008), using probes from the genome of *Megaleporinus elongatus* (Valenciennes, 1850) (Martins & Galetti Jr. 1999) and *Prochilodus argenteus* Spix & Agassiz, 1829 (Hatanaka & Galetti Jr. 2004), respectively. The probes were labelled through nick translation, with digoxigenin-11-dUTP (5S rDNA) and biotin-16-dUTP (18S rDNA) (Roche). Detection and amplification of the hybridization signal were carried out using avidin-FITC

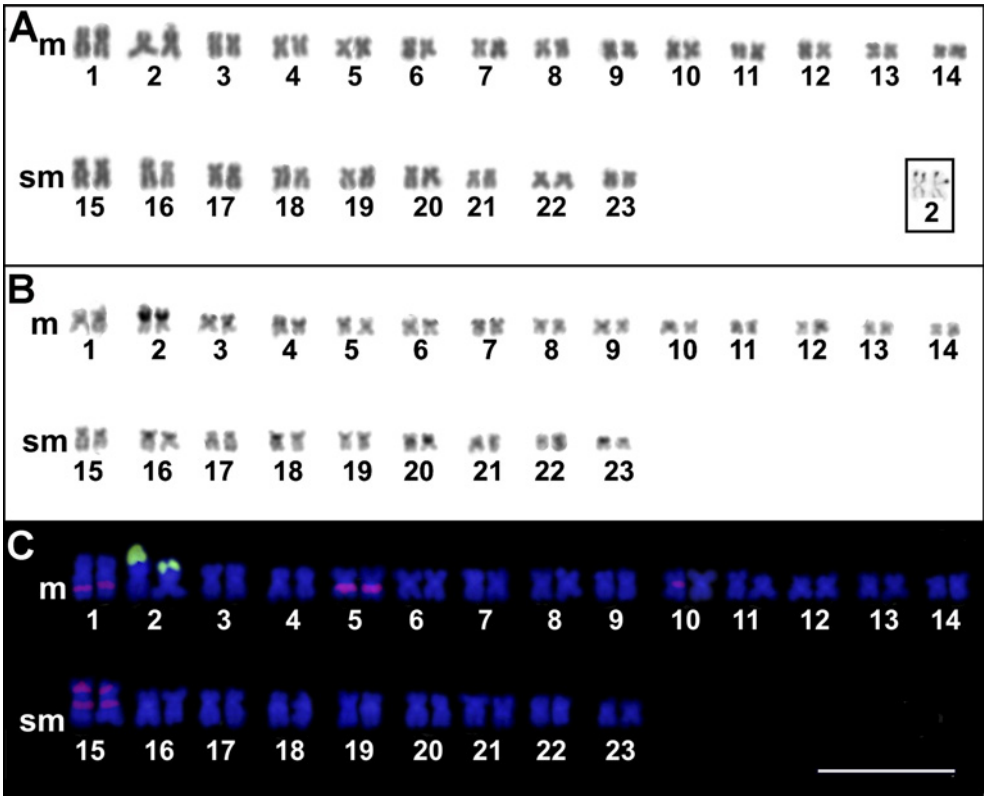

Fig. 2. Karyotypes of *Sternopygus macrurus* arranged from Giemsa-stained (A), C-banded (B) and after double FISH with 5S rDNA probes (red) and 18S rDNA (green) (C). The NOR-bearing chromosomes (pair No. 2) are boxed. Scale 10 μm.

and anti-avidin biotin (Sigma) for probes labelled with biotin, and anti-digoxigenin rhodamine (Roche) for probes labelled with digoxigenin. Slides were counterstained with DAPI (50 μg ml^{-1}) and analyzed in epifluorescence microscope (Olympus BX61). The images were captured using the software DP controller (Media Cybernetics) and the image composition with Adobe Photoshop CS6.

Results

All the 16 individuals of *S. macrurus* from the two sites had an invariably diploid chromosome number $2n = 46$ with the karyotype composed of 28 metacentric and 18 submetacentric chromosomes and NF value 92 in both sexes (Fig. 2A). Heteromorphic sex chromosomes were not identified. A secondary constriction was observed in the terminal region of the p arm of the m pair No. 2, which corresponded to the Ag-NORs signals (Fig. 2A, in box) and also had a clear size heteromorphism (Fig. 2C).

The heterochromatin was detected on the pericentromeric region of the pairs Nos. 3, 16, 18, 20 and 23 and also a large heterochromatic block in the p arm of the pair No. 2, close to the terminal secondary constriction (Fig. 2B).

The mapping of 18S rDNA cluster showed single positive site, corresponding to the Ag-NORs. Multiple 5S rDNA sites were observed in interstitial position of the q arm of the pair No. 1, in pericentromeric region of the q arm of the pair No. 5 and in one of the homologues of the pair No. 10. The pair No. 15 showed two sites – one on the p arm and another one on the q arm, both in pericentromeric position (Fig. 2C). The double FISH with both probes demonstrated that both ribosomal gene clusters were located in different chromosomes.

The base-specific fluorochromes revealed CMA_3^+ and $DAPI^-$ regions corresponding to the NOR sites, showing that those regions possessed GC-rich DNA segments (Fig. 3). The large heterochromatic block in the p arm of the pair No. 2 did not present positive sites for CMA_3^+ and $DAPI^+$.

The chromosome markers observed in the present study for *S. macrurus* are summarized in Fig. 4A.

Discussion

Our results showed that individuals from the populations of *S. macrurus* from the two analyzed locations had the same $2n = 46$, FN = 92, and identical karyotypes composed of 28m+18sm chromosomes as well as other chromosomal characteristics revealed by Ag-NOR, C-banding and FISH mapping ribosomal clusters location. Moreover, $2n = 46$ chromosomes and FN = 92 were equally reported in individuals from other populations of *S. macrurus* – the Amazon (Almeida-Toledo et al. 1993; Silva et al. 2008), São Francisco and Paraná River basins (Almeida-Toledo et al. 1993) but with different karyotype composition. The individuals from Amazon River basin had 30m+16sm, those from São Francisco River basin had 32m+14sm and those from Paraná River basin had 28m+18sm. Other populations from upper Paraná River basin had the same karyotype

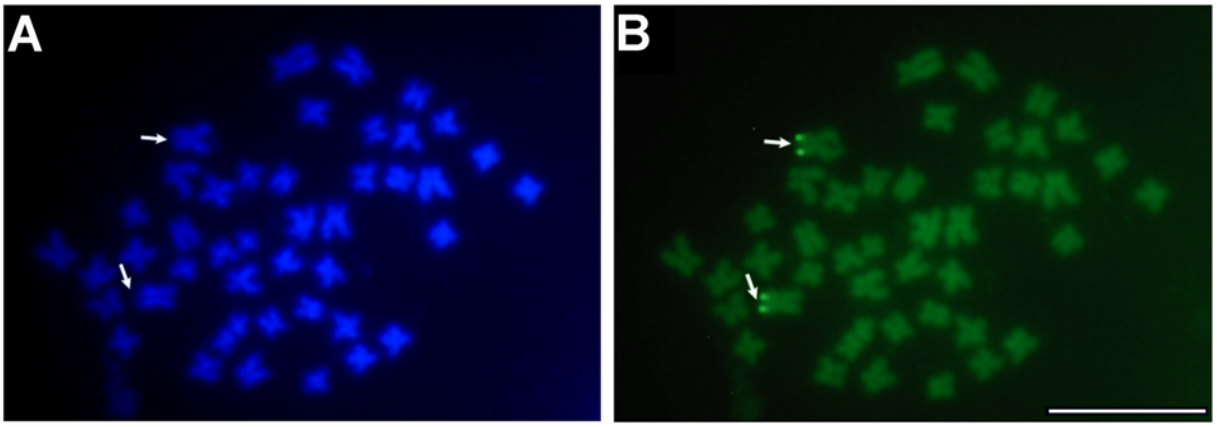

Fig. 3. Sequential metaphases of *Sternopygus macrurus* stained with DAPI (A) and Chromomycin A_3 (B). Arrows indicate NOR-bearing pair No. 2. Scale 10 μm.

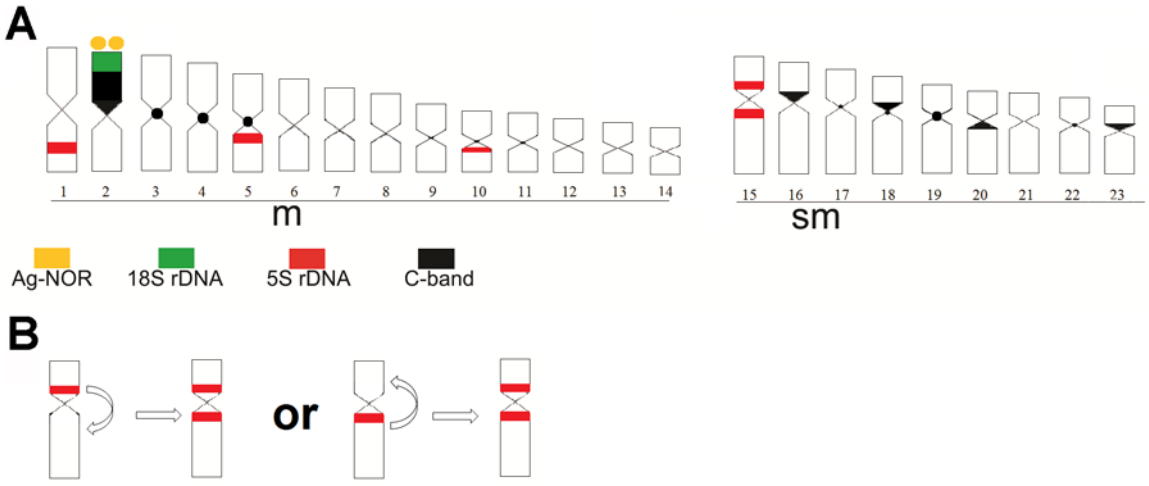

Fig. 4. Ideogram of *Sternopygus macrurus* karyotype, showing the heterochromatin, Ag-NORs, 18S and 5S rDNA distribution patterns (A). Scheme with probable pericentromeric inversion that transferred part of the 5S rDNA cluster to another arm of the same chromosome (B).

according to Almeida-Toledo et al. (1993), showing that this species has an identical karyotype in this region. This karyotype stability suggests that there is gene flow among upper Paraná River basin populations, associated with potamodromy of this species (Mago-Leccia 1978).

The present study also detected two positive NORs in karyotype of *S. macrurus* but with different position as compared to those of the other populations discussed above. Herein, the signals were terminal on the p arm of the pair No. 2, whereas in karyotype of individuals from Amazon River basin populations it was terminal on the p arm of the pair No. 1 (Silva et al. 2008) and terminal on long q arm of the pair No. 1 (Almeida-Toledo et al. 1993); while in the individuals from São Francisco River basin population it was interstitial on the q arm of the pair No. 1 and in individuals from Paraná River basin it was terminal on the p arm of the pair No. 19 (Almeida-Toledo et al. 1993). According to Almeida-Toledo et al. (1993), the differences in NOR patterns between the São Francisco and Paraná populations are probably due to the partial reduction of the heterochromatin block adjacent to the NOR in the latter. This hypothesis could also explain the difference in NOR position between the populations of the Paraná River basin (present study) and analyzed by Almeida-Toledo et al. (1993).

Our study also revealed that NORs were CMA_3 positive$^+$, demonstrating that the 5,8S, 18S and 28S rDNA sequences are interspaced by GC-rich sequences. This was also observed in karyotype of *S. macrurus* described by Silva et al. (2008). On the other hand, NOR-bearing chromosomes in the karyotype of individuals from Amazon population (Silva et al. 2008) were almost twice larger than the second pair, whereas in the present study NOR-bearing chromosomes were slightly smaller than the first pair of chromosomes.

Pattern of heterochromatin distribution described here is in agreement with that of karyotypes of representatives of seven populations studied by Almeida-Toledo et al. (1993) and Silva et al. (2008) except the large heterochromatic block in the p arm of pair No. 2 did not possesses AT-rich sequences as detected in the karyotypes of the Amazonian *S. macrurus* (Silva et al. 2008).

Physical mapping of 18S rDNA in genome of *S. macrurus* demonstrated only one chromosome pair bearing these clusters in terminal position on the p arm. Similar patterns were observed in karyotypes of *Eigenmannia* sp. 1, *Eigenmannia* cf. *trilineata*, *Eigenmannia* sp., *Eigenmannia virescens* and *Eigenmannia virescens* - XY, except for *Eigenmannia* sp. 2 that had these sites located in interstitial position (Sene et al. 2014). Thus, simple NORs is possibly a plesiomorphic characteristics for genome of Sternopygidae representatives.

Physical mapping of 5S rDNA in genome of *S. macrurus* showed these clusters located in 7 chromosomes. Multiple 5S rDNA sites were also observed in karyotypes of six species of *Eigenmannia*, ranging from 2 to 10 signals in karyotypes of different species (Sene et al. 2014). Thus, this variation of the number of 5S rDNA cistrons among different species of Sternopygidae could be related to insertion of transposable elements into 5S rDNA sequences. An example of such transposition would be the presence of 5S rDNA cistrons in one of the homologues of pair No. 10, which could be the result of insertion of transposable elements into 5S rDNA sequences of the other carrier pairs that could have led to the dispersion of these sequences to the m chromosome No. 10 of *S. macrurus*. Recent studies have proposed that the activity of transposable elements is one possible source for rDNA movement in plants (Jiang et al. 2004; Lai et al. 2005; Raskina et al. 2008) and animals (Cioffi et al. 2010; Piscor et al. 2013).

The pattern where two or more 5S rRNA gene clusters are localized on the same chromosome is quite rare, however, such situation has been described in a few plant species (Appels et al. 1980; Mukai et al. 1990; Lee et al. 1999; Monkheang et al. 2016), the fruit fly *Drosophila melanogaster* Meigen, 1830 (Kress et al. 2001) and the fish *Upsilodus* sp. (Kavalco et al. 2004), *Hippoglossus hippoglossus* (L., 1758) (Ocalewicz et al. 2008) and *Trachydoras paraguayensis* (Eigenmann & Ward, 1907) (Baumgärtner et al. 2016) and *Apteronotus albifrons* (L., 1766) (Fernandes et al. 2017). Double 5S rDNA sites (on p and q arms) in the same chromosome observed in the genome of *S. macrurus*, suggested that pericentric inversion might have caused a breakage of the 5S rDNA cluster, transferring part of the site to the other arm of the same chromosome (Fig. 4B). This situation was found in the genomes of two *S. macrurus* populations and may represent a chromosome marker for this species in the upper Paraná River basin.

The present data demonstrated that the individuals of *S. macrurus* analysed here and those studied by Almeida-Toledo et al. (1993) and Silva et al. (2008) possess different karyotypes and chromosomal markers, indicating that individuals identified as *S. macrurus* from the three river basins can be distinct species warranting detailed taxonomic analysis. Thus, new information on the location of 5S and 18S rDNA sites in genomes of analyzed *S. macrurus* populations could confirm this hypothesis.

Acknowledgements

The authors thank the Brazilian agency Fundação de Apoio ao Desenvolvimento do Ensino, Ciencia e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) for financial support and Dr. Weferson Júnio da Graça for taxonomic identification of the individuals. As well, we are grateful to Ministério do Meio Ambiente/ Instituto Chico Mendes de Conservação da Biodiversidade (MMA / ICMBio – License number 54199-1) for authorization to collect the biological material.

References

Albert J.S. 2003. Family Sternopygidae, pp. 493–497. In: Reis R.E., Kullander S.O. & Ferraris J.C.J. (eds), Checklist of the Freshwater Fish of South and Central America, Edipucrs, Porto Alegre, 742 pp. ISBN: 8574303615, 9788574303611

Albert J.S. & Crampton W.G.R. 2005. Diversity and phylogeny of Neotropical electric fishes (Gymnotiformes), pp. 360–409. DOI: 10.1007/0-387-28275-0_13 In: Bullock T.H., Hopkins C.D., Popper A.N. & Fay R.R. (eds), Eletroreception, Springer Handbook of Auditory Research, New York, 472 pp. ISBN: 978-0-387-23192-1

Almeida-Toledo L.F., Foresti F., Daniel M.F.Z. & de Almeida Toledo-Filho S. 1993. Nucleolar chromosome variants in *Sternopygus macrurus* (Pisces, Sternopygidae) from three Brazilian river basins. Caryologia **46:** 53–61. DOI: 10.1080/00087114.1993.10797246

Almeida-Toledo L.F., Foresti F., Péquignot E.V. & Daniel-Silva M.F. 2001. XX:XY sex chromosome system with X heterochromatinization: an early stage of sex chromosome differentiation in the Neotropic electric eel *Eigenmannia virescens*. Cytogenet. Cell Genet. **95 (1-2):** 73–78. DOI: 10.1159/000057020

Appels R., Gerlach W.L., Dennis E.S., Swift H. & Peacock W.J. 1980. Molecular and chromosomal organization of DNA sequences coding for the ribosomal RNAs in cereals. Chromosoma **78 (3):** 293–311. DOI: 10.1007/BF00327389

Baumgärtner L., Paiz L.M., Margarido V.P. & Portela-Castro A.L.B. 2016. Cytogenetics of the Thorny Catfish *Trachydoras paraguayensis* (Eigenmann & Ward, 1907), (Siluriformes, Doradidae): Evidence of Pericentric Inversions and Chromosomal Fusion. Cytogenet. Genome Res. **149:** 201–206. DOI: 10.1159/000448126

Bertollo L.A.C., Takahashi C.S. & Moreira-Filho O. 1978. Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidade). Brazil. J. Genet. **1:** 103–120.

Cioffi M.B., Martins C. & Bertollo L.A.C. 2010. Chromosomal spreading of associated transposable elements and ribosomal DNA in the fish *Erythrinus erythrinus*. Implications for genome change and karyoevolution in fish. BMC Evol. Biol. **10:** 271. DOI: 10.1186/1471-2148-10-271

Fernandes C.A., Bailly D., Silva V.F.B. & Martins-Santos I.C. 2010. System of multiple sex chromosomes in *Eigenmannia trilineata* López & Castello, 1966 (Sternopygidae, Gymnotiformes) from Iguatemi River Basin, MS, Brazil. Cytologia **75 (4):** 463–466. DOI: 10.1508/cytologia.75.463

Fernandes C.A., Paiz L.M., Baumgärtner L., Margarido V.P. & Vieira M.M.R. 2017. Comparative cytogenetics of the black ghost knifefish (Gymnotiformes: Apteronotidae): Evidence of chromosomal fusion and pericentric inversions in karyotypes of two *Apteronotus* species. Zebrafish. DOI: 10.1089/zeb.2017.1432

Froese R. & Pauly D. (eds). 2017. FishBase. Word Wide Web Eletronic Publication. http://www.fishbase.org/ (accessed 22.04.2017).

Griffiths S. 2000. The use of clove oil as an anaesthetic and method for sampling intertidal rockpool fishes. J. Fish Biol. **57 (6):** 1453–1464. DOI: 10.1111/j.1095-8649.2000.tb02224.x

Hatanaka T. & Galetti Jr. P.M. 2004. Mapping of the 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae). Genetica **122 (3):** 239–244. DOI: 10.1007/s10709-004-2039-y

Henning F., Trifonov V. & Almeida-Toledo L.F. De 2008. Use of chromosome microdissection in fish molecular cytogenetics. Genet. Mol. Biol. **31 (1) Suppl.:** 279–283. DOI: 10.1590/S1415-47572008000200022

Howell W.M. & Black D.A. 1980. Controlled silver-staining of nucleolus organizer regions with the protective coloidal developer: a 1-step method. Experientia **36:** 1014–1015. DOI: 10.1007/BF01953855

Kavalco K.F., Pazza R., Bertollo L.A.C. & Moreira-Filho O. 2004. Gene mapping of 5S rDNA sites in eight fish species from the Paraíba do Sul river basin, Brazil. Cytogenet. Genome Res. **106 (1):** 107–110. DOI: 10.1159/000078567

Kress H., Bechler K., Swida U. & Maletz S. 2001. Evolution of 5S rRNA gene families in *Drosophila*. Chromosom. Res. **9 (5):** 403–415. DOI: 10.1023/A:1016787602583

Jiang N., Bao Z., Zhang X., Eddy S.R. & Wessler S.R. 2004. Pack-MULE transposable elements mediate gene evolution in plants. Nature **431(7008):** 569–573. DOI: 10.1038/nature02953

Lai Z., Nakazato T., Salmaso M., Burke J.M., Tang S., Knapp S.J. & Rieseberg L.H. 2005. Extensive chromosomal repatterning and the evolution of sterility barriers in hybrid sunflower species. Genetics **171 (1):** 291–303. DOI: 10.1534/genetics.105.042242

Lee S.H., Do G.S. & Seo B.B. 1999. Chromosomal localization of 5S rRNA gene loci and the implications for relationships within the *Allium* complex. Chromosom. Res. **7 (2)**: 89–93. DOI: 10.1023/A:1009222411001

Levan A., Fredga K. & Sandberg A.A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52 (2):** 201–220. DOI: 10.1111/j.1601-5223.1964.tb01953.x

Mago-Leccia F. 1978. Los peces de la família Sternopygidae de Venezuela. Acta Científica Venez. **29 (Suppl. 1):** 1–91.

Margarido V.P. & Moreira-filho O. 2008. Karyotypic differentiation through chromosome fusion and number reduction in *Imparfinis hollandi* (Ostariophysi , Heptapteridae). Genet. Mol. Biol. **31 (1) Suppl.:** 235–238. DOI: 10.1590/S1415-47572008000200012

Martins C. & Galetti Jr. P.M. 1999. Chromosomal localization of 5S rDNA genes in *Leporinus* fish (Anostomidae, Characiformes). Chromosome Res. **7 (5):** 363–367. DOI: 10.1023/A:1009216030316

Monkheang P., Chaveerach A., Sudmoon R. & Tanee T. 2016. Karyotypic features including organizations of the 5S, 45S rDNA loci and telomeres of *Scadoxus multiflorus* (Amaryllidaceae). Comp. Cytogenet. **10 (4):** 637–646. DOI: 10.3897/CompCytogen.v10i4.9958

Mukai Y., Endo T.R. & Gill B.S. 1990. Physical mapping of the 5S rRNA multigene family in common wheat. J. Hered. **81 (4):** 290–295. DOI: 10.1093/oxfordjournals.jhered.a110991

Ocalewicz K., Penman D.J. & Babiak I. 2008. Variation in size and location of the Ag-NOR in the Atlantic halibut (*Hippoglossus hippoglossus*). Genetica **133 (3):** 261–267. DOI: 10.1007/s10709-007-9209-7

Pinkel D., Straume T. & Gray J.W. 1986. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc. Natl. Acad. Sci. USA **83 (9):** 2934–2938.

Piscor D., Ribacinko-Piscor D.B., Fernandes C.A. & Parise-Maltempi P.P. 2013. Cytogenetic analysis in three *Bryconamericus* species (Characiformes, Characidae): first description of the 5S rDNA-bearing chromosome pairs in the genus. Mol. Cytogenet. **6 (1):** 13. DOI: 10.1186/1755-8166-6-13

Raskina O., Barber J.C., Nevo E. & Belyayev A. 2008. Repetitive DNA and chromosomal rearrangements: speciation-related events in plant genomes. Cytogenet. Genome Res. **120 (3-4):** 351–357. DOI: 10.1159/000121084

Schmid M. 1980. Chromosome banding in Amphibia. IV. Differentiation of GC- and AT-rich chromosome region in Anura. Chromosoma **77 (1):** 83–103. DOI: 10.1007/BF00292043

Sene V.F., Pansonato-Alves J.C., Utsunomia R., Oliveira C. & Foresti F. 2014. Karyotype diversity and patterns of chromosomal evolution in *Eigenmannia* (Teleostei, Gymnotiformes, Sternopygidae). Comp. Cytogenet. **8 (4):** 301–311. DOI: 10.3897/CompCytogen.v8i4.8396

Silva D.S., Milhomem S.S.R., Pieczarka J.C. & Nagamachi C.Y. 2009. Cytogenetic studies in *Eigenmannia virescens* (Sternopygidae, Gymnotiformes) and new inferences on the origin of sex chromosomes in the *Eigenmannia* genus. BMC Genet. **10:** 74. DOI: 10.1186/1471-2156-10-74

Silva D. dos S., Milhomem S.S.R., de Souza A.C.P., Pieczarka J.C. & Nagamachi C.Y. 2008. A conserved karyotype of *Sternopygus macrurus* (Sternopygidae, Gymnotiformes) in the Amazon region: differences from other hydrographic basins suggest cryptic speciation. Micron **39 (8):** 1251–1254. DOI: 10.1016/j.micron.2008.04.001

Sumner A.T. 1972. A simple technique for demonstrating centromeric heterochromatin. Explor. Cell Res. **75 (1):** 304–306. DOI: 10.1016/0014-4827(72)90558-7

Received January 18, 2017
Accepted May 12, 2017