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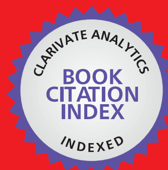

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Chapter 4

Cytogenetic Tools to Study the Biodiversity of Neotropical Fish: From the Classic to the Advent of Cell Culture

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Abstract

Neotropical Ichthyofauna is considered the richest and most diverse in the world. All this biodiversity has attracted attention from researchers from different areas of study, including the cytogenetics. Many cytogenetics studies have search to understand the evolution of macro and micro karyotype structure of these different groups of fish, and classical and molecular cytogenetics techniques have contributed significantly for all knowledge of this karyotypic diversity. Recently, the use of cell cultures as an alternative to obtaining mitotic chromosomes opening up new opportunities to study groups that have not been explored or have not yet been cytogenetically investigated. In this work, we take a chronological overview of the advances of different cytogenetic techniques ("*in vivo*" and "*in vitro*" methods to obtain the chromosome, C-banding, the detection of nucleolar organizer regions (Ag-RON), fluorescent *in situ* hybridization (FISH) with several repetitive probes and paint chromosome) over the decades and how these techniques helped elucidate questions of the organization and function of the fish genome.

Keywords: chromosome, karyotype evolution, molecular cytogenetics, fibroblast cells

1. Introduction

The Neotropical region includes the area between the north of Mexico and the south of South America. This is the richest and more diversity freshwater fish fauna in the world with approximately 5160 freshwater fish species, distributed in 739 genera, 69 families and 20 orders, which

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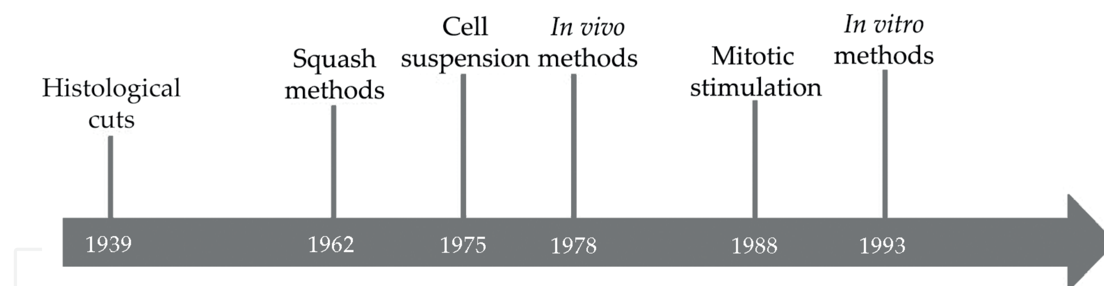

Figure 1. Timeline showing the major technical innovations that contribute to the development of fish cytogenetic.

represents one-third of all fishes on the planet [1]. A larger part of this diversity is grouped in Characiformes and Siluriformes, but there are still gaps in information in many groups [2].

All this diversity has been studied in different areas, including Cytogenetics. The refinement of cytogenetic techniques (**Figure 1**) provided the obtain of quality chromosome preparations that significantly increased the number of species studied and the description of their chromosomal characteristics, which contributed significantly to a better understanding of the genetic structures, evolution and systematic of the fishes [3–7].

2. Classical methods of cytogenetics to obtain metaphase chromosomes and their adaptations

Obtaining metaphase chromosomes is the most important point for cytogenetic studies, since any study to understand evolution and structure of the karyotypes of the species depends on this initial stage. It is known that many adjustments were made to improve the different techniques which it have arisen over the years in research within the fish cytogenetics.

The first studies with fish cytogenetics used fragments of testis previously fixed, included in paraffin and then submitted to cuts, like the experiments of Makino [8]. This methodology generated a certain doubt in its results, due to the uncertainty of the exact diploid number of each cell and was not employed with a significant number of species.

Subsequently, studies where the obtainment of chromosome depended on the squash technique were developed. In this case, a small fragment of tissue was directly crushed on a glass slide and fixed with acetic acid [9]. This technique often produced overlapping chromosomes, making it difficult to visualize the morphology and diploid number. Anyway, researches using this methodology continued and resulted in the creation of the "crushing machine" invented by Orlando Moreira Filho to minimize the injuries in the researcher's fingers [10].

The use of tissues to obtain metaphase chromosomes was not considered easily applied, because it was not easy to develop studies in the field [11]. Another relevant point is that it was not possible to regulate the rate of mitotic division and the condensation of the chromosomal arms. However, if it was known about the high hematopoietic activity of the anterior kidney in fishes [12] and from this organ, it was possible to obtain good metaphases, especially when subcutaneous or intraperitoneal stimulation of a mitogenic agent was performed [13, 14].

In 1971, Cole and Leavens [15] were the first to suggest the use of yeast as mitotic stimulating agents in hematopoietic tissues of reptiles and amphibians, but Lee and Elder [16] adapted this protocol for small mammals using a suspension of bread yeasts injected into the animal, observing that the chromosomes spread better and responded more effectively to banding treatments. For fishes, this methodology was adapted by Oliveira et al. [17], and it has been widely used over the years [18–20]. Other mitogenic agents were also employed in work with freshwater fish, such as phytohemagglutinin [21, 22], horse serum [23], parasitic infection as *Ichthyophthirius multifiliis*, or pharmaceutical agents [24, 25]. However, the use of enriched glucose solution of *Saccharomyces cerevisiae* (yeast activated suspension) is still the most used by its efficiency and low cost.

Since 1956, the works of Tjio, Levan, Ford, and Hamerton [26, 27] have reported about treating cytogenetic preparations with colchicine and hypotonic solutions, and the chromosomes have shown morphologically well-defined and that spread easily on the glass slide. Only in 1975, with the publication of a paper by McPhail and Jones, the chromosome preparations for cytogenetic studies in fish began to use this methodology [10].

The advances of chromosomal preparations in fishes have been boosted from the "air-drying" technique developed for mammals and later adapted for fish in "*in vivo*" [28] and *"in vitro"* [29] protocols. Both methods involved pre-treatment with colchicine. The use of this drug has enabled a direct control of chromosome condensation, which favored a more detailed study of the morphology of the chromosomes.

Another aspect that contributed to improving the quality of chromosome preparations was hypotonization process. Substances such as sodium citrate, distilled water and potassium chloride are used in the hypotonic treatment of the material; however, the potassium chloride is the most used in fish. In addition, the incubation temperature and hypotonization time should be adjusted according to the organism (e.g., in freshwater fish is common the hypotonization time of 21 min, while for marine fish is used from 30–35 min). After hypotonization, the cells are fixed in Carnoy's solution [21] and the cell suspension obtained is dropped into a glass slide for the rupture of the nuclear envelope [11] and thereby spreading the chromosomes for visualization of the diploid number and morphology.

Alternative methodologies have been published to improve chromosomal preparations in fish. Such methodologies, as proposed by Netto et al. [30], describe new proposed methodologies based on previously published protocols that allow cytogenetic analysis in individuals after death or that described by Blanco et al. [31], who proposed a protocol to be conducting in the field, where it eliminates the need for transportation of the specimens to the laboratory, but it is still not as common as the methodologies of Bertollo et al. [28] and Foresti et al. [29].

3. Chromosomal banding techniques and their contributions to the understanding of karyotypic macrostructure in several fish groups

Major breakthroughs in cytogenetic fish were possible with the development of differential staining techniques in the early 1970s that made it possible to understand the evolutionary

relationships in many fish groups. These methods allowed a better characterization of the chromosomal structure of the fish with appearance of markings along the chromosomes that before these techniques were based only in description of the number and chromosomal morphology. The main techniques used for chromosomal characterization in fish include the C and G banding techniques (not so usual due to compartmentalization of genomics) and silver nitrate staining.

The C-banding technique described by Summer [32] shows the patterns of the constitutive heterochromatin, and it has been widely used in cytogenetic studies of fish for characterization of similar karyotypes, especially to identify variations among species or populations of the same species [19, 33–36]. It was applied for first time in salmonid species [37, 38]. In fishes from the Neotropical region, the first studies were conducted in *Prochilodus* [39], *Eigenmannia* [40], and *Leporinus* [41], and since then, several studies have reported C bands in different fish species.

Most of this heterochromatin has been reported in centromeric and terminal regions of the chromosomes of most Neotropical fish species [34, 36, 42], while in some Loricariidae species it is possible to observe many heterochromatin blocks in the interstitial region [35], which appear to be a common feature for this group. In some species, heterochromatin can be more abundant [35, 43], whereas in other species these heterochromatin blocks are reduced [42]. Other studies have emphasized the importance of heterochromatin as a major source of karyotype diversification within and among some fish groups (e.g. 19). In some groups, it is possible to observe trends in relation to the behavior of heterochromatin, for example in Hypostominae, in which there is a relationship between the amount of heterochromatin and chromosome number of the species of this subfamily [44].

Not only did the C-banding technique provided a better characterization of the karyotypes but also the use of the silver nitrate staining technique that identifies the nucleolar organizing regions (NORs) became routine since the 1980s [45]. The NORs are chromosomal regions where the ribosomal RNA genes (45S = 18S + 5.8S + 28S) are located [46]. The first works using the technique in Neotropical fishes were in the species of Gymnotiformes [40, 47].

In general, two distribution patterns of NORs can be observed in fish, the first being the occurrence of only a single chromosome pair with NORs [33, 48, 49], while in other groups of fish more sites with NORs distributed in different chromosomes of the karyotype [36, 50, 51]. In fact, a single pair of NOR has been arbitrarily considered a plesiomorphic condition in fish [52]. Although this technique has been widely used, for the cost and ease, only 1.3% of the fish species had their NORs distribution investigated [46]. In some fish groups, it has been considered an excellent cytotaxonomic marker, as in *Apareidon* and *Paradon* of the Parondontidae family [53, 54]. In addition, polymorphism NORs have been evidenced with variation and size differences between homologous NORs [47].

4. How fluorescent *in situ* hybridization (FISH) and its variations have helped in the understanding of the evolution and organization of the fish genome?

The technique of fluorescence *in situ* hybridization (FISH) made it possible to physically map specific nucleotide sequences in the chromosomes of the species or group in study [55]. It was

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first used by Buongiorno-Nardeli and Amaldi [56] in histological cuts and by Gall and Pardue [57] in chromosomes, but the adjustments to the protocols used to this day for fish studies are basically small changes from the original protocol proposed by Pinkel et al. [58]. This technique provided better results to investigate how chromosome diversity and organization of genomic segments occurred in fish chromosomes [59].

For example, cichlids are an interesting group of fish to be studied to explore different ecological niches and to report varied life strategies, morphology and behavior [60, 61], besides species important for fishing and aquarism [62]. Thus, many studies have search to understand more about the karyotypic macrostructure of this group of fish [66, 67], and the physical mapping of repetitive sequences has showing that such portions of the DNA may be involved in several chromosomal rearrangements in Cichlinae [63–67].

4.1. Ribosomal genes

In the genome of the eukaryotes, ribosomal genes are organized into two multigenic families, the 45S rDNA responsible for encoding the 18S, 5.8S and 26S/28S rRNAs and the other 5S rDNA, which encodes the 5S rRNA [59]. They are repeating sequences in *tandem*, and these genes are easily identified by FISH [68]. Several studies have searched to understand a little of the evolutionary dynamics of these repetitive sequences in the fish genome [5, 64, 67].

The 5S rRNA gene has been described in many fish groups, and it is located mainly in the interstitial region of the chromosome [59, 69–72], which may not only be a coincidence, but rather that this ribosomal minor distribution brings some advantage to the carrier genome [73]. It is known that the 5S rRNA is composed of a conserved region of 120 base pairs, separated from each other by the NTSs (not transcribed portions, which may vary in size or sequence). These variations have become important markers for specific species or specific populations.

Some studies with physical mapping of 5S rRNA in Anostomidae species have shown that the sites marked by the smaller ribosomal have been conserved during the karyotype evolution of the fish of this family [59, 70, 71, 74]. In *Brycon*, the physical mapping of 5S rRNA sequences was considered an important cytogenetic marker in the evolution of this group [75]. There is a variation in the number of chromosomes marked with the 5S rRNA in the genus *Astyanax*, with species with 1 pair [76, 77], species with 2 pairs [35, 76], until populations with 4 pairs, as in *A. scabripinnis* [78], and the distribution of these repetitive clusters seems to have been conserved in the group [76, 79, 80]. *Characidium* also have differences in relation to the number and location of the 5S rRNA clusters [81–83], and these variations are probably a reflection of the allopatric speciation occurred in populations of this genus.

In some fish species, more than one class of 5S rRNA gene has been identified, as reported in *Leporinus* [59]. This variation was due to differences in the sequences of portions not transcribed, and also it was reported in *Oreochromis niloticus* [84]. These sequences were found in pseudogenes and the 5S rRNA gene inverted; but in both works, the technique of FISH was contributed to identify the chromosomal location of the two classes of 5S rRNA. In the species, *Gymnotus sylvius* and *G. inaequilabiatus* were also detected two smaller classes, and with FISH, it was possible to observe that the two clusters of rRNA 5S are co-located in a chromosome pair, while the second class showed too marked in distinct chromosomes [85].

Many species of fish have the 18S rRNA gene co-located with the 5S rRNA gene [76, 86–89]; however, from the functional point of view, it would be more advantageous for two ribosomal classes to be on separate chromosomes since the transcription of them is made by distinct RNA polymerases, and the non-synteny is a way of ensuring that the 5S rRNA is not translocated to the rRNA 45S [70, 71], and allows the independent evolution of these genes [71].

Almeida-Toledo et al. [76] found that the genes 5S and 18S rRNA are co-located in five species of *Astyanax*, and such sequence was considered important markers for studying the evolutionary history of the group, including *A. altiparanae* and *A. lacustris*. This fact can be a sign of the recent separation of species, which previously belonged to a taxonomic unit of *A. bimaculatus* [90].

In the family Loricariidae, the FISH showed that most species have ribosomal sites in distinct chromosomes [91–95]. However, in the subfamily Neoplecostominae and Hypoptopomatinae [95], Hypostominae [92] and Loricariinae [91], these genes are in synteny condition, which is considered a primitive condition for the family, since it was found in the outgroup Trichomycteridae [19, 95]. According to Oliveira [19], the co-localization of 5S rDNA and 18S sites in Trichomycterus species is considered a plesiomorphic condition of the group, however the smaller ribosomal is more variable, since more labeled chromosomal pairs were observed, whereas the larger ribosomal was kept in only a couple, which according to the authors are homeologous.

Investigations using the genes rRNA 5S and 18S rRNA by Scacchetti et al. [83] showed that these genes are present in the sex chromosomes of some species of *Characidium*, indicating that the ribosomal can also participate in the differentiation process by chromosomes linked to sex in this group of fishes. In some fish, genome sequences of 18S rRNA 28S associated to heterochromatin have also been reported [69, 86], which seems to indicate that the constitutive heterochromatin may be involved in both the structural maintenance of the nucleolus and integrity of repetitions of ribosomal DNA [96].

4.2. Histones

The histone genes are composed of a genetic complex of a multigenic family (H2A, H2B, H3 and H4), which can vary in number of copies and organization genome [97]. In addition, they may be configured by H1 histone or spread throughout the genome [98]. In fishes, there are still a few studies that investigated the location and organization of these sequences, but in some of these studies histones are associated with ribosomal genes [85, 98, 99], and the genes H1, H3 and H4 are grouped in species of *Astyanax* [100, 101], as well as in the case of *Synbranchus*, where H3 and H4 are associated and spread throughout the genomes, likely to transposable elements [102]. This conformation was also observed in *Orestias ascotanensis* [103], where these sequences are organized into small copies. In *Characidium alipioi* [104], the H3 and H4 genes were mapped in a single chromosomal pair, which seems to be a conservative characteristic of the group [105].

4.3. snRNA

SnRNA genes are characterized in five RNA types (U1, U2, U4, U5, U6), non-coding, that are part of a large RNA-protein complex known as spliceosome machinery [106, 107]. The U2

gene is highly conserved in the genome of eukaryotes; however, the number of sites of these sequences may be different among species. This is because multigenic families may adopt different conservation strategies for their sequences [108].

Merlo et al. [109] and Úbeda-Manzanaro et al. [110] investigated the location of rRNA sequences U2 in species of the families Batrachoididae and Moronidae, while Manchado et al. [111] described U1 sites linked to smaller ribosomal in the genome of *Solea senegalensis*. However, few studies have been performed to map these sequences in Neotropical fishes. Study conducted by Cabral-de-Melo et al. [112] showed that the U1 snRNA gene in cichlids is found in just one chromosome pair, probably being a conserved feature in this group since the fragmentation of Gondwana [113]. On the other hand, the technique of FISH showed that the position of the snRNA U1 clusters can vary between distant species, and this is due to chromosomal rearrangements such as inversions and transpositions that modify and restructure the karyotypes of cichlids. The snRNA U1 sites were more variable between South American Cichlids than among the African species [112].

In *Gymnotus*, physical mapping of U2 snRNA sequences showed differences in the distribution of this gene, which can be clustered in homologous chromosomes as in most species or spread in several sites as in *G. pantanal*, an apomorphic condition [102]. In addition, the technique of FISH showed the U2 snRNA marked in a chromosome linked to sex in the species *G. pantanal* [102]. In other Neotropical fishes, these two configurations of the location of U2 snRNA gene can be found [83, 102, 103, 113, 114].

4.4. Telomeric probes

The telomere portions of the chromosomes are composed of repetitive sequences in tandem, which in vertebrates have been reported by sequence (TTAGGG)n [115]. In fish, these sites have already been mapped occupying regions of the telomeres [116, 117] and non-telomeric chromosome portions [118]. These interstitial marks contribute to studies about organization and macrostructural evolution of karyotypes, since they may answer some questions as fusion or inversions that modify the chromosomal structure of some species [117, 119]. Sometimes, these interstitial sites are the result of fusions but are not easily mapped because the karyotype in study may evolve and the telomeric sequences lose its function [55]. Another relevant point investigated in fish with a FISH technique using telomeric sequences is associated with satellite DNA [120, 121], which would be a response to the spreading of these regions in the interstitial regions. Scacchetti et al. [121] made it through the physical mapping of telomeric sequences in *Characidium* species, find interstitial markings in the chromosomes of some populations and, from there, carried out analyses that allowed establishing monophyletic group conditions. In Cioffi and Bertollo [122], telomeric interstitial markings were also observed in the neo-Y genome chromosome *Hoplias malabaricus*, which contributed to answer questions about the origins of the sexual system in this group of fishes.

4.5. Satellite DNA

Satellite DNA is composed of repetitive sequences that tend to accumulate in the chromosomes, especially in heterochromatic regions [123]. They are not protein coding and can form clusters on

the chromosome arms [123], which facilitate their physical mapping in the karyotype of interest. In the 1980s, satellite DNA families were first described in fish, and many works showed that they accumulate in the centromeric portion of the chromosomes and they may be related to the structural and functional roles of the centromere [124–126]. Some events such as unequal crossing over, transpositions and duplications may contribute to repetitive sequences including satellite DNA accumulating in heterochromatic regions, where they undergo less selective pressures and may thus evolve in the genome [127]. Some studies have used different satellite DNA probes to investigate the composition of supernumerary chromosomes in some species of fish [128–131].

4.6. Sex chromosome

Several studies attempted to understand the origin, evolution and maintenance of the sex-linked chromosomes [103], and fishes have become excellent models of studies because they have a wide and varied sexual system [122]. The sex chromosomes have been described in more than 7% of the fish karyotypes [132], and with the FISH technique, many satellite DNA sequences have been isolated and mapped in different species [133–137]. In some species of fishes, FISH technique has contributed to map sequences that characterize sex chromosomes undifferentiated by morphology or conventional staining, as is the case with guppy, within the family Poeciliidae [133, 138, 139]. And in other cases, the mappings of satellites sequences were important in work with morphologically differentiated sex chromosomes [135, 140]. Chromosome painting using W-chromosome-specific probe helped to answer about the common origin of this chromosome linked to sex in *Characidium* species [83, 141].

4.7. B chromosome

Many studies search to understand more about the origin, function and evolution of B chromosomes in fishes, since these are considered expendable parasites to supernumerary genome [142]. With the technique of FISH and advances in chromosomal painting, studies using themselves as probes it was possible to examine if there is homology of these extra chromosomes with the normal chromosomes of the karyotype, and from this understand possible answers about the origin and evolution of these chromosomes [51, 104, 130, 143, 144].

4.8. Fiber-FISH

The Fiber-FISH technique contributed greatly to the investigation of specific sites in the genome of Neotropical fish, since it allowed to determine the position of the genes in the chromatin fiber and to verify the organization of the gene sequences [145].

5. Culture of cells in fish: alternative tools for obtaining metaphase chromosomes

Cell culture is an *in vitro* technique widely used to isolate and maintain cells outside their original environment [146]. Briefly, a tissue fragment is aseptically removed from the individual and then mechanically and enzymatically dissociated or both. The isolated cells are

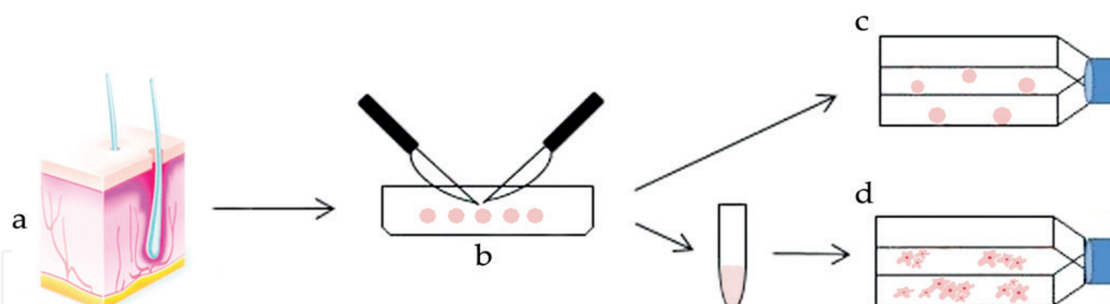

Figure 2. Scheme of obtaining cell culture. (a) Tissue; (b) disaggregation of the tissue mechanically; (c) tissue fragments (explant) grown in flasks with medium culture and (d) cells cultured in flasks after enzymatic disaggregation.

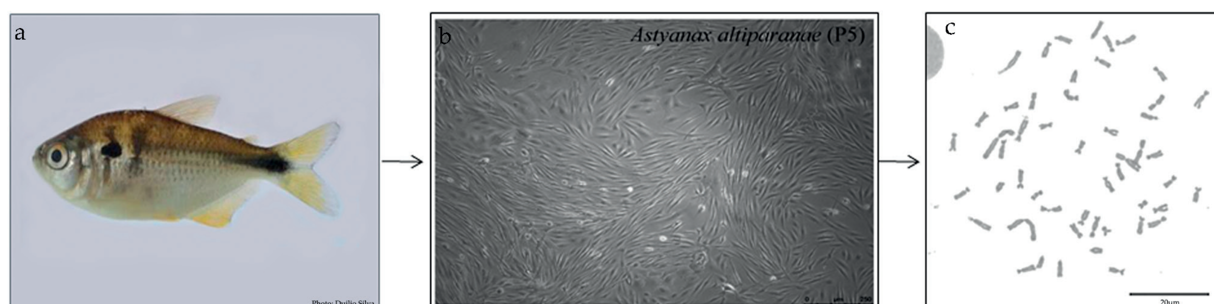

Figure 3. (a) *Astyanax altiparanae* (Characidae); (b) fibroblast cell line of *A. altiparanae* in the fifth passages and (c) mitotic chromosome of *A. altiparanae* obtain a cell line with diploid number of 2n = 50 chromosomes.

cultured in flasks with suitable medium with adjusted pH, antibiotic/antimycotic agents and fetal bovine serum (**Figure 2**). Cell culture is maintained at the appropriate temperature to the species under study and monitored daily for cell growth and possible contamination. When these cells cover the entire bottom of the flask (cell confluence), these cells are trypsinized and cultured in new vials (a process known as subculture or passage). These cells are treated with colchicine and after detached from the bottom of the flask are hypotonized, fixed with Carnoy's solution and then dropped onto slides (**Figure 3**) [147, 148].

Cell culture is still little used as a tool in fish cytogenetic studies [149–151], mainly by the difficulty of standardization of the technique of isolation and maintenance of cell cultures. Nevertheless, this technology is an excellent alternative to obtain good quality chromosome preparations, since it can be applied in cytogenetic studies of small and large species, in which it is difficult to work with direct methods of chromosome preparation or also in species used in aquaculture or endangered, when there is no possibility of sacrifice of animals [149]. Another advantage is that the methodology can provide the establishment of cell bank available at any time, so, in case of repetition of cytogenetic methodologies, it is not necessary to go back to the field for new individuals.

6. Conclusion

The advances of cytogenetic techniques have contributed directly in studies that search to investigate and understand the macro and micro karyotype structure of the most diverse

groups of Neotropical fish, and many questions have been answered with the use of these technologies, as well as new problems have arisen that it was not possible to investigate because of the difficulties of the techniques. It is known that there are still many gaps to be filled, but cytogenetics has grown a lot in recent years and morphological and /or phytogenetic tools have played an important role in cytogenetic advances.

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References

[1] Reis RE, Albert JS, Dario FD, Mincarone MM, Petry P, Rocha LA. Fish biodiversity and conservation in South America. Journal of Fish Biology. 2016;**89**:1-39. DOI: 10.1111/jfb.13016

[2] Albert JS, Reis RE. Historical Biogeography of Neotropical Freshwater Fishes. Berkeley: University of California Press; 2011

[3] Porto-Foresti F, Hashimoto DT, Alves AL, Almeida RBC, et al. Cytogenetic markers as diagnoses in the identification of the hybrid between piauçu (*Leporinus macrocephalus*) and piapara (*Leporinus elongatus*). Genetics and Molecular Biology. 2008;**31**(1):195-202

[4] Hashimoto DT, Laudicina A, Bortolozzi J, Foresti F, Porto-Foresti F. Chromosomal features of nucleolar dominance in hybrids between the Neotropical fish *Leporinus macrocephalus* and *Leporinus elongatus* (Characiformes, Anostomidae). Genetica. 2009;**137**:135-140. DOI: 10.1007/s10709-009-9366-y

[5] Vicari MR, Nogaroto V, Noleto RB, Cestari MM, Cioffi MB, et al. Satellite DNA and chromosomes in Neotropical fishes: Methods, applications and perspectives. Journal of Fish Biology. 2010;**76**:1094-1116. DOI: 10.1111/j.1095-8649.2010.02564.x

[6] Tenório RCCO, Vitorino CA, Souza IL, Oliveira C, Venere PC. Comparative cytogenetics in *Astyanax* (Characiformes: Characidae) with focus on the cytotaxonomy of the group. Neotropical Ichthyology. 2013;**11**(3):553-564

[7] Ramirez JL, Birindelli JLO, Galetti PM. A new genus of Anostomidae (Ostariophysi: Characiformes): Diversity, phylogeny and biogeography based on cytogenetic, molecular and morphological data. Molecular Phylogenetics and Evolution. 2017;**107**:308-323. DOI: 10.1016/j.ympev.2016.11.012

[8] Makino S. The chromosomes of the Carp, *Cyprinus caypio*, including those of some related species of Cyprinidae for comparison. Cytologia. 1939;**9**:430-440

[9] Bstergren G, Heneen WK. A Squash Technique for Chromosome Morphological Studies. Sweden: Institute of Genetics, University of Lund; 1962. pp. 332-341

[10] Foresti F. A brief history of Fish genetics in Brazil. Genetics and Molecular Biology. 2008;**31**(1 (suppl)):1-4

[11] Sessionss K. Chromosomes: Molecular cytogenetics. In: Hillis DM, Moritz C, editors. Molecular Systematics. Sunderland, Massachusetts: Sinauer Associates; 1990. pp. 156-203

[12] Catton WT. Blood cell formation in certain teleost fishes. Blood. 1951;**6**:39-60

[13] Ojima Y, Kurishita A. A new method to increase the number of mitotic cells in the kidney tissue for fish chromosome studies. Proceedings of the Japan Academy. 1967;**56** (10):610-615

[14] Ozouf-Costaz C, Foresti F. Fish cytogenetic research: Advances, applications and perspectives. Netherlands Journal of Zoology. 1992;**42**(2-3):277-290

[15] Cole CI, Leavens CR. Chromosome preparations of amphibians and reptiles: Improved techniques. Herpet. 1971;**3**(6):102

[16] Lee MR, Elder FFB. Yeast stimulation of bone marrow mitosis for cytogenetic investigations. Cytogenetics and Cell Genetics. 1980;**26**:36-40

[17] Oliveira C, Almeida-Toledo LF, Foresti F, Toledo Filho SA. Supernumerary chromosomes, Robertsonian rearrangement and multiple NORs in *Corydoras aeneus* (Pisces, Siluriformes, Callichthyidae). Caryologia. 1988;**41**(36):227-236

[18] Nirchio M, Rondón R, Oliveira C, et al. Cytogenetic studies in three species of *Lutjanus* (Perciformes: Lutjanidae: Lutjaninae) from the Isla Margarita, Venezuela. Neotropical Ichthyology. 2008;**6**(1):101-108

[19] Oliveira MLM, Utsunomia R, Pansonato-Alves JC, et al. Microstructural chromosome reorganization in the genus *Trichomycterus* (Siluriformes: Trichomycteridae). Neotropical Ichthyology. 2016;**14**(2):e150084. DOI: 10.1590/1982-0224-20150084

[20] Paim FG, Almeida LAH, Affonso PRAM, Sobrinho-Scudeler PE, et al. Chromosomal stasis in distinct families of marine Percomorpharia from South Atlantic. Comparative Cytogenetics. 2017;**11**(2):299-307. DOI: 10.3897/CompCytogen.11(2).11942

[21] Gold JR, Li YC, Shipey NS, Powers PK. Improved methods for working with fish chromosomes with a review of metaphase chromosome banding. Journal of Fish Biology. 1990;**37**:563-575

[22] Ren X, Qixing Y, Ping W. Polymorphisms of silver stained NORs in rice-fish eels. Acta Genetica Sinica. 1991;**18**:304-311

[23] Ojima Y, Kurishita A. A new method to increase the number of mitotic cells in the kidney for fish chromosome study. Proceedings of the Japan Academy, Series B. 1980;**56**:610-615

[24] Molina WF. An alternative method for mitotic stimulation in fish cytogenetics. Chromosome Science. 2001;**5**:149-152

[25] Molina WF, Alves DEO, Araújo WC, Martinez PA, et al. Performance of human immunostimulating agents in the improvement of fish cytogenetic preparations. Genetics and Molecular Research. 2010;**9**(3):1807-1814

[26] Tjio JH, Levan A. The Chromosome Number of Man. Lund: Institute of Genetics; 1956. pp. 1-6

[27] Ford CE, Hamerton JL. A colchicine, hypotonic citrate squash sequence for mammalian chromosomes. Stain Technology. 1956;**31**:247-251

[28] Bertollo LAC, Takahashi CS, Moreira-Filho O. Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). Brazilian Journal of Genetics. 1978;**2**:103-120

[29] Foresti F, Oliveira C, Almeida-Almeida-Toledo LF. A method for chromosome preparations from large fish specimens using in vitro short-term treatment with colchicine. Experientia. 1993;**49**:810-813

[30] Netto MRCB, Pauls E, Affonso PRAMA. Standard protocol for obtaining fish chromosomes under post-mortem conditions. Micron. 2007;**38**:214-217. DOI: 10.1016/j.micron.2006.07.019

[31] Blanco DR, Vicari MR, Lui RL, Artoni RF, Almeida MC, et al. Origin of the X1X1X2X2/X1X2Y sex chromosome system of *Harttia punctata* (Siluriformes, Loricariidae) inferred from chromosome painting and FISH with ribosomal DNA markers. Genetica. 2014;**142**:119-126. DOI: 10.1007/s10709-014-9759-4

[32] Sumner AT. A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research. 1972;**75**:304-306. DOI: 10.1016/0014-4827(72)90558-7

[33] Galetti PM Jr, Cesar ACG, Venere PC. Heterochromatin and NORs variability in *Leporinus* fish (Anostomidae, Characiformes). Caryologia. 1991;**44**(3-4):287-292. DOI: 10.1080/00087114.1991.10797193

[34] Balen RE, Noleto RB, Vicari MR, Artoni RF, Cestari MM. Comparative cytogenetics among populations of *Hollandichthys multifasciatus* (Teleostei: Characidae). Zoological Science. 2013;**30**(2):105-109. DOI: 10.2108/zsj.30.105

[35] Kavalco KF, Pazza R, Almeida-Toledo LF. *Astyanax bockmanni* Vari and Castro, 2007: An ambiguous karyotype in the Astyanax genus. Genetica. 2009;**136**:135-139

[36] Kamei MCSL, Baumgärtner L, Paiva S, Zawadzki CH, et al. Chromosomal diversity of three species of *Hypostomus* Lacépède, 1803 (Siluriformes, Loricariidae), from the Paraná River Basin, Brazil: A species complex in *Hypostomus ancistroides* reinforced by a ZZ/ZW sex chromosome system. Zebrafish. 2017;**14**:4. DOI: 10.1089/zeb.2017.1429

[37] Abe S, Muramoto J. Differential staining of chromosomes of two Salmonoid species, *Salvelinus leucomaenis* (Pallas) and *Salvelinus malma* (Walbaum). Proceedings of the Japan Academy. 1974;**50**:507-511

[38] Zenzes MT, Voiculescu O. C-banding of patterns in *Salmo trutta* a species of tetraploid origin. Genetica. 1975;**45**:531-536

[39] Pauls E, Bertollo LAC. Evidence for a system of supernumerary chromosomes in *Prochilodus scrofa* Steindachner, 1881 (Pisces, Prochilodontidae). Caryologia. 1983;**36**(4): 307-314. DOI: 10.1080/00087114.1983.10797671

[40] Almeida-Toledo LF, Foresti H, Toledo Filho SA. Complex sex chromosome system in *Eigenmannia* sp. (Pisces, Gymnotiformes). Genetica. 1984;**64**:165-169

[41] Galetti PM, Foresti F. Evolution of the ZZ/ZW system in *Leporinus* (Pisces, Anostomidae). Cytogenetics and Cell Genetics. 1986;**43**:43-46

[42] Artoni RF, Bertollo LAC. Evolutionary aspects of the ZZ/ZW sex chromosome system in the Characidae fish, genus *Triportheus*. A monophyletic state and NOR location on the W chromosome. Heredity. 2002;**89**:15-19. DOI: 10.1038/sj.hdy.6800081

[43] Galetti Jr PM, Mestriner CA, Venere PC, Foresti F. Heterochromatin and karyotypic reorganization in fish of family Anostomidae (Characiformes). Cytogenetics and Cell Genetics. 1991;**56**:116-121

[44] Artoni RF, Bertollo LAC. Trends in the karyotype evolution of Loricariidae fish (Siluriformes). Hereditas. 2001;**134**:201-210

[45] Howell WM, Black DA. Controlled silver staining of nucleolus organizer region with protective colloidal developer: A 1-step method. Experientia. 1980;**36**:1014-1015. DOI: 10.1007/BF01953855

[46] Gornung E. Twenty years of physical mapping of major ribosomal RNA genes across the teleosts: A review of research. Cytogenetic and Genome Research. 2013;**141**:90-102. DOI: 10.1159/000354832

[47] Foresti F, Almeida Almeida-Toledo LF, Almeida-Toledo FSA. Polymorphic nature of nucleolus organizer regions in fishes. Cytogenetics and Cell Genetics. 1981;**31**:137-144. DOI: 10.1159/000131639

[48] Oliveira C, Almeida-toledo LF, Almeida-Toledo FSA. Comparative cytogenetic analysis of three cytotypes of *Corydoras nattereri* (Pisces, Siluriformes, Callichthyidae). Cytologia. 1990;**55**:21-26

[49] Martinez ERM, Alves AL, Silveira SM, Foresti F, Oliveira C. Cytogenetic analysis in the incertae sedis species *Astyanax altiparanae* Garutti and Britzki, 2000 and *Hyphessobrycon eques* Steindachner, 1882 (Characiformes, Characidae) from the upper Paraná river basin. Comparative Cytogenetics. 2012;**6**(1):41-51. DOI: 10.3897/CompCytogen. v6i1.1873

[50] Born GG, Bertollo LAC. A new sympatric region for distinct karyotypic forms of *Hoplias malabaricus* (Pisces, Erythrinidae). Brazilian Journal of Biology. 2006;**66**(1B):205-210

[51] Silva DMZ, Pansonato-Alves JC, Utsunomia R, Araya-Jaime C, Ruiz-Ruano FJ, et al. Delimiting the origin of a B chromosome by FISH mapping, chromosome painting and

DNA sequence analysis in *Astyanax paranae* (Teleostei, Characiformes). PLoS One. 2014; **9**(4):e94896. DOI: 10.1371/journal.pone.0094896

[52] Amemiya CT, Gold JR. Chromosomal NORs as taxonomic and systematic characters in North American cyprinid fishes. Genetica. 1998;**76**:81-90

[53] Bellafronte E, Margarido VP, Moreira-Filho O. Cytotaxonomy of *Parodon nasus* and *Parodon tortuosus* (Pisces, Characiformes). A case of synonymy confirmed by cytogenetic analyses. Genetics and Molecular Biology. 2005;**28**(4):710-716

[54] Jesus CM, Moreira-Filho O. Karyotypes of three species of *Parodon* (Teleostei, Parodontidae). Ichthyological Exploration of Freshwaters. 2000;**11**:75-80

[55] Guerra M. FISH: Conceitos e aplicações na citogenética. Ribeirão Preto, SP, BR: Sociedade Brasileira de Genética; 2004. 184 pp

[56] Buongiorno-Nardelli M, Amaldi F. Autoradiographic detection of molecular hybrids between rRNA and DNA in tissue sections. Nature. 1969;**225**:946-947

[57] Gall J, Pardue ML. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proceedings of the National Academy of Sciences. 1969;**63**:378-383

[58] Pinkel D, Straume T, Gray JW. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proceedings of the National Academy of Sciences. 1986;**83**:2934-2938. DOI: 10.1073/pnas.83.9.2934

[59] Martins C, Galetti PM Jr. Organization of 5S rDNA in *Leporinus* fish species: Two different genomic locations are characterized by distinct nontranscribed spacers. Genome. 2001;**44**:903-910

[60] Lowe-McConnell RH. Ecology of cichlids in South American and African waters, excluding the African Great Lakes. In: Keenleyside MHA, editor. Cichlid Fishes: Behavior, Ecology and Evolution. London: Chapman and Hall; 1991. pp. 60-85

[61] López-Fernández H, Honeycutt RL, Winemiller KO. Molecular phylogeny and evidence for an adaptive radiation of geophagine cichlids from South America (Perciformes: Labroidei). Molecular Phylogenetics and Evolution. 2005;**34**:227-244. DOI: 10.1016/j.ympev.2004.09.004

[62] Schnider CH, Gross MC, Terencio ML, Artoni RF, et al. Chromosomal evolution of neotropical cichlids: The role of repetitive DNA sequences in the organization and structure of karyotype. Reviews in Fish Biology and Fisheries. 2013;**23**:201-214. DOI: 10.1007/s11160-012-9285-3

[63] Gross MC, Schneider CH, Valente GT, et al. Comparative cytogenetic analysis of the genus *Symphysodon* (Discus Fishes, Cichlidae): Chromosomal characteristics of Retrotransposons and minor ribosomal DNA. Cytogenetic and Genome Research. 2009;**127**:43-53. DOI: 10.1159/000279443

[64] Gross MC, Schneider CH, Valente GT, et al. Variability of 18S rDNA locus among Symphysodon fishes: Chromosomal rearrangements. Journal of Fish Biology. 2010;**76**: 1117-1127. DOI: 10.1111/j.1095-8649.2010.02550.x

[65] Teixeira WG, Ferreira IA, Cabral-de-Mello DC, et al. Organization of repeated DNA elements in the genome of the cichlid fish Cichla kelberi and its contributions to the knowledge of fish genomes. Cytogenetic and Genome Research. 2009;**125**:224-234. DOI: 10.1159/000230006

[66] Mazzuchelli J, Martins C. Genomic organization of repetitive DNAs in the cichlid fish *Astronotus ocellatus*. Genetica. 2009;**136**:461-469. DOI: 10.1007/s10709-008-9346-7

[67] Valente GT, Mazzuchelli J, Ferreira IA, Poletto AB, et al. Cytogenetic mapping of the retroelements Rex1, Rex3 and Rex6 among Cichlid fish: New insights on the chromosomal distribution of transposable elements. Cytogenetic and Genome Research. 2011;**133**:34-42. DOI: 10.1159/000322888

[68] Rebordinos L, Cross I, Merlo A. High evolutionary dynamism in 5S rDNA of fish: State of the art. Cytogenetic and Genome Research. 2013;**141**:103-113

[69] Fujiwara A, Abe S, Yamaha E, Yamazaki F, Yoshida MC. Chromosomal localization and heterochromatin association of ribosomal RNA genes loci and silver stained nucleolar organizer regions in salmonid fishes. Chromosome Research. 1998;**6**:463-471

[70] Martins C, Galetti PM Jr. Chromosomal localization of 5S rDNA genes in *Leporinus* fish (Anostomidae, Characiformes). Chromosome Research. 1999;**7**:363-367

[71] Martins C, Galetti PM Jr. Conservative distribution of 5S rDNA loci in *Schizodon* (Pisces, Anostomidae) chromosomes. Chromosome Research. 2000;**8**:353-355

[72] Born GG, Bertollo LAC. An XX/XY sex chromosome system in a fish species, *Hoplias malabaricus* with a polymorphic NOR bearing X chromosome. Chromosome Research. 2000;**8**:111-118

[73] Martins C, Wasko AP. Organization and evolution of 5S ribosomal DNA in the fish genome. In: Williams CR, editor. Focus on Genome Research. Hauppauge: Nova Science Publishers; 2004. pp. 335-363

[74] Aguilar CT. Estudos citogenéticos e moleculares em populações brasileiras de Leporellus vittatus (Characiformes, Anostomidae). Rio de Janeiro: Tese (Doutorado)—Universidade Federal do Rio de Janeiro; 2001

[75] Wasko AP, Martins C, Wright JM, Galetti PM Jr. Molecular organization of 5S rDNA in fishes of the genus *Brycon*. Genome. 2001;**44**:893-902

[76] Almeida-Toledo LF, Ozouf-Costaz C, Foresti F, Bonillo C, et al. Conservation of the 5S bearing chromosome pair and co-localization with major rDNA clusters in five species of *Astyanax* (Pisces, Characidae). Cytogenetic and Genome Research. 2002;**97**:229-233

[77] Vicari MR, Artoni RF, Moreira-Filho O, Bertollo LAC. Diversification of a ZZ/ZW sex chromosome system in Characidium fish (Crenuchidae, Characiformes). Genetica. 2008;**134**:311-317. DOI: 10.1007/s10709-007-9238-2

[78] Ferro DAM, Neo DM, Moreira-Filho O, Brtollo LA. Nucleolar organizing regions, 18S and 5S rDNA in *Astyanax scabripinnis* (Pisces, Characidae): Populations distribution and functional diversity. Genetica. 2001;**110**:55-62

[79] Mantovani M. Citogenetica comparativa entre populações de Astyanax scabripinnis (Pisces, Characidae) da bacia do rio Paranapanema [Dissertação (Mestrado)]. Universidade Federal de São Carlos; 2001

[80] Hashimoto DT, Ferguson-Smith MA, Rens W, Foresti F, Porto-Foresti F. Chromosome mapping of H1 histone and 5S rRNA gene clusters in three species of *Astyanax* (Teleostei, Characiformes). Cytogenetic and Genome Research. 2011;**134**:64-71. DOI: 10.1159/000323512

[81] Pansonato-Alves JC, Oliveira C, Foresti F. Karyotypic conservatism in samples of *Characidium* cf. *zebra* (Teleostei, Characiformes, Crenuchidae): Physical mapping of ribosomal genes and natural triploidy. Genetics and Molecular Biology. 2011;**34**:208-213. DOI: 10.1590/S1415-47572011005000005

[82] Pucci MB, Barbosa P, Nogaroto V, Almeida MC, Artoni RF, Pansonato-Alves JC, et al. Population differentiation and speciation in the genus *Characidium* (Characiformes: Crenuchidae): Effects of reproductive and chromosomal barriers. Biological Journal of the Linnean Society. 2014;**111**:541-553. DOI: 10.1111/bij.12218

[83] Scacchetti PC, Utsunomia R, Pansonato-Alves JC, Da Silva GJC, et al. Repetitive DNA sequences and evolution of ZZ/ZW sex chromosomes in *Characidium* (Teleostei: Characiformes). Plos One. 2015;**10**:e0137231. DOI: 10.1371/journal.pone.0137231

[84] Martins C, Wasko AP, Oliveira C, Porto-Foresti F, Parise-Maltempi PP, et al. Dynamics of 5S rDNA in the tila (*Oreochromis niloticus*) gnome: Repeat units, inverted sequences, pseudogenes and chromosome loci. Cytogenetic and Genome Research. 2002;**98**:78-85

[85] Scacchetti PC, Alves JCP, Utsunomia R, Claro FL, de Toledo LFA, et al. Molecular characterization and physical mapping of two classes of 5S rDNA in the genomes of *Gymnotus sylvius* and *G. inaequilabiatus* (Gymnotiformes, Gymnotidae). Cytogenetic and Genome Research. 2012;**136**:131-137. https://doi.org/10.1159/000335658

[86] Pendás AM, Móran P, Freije JP, Garcia-Vásquez E. Chromosomal location and nucleotide sequence of two tandem repeats of the Atlantic salmon 5S rDNA. Cytogenetics and Cell Genetics. 1994;**67**:31-36

[87] Hatanaka T, Galetti PM Jr. Mapping of the 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae). Genetica. 2004;**122**:239-244

[88] Móran P, Martínez JL, Garcia-Vásquez E, Pendás AM. Sex linkage of 5S rDNA in rainbow trout (*Oncorhynchus mykiss*). Cytogenetics and Cell Genetics. 1996;**75**:145-150

[89] Moraes-Neto A, Silva M, Matoso DA, Vicari MR, et al. Karyotype variability in neotropical catfishes of the family Pimelodidae (Teleostei: Siluriformes). Neotropical Ichthyology. 2011;**9**:97-105

[90] Garutti V, Britski HA. Descrição de uma espécie novade *Astyanax* (Teleostei: Characidae) da Bacia do Alto Rio Paraná e consideraço˜es sobre as demais espécies do gênero na bacia. Comunicações do Museu de Ciencias da PUCRS, Série Zoologia. 2000

[91] Kavalco KF, Pazza R, Bertollo LA, Moreira-Filho O. Gene mapping of 5S rDNA sites in eight fish species from the Paraiba do Sul river basin, Brazil. Cytogenetic and Genome Research. 2004;**106**:107-110

[92] Mariotto S, Centofante L, Vicari MR, Artoni RFA, et al. Chromosomal diversification in ribosomal DNA sites in *Ancistrus* Kner, 1854 (Loricariidae, Ancistrini) from three hydrographic basins of Mato Grosso, Brazil. Comparative Cytogenetics. 2011;**5**:289-300

[93] Mendes-Neto EO, Vicari MR, Artoni RF, Moreira-Filho O. Description of karyotype in *Hypostomus regani* (Ihering, 1905) (Teleostei, Loricariidae) from the Piumhi river in Brazil with comments on karyotype variation found in *Hypostomus*. Comparative Cytogenetics. 2011;**5**:133-142

[94] Rosa KO, Ziemniczak K, Barros AV, Nogaroto V, et al. Numeric and structural chromosome polymorphism in *Rineloricaria lima* (Siluriformes: Loricariidae): Fusion points carrying 5S rDNA or telomere sequence vestiges. Reviews in Fish Biology and Fisheries. 2011;**22**:739-749

[95] Ziemniczak K, Barros AV, Rosa KO, Nogaroto V, et al. Comparative cytogenetics of Loricariidae (Actinopterygii: Siluriformes): Emphasis in Neoplecostominae and Hypoptopomatinae. The Italian Journal of Zoology. 2012;**79**:1

[96] McStay B, Grummt I. The epigenetics of rRNA genes: From molecular to chromosome biology. Annual Review of Cell and Developmental Biology. 2008;**24**:131-157. DOI: 10.1146/annurev.cellbio.24.110707.175259

[97] Kedes LH. Histone genes and histone messengers. Annual Review of Biochemistry. 1979;**48**:837-870

[98] Childs G, Maxson R, Cohn RH, Kedes L. Orphons: Dispersed genetic elements derived from tandem repetitive genes of eukaryotes. Cell. 1981;**23**:651-663

[99] Lima-Filho P, Cioffi M, Bertollo L, Molina WF. Chromosomal and morphological divergences in Atlantic populations of the frillfin goby *Bathygobius soporator* (Gobiidae, Perciformes). Journal of Experimental Marine Biology and Ecology. 2012;**434-435**:63-70. DOI: 10.1016/j.jembe.2012.08.004

[100] Pansonato-Alves JC, Hilsdorf AWS, Utsunomia R, et al. Chromosomal mapping of repetitive DNA and cytochrome C oxidase I sequence analysis reveal differentiation among sympatric samples of *Astyanax fasciatus* (Characiformes, Characidae). Cytogenetic and Genome Research. 2013;**141**:133-142. DOI: 10.1159/000354885

[101] Silva DMZA, Pansonato-Alves JC, Utsunomia R, Daniel SN, et al. Chromosomal organization of repetitive DNA sequences in *Astyanax bockmanni* (Teleostei, Characiformes): Dispersive location, association and co-localization in the genome. Genetica. 2013;**141**: 329-336

[102] Utsunomia R, Pansonato-Alves JC, Scacchetti PC, Oliveira C, Foresti F. Scattered organization of the histone multigene family and transposable elements in *Synbranchus*. Genetics and Molecular Biology. 2014;**37**:30-36. DOI: 10.1590/S1415-47572014000100007

[103] Araya-Jaime C, Lam N, Pinto IV, Méndez MA, Iturra P. Chromosomal organization of four classes of repetitive DNA sequences in killifish *Orestias ascotanensis* Parenti, 1984 (Cyprinodontiformes, Cyprinodontidae). Comparative Cytogenetics. 2017;**11**(3):463-475. DOI: 10.3897/CompCytogen.v Iii3.11729

[104] Serrano EA, Utsunomia R, Scudeller PS, Oliveira C, Foresti F. Origin of B chromosomes in *Characidium alipioi* (Characiformes, Crenuchidae) and its relationships with supernumerary chromosomes in other *Characidium* species. Comparative Cytogenetics. 2017;**11**(1):81-95. DOI: 10.3897/CompCytogen.v11i1.10886

[105] Oliveira et al. Physical mapping of repeating sequences in the genome of *Characidium* and investigation of two classes of 5S rDNA in the species (in preparation)

[106] Bringmann P, Lührmann R. Purification of the individual snRNPs U1, U2, U5 and U4/U6 from HeLa cells and characterization of their protein constituents. EMBO Journal. 1986;**5**(13):3509-3516

[107] Valadkhan S. snRNAs as the catalysts of pre-mRNA splicing. Current Opinion in Chemical Biology. 2005;**9**:603-608

[108] Matera AG, Weiner AM, Schmid CW. Structure and evolution of the U2 small nuclear RNA multigene family in primates: Gene amplification under natural selection? Molecular and Cellular Biology. 1990;**10**:5876-5882

[109] Merlo MA, Cross I, Chairi H, Manchado M, Rebordinos L. Analysis of three multigene families as useful tolls in species characterization of two closely-related species, *Dicentrarchus labrax*, *Dicentrarchus punctatus* and their hybrids. Genes & Genetic Systems. 2010;**85**:341-349

[110] Úbeda-Manzanaro M, Merlo MA, Palazón JL, Cross I, Sarasquete C, Rebordinos L. Chromosomal mapping of the major and minor ribosomal genes, (GATA)n and U2 snRNA gene by double-colour FISH in species of the Batrachoididae family. Genetica. 2010;**138**:787-794

[111] Manchado M, Zuasti E, Cross I, Merlo A, Infante C, Rebordinos L. Molecular characterization and chromosomal mapping of the 5S rRNA gene in Solea senegalensis: A new linkage to the U1, U2, and U5 small nuclear RNA genes. Genome. 2006;**49**:79-86

[112] Cabral-de-Mello DC, Valente GT, Nakajima RT, Martins C. Genomic organization and comparative RNA gene in cichlid fish, with an emphasis in *Oreochromis niloticus*. Chromosome Research. 2012;**20**:279-292

[113] Turner GF. Adaptive radiation of cichlid fish. Current Biology. 2007;**17**:827-831

[114] Silva DMZA, Utsunomia R, Pansonato-Alves JC, Oliveira C, Foresti F. Chromosomal mapping of repetitive DNA sequences in five species of *Astyanax* (Characiformes, Characidae) reveals independent location of U1 and U2 snRNA sites and association of U1 snRNA and 5S rDNA. Cytogenetic and Genome Research. 2015;**146**(2):1-9. DOI: 10.1159/000438813

[115] Meyne J, Ratliff RL, Moyzis RK. Conservative of the human telomere sequences (TTAGGG)n among vertebrates. Proceedings of the National Academy of Sciences of the United States of America. 1989;**86**:7049-7053

[116] Meyne J, Baker RJ, Hobart HH, Hsu TC, Ryder OA, Ward OG, et al. Distribution of non-telomeric sites of the (TTAGGG)n telomeric sequence in vertebrate chromosomes. Chromosoma. 1990;**99**:3-10

[117] Chew JSK, Oliveira C, Wright JM, Dobson MJ. Molecular and cytogenetics analysis of the telomeric (TTAGG)n repetitive sequence in the Nile tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae). Chromosoma. 2002;**111**:45-52

[118] Reed KM, Phillips RB. Molecular cytogenetic analysis of the couble-CMA3 chromosome of lake trout, *Salvelinus namaycush*. Cytogenetics and Cell Genetics. 1995;**70**:104-107

[119] Albuín M, Martinez P, Sánchez L. Localization of the telomeric sequence (TTAGGG)n in four salmonid species. Genome. 1996;**39**:1035-1038

[120] Garrido-Ramos MA, Herran R, Ruiz Rejon C, Ruiz Rejon M. A satellite DNA of Sparidae family (Pisces, Perciformes) associated with telomeric sequences. Cytogenetics and Cell Genetics. 1998;**83**:3-9

[121] Scacchetti PC, Utsunomia R, Pansonato-Alves JC, et al. Extensive spreading of interstitial telomeric sites on the chromosomes of *Characidium* (Teleostei, Characiformes). Genetica. 2015;**143**:263-270. DOI: 10.1007/s10709-014-9812-3

[122] Cioffi MB, Bertollo LAC. Initial steps in XY chromosome differentiation in *Hoplias malabaricus* and the origin of an X 1 X 2 Y sex chromosome system in this fish group. Heredity. 2010;**105**:554-561

[123] Ugarkovic D, Plohl M. Variation in satellite DNA profiles—Causes and effects. The EMBO Journal. 2002;**21**:5955-5959

[124] Datta U, Dutta P, Manda K. Cloning and characterization of a highly repetitive fish nucleotide sequence. Gene. 1998;**62**:331-336

[125] Moyer SP, Ma DP, Thomas TL, Gold JR. Characterization of a highly repeated satellite DNA from the cyprinidae fish *Notropis lutrensis*. Comparative Biochemistry and Physiology. 1988;**91B**:639-646

[126] Monaco PJ, Swan KF, Rasch EM, Musich PR. Characterization of a repetitive DNA in the unisexual fish *Poecilia formosa*. I. Isolation and cloning of the MboI family. Evolution and Ecology of Unisexual Vertebrates. 1989;**466**:123-131

[127] Grewal SIS, Jia S. Heterochromatin revised. Nature Reviews. Genetics. 2007;**8**:35-46. DOI: 10.1038/nrg2008

[128] Mestriner CA, Bertollo LAC, Galetti PM Jr. Chromosome banding and synaptonemal complexes in *Leporinus lacustris* (Pisces, Anostomidae): Analysis of a sex system. Chromosome Research. 1995;**3**:440-443

[129] Jesus CM, Galetti PM Jr, Valentini SR, Moreira-Filho O. Molecular characterization and chromosomal localization of two families of satellite DNA in *Prochilodus lineatus* (Pisces, Prochilodontidae), a species with B chromosomes. Genetica. 2003;**118**:25-32

[130] Utsunomia R, Silva DMZA, Ruiz-Ruano FJ, Araya-Jaime C, et al. Uncovering the ancestry of B chromosomes in *Moenkhausia sanctaefilomenae* (Teleostei, Characidae). PLoS One. 2016;**11**(3):e0150573. DOI: 10.1371/journal. pone.0150573

[131] Utsunomia R, Ruiz-Ruano FJ, Silva DMZA, Serrano EA, Rosa IF, et al. A glimpse into the satellite DNA library in Characidae Fish (Teleostei, Characiformes). Frontiers in Genetics. 2017;**8**:103. DOI: 10.3389/fgene.2017.00103

[132] Arai R. Fish Karyotypes: A Check List. New York: Springer Science and Business Media; 2011

[133] Nanda I, Feichtinger W, Schmid M, Schroeder JH, Zischler H, Epplen JT. Simple repetitive sequences are associated with differentiation of the sex chromosomes in the guppy fish. Journal of Molecular Evolution. 1990;**30**:456-462

[134] Devlin RH, McNeil BK, Donaldson EM. Isolation of a Y-chromosomal DNA probe capable of determining sex in Chinook Salmon. Canadian Journal of Fisheries and Aquatic Sciences. 1991;**48**:1606-1612

[135] Nakayama I, Foresti F, Tewari R, Schartl M, Chourrout D. Sex chromosome polymorphism and heterogametic males revealed by two cloned DNA probes in the ZW/ZZ fish *Leporinus elongatus*. Chromosoma. 1994;**103**:31-39

[136] Capriglione T, Morescalchi A, Olmo E, Rocco L, Stingo L, Manzo S. Satellite DNAs heterochromatin and sex chromosomes in *Chionodraco hamatus* (Channichthyidae, Perciformes). Polar Biology. 1994;**14**:285-290

[137] Stein J, Phillips RB, Devlin RH. Identification of the Y chromosome in Chinook Salmon (*Oncorhynchus tshawytscha*). Cytogenetics and Cell Genetics. 2001;**92**:108-110

[138] Haaf T, Schmid M. An early stage of ZZ/ZW sex chromosome differentiation in *Poecilia sphenops* var. *melanisticta* (Poeciliidae, Cyprinodontiformes). Chromosoma. 1984;**89**:37-41

[139] Nanda I, Schartl M, Feichtinger W, Epplen JT, Schmid M. Early stages of sex chromosome differentiation in fish as analysed by simple repetitive DNA sequences. Chromosoma. 1992;**101**:301-310

[140] Vicente VE, Jesus CM, Moreira-Filho O. Chromosomal localization of 5S and 18S rRNA genes in three *Parodon* species (Pisces, Parodontidae). Caryologia. 2001;**54**(4):365-369

[141] Pansonato-Alves JC, Serrano EA, Camacho JPM, Utsunomia R, et al. Single origin of sex chromosomes and multiple origins of B chromosomes in fish of the genus *Characidium*. PLoS One. 2014;**9**(9):e107169. DOI: 10.1371/journal.pone.0107169

[142] Camacho JP, Sharbel TF, Beukeboom LW. B-chromosome evolution. Philosophical Transactions of the Royal Society B: Biological Sciences. 2000;**355**:163-178

[143] Sampaio TR, Gouveia JG, da Silva CRM, Dias AL, da Rosa R. Molecular analysis of the B microchromosomes in *Steindacnerina insculpita* (Characiformes: Curimatidae) by microdissection. Cytogenetic and Genome Research. 2015;**146**(1):51-57. https://doi.org/10.1159/000381932

[144] Silva DMZA, Daniel SN, Camacho JPM, Utsunomia R, et al. Origin of B chromosomes in the genus *Astyanax* (Characiformes, Characidae) and the limits of chromosome painting. Molecular Genetics and Genomics. 2016:**291**(3):1407-1418. DOI: 10.1007/s00438-016-1195-y

[145] Barros AV, Sczepanski TS, Cabrero J, Camacho JP, Vicari MR, Artoni RF. Fiber FISH reveals different patterns of high-resolution physical mapping for repetitive DNA in fish. Aquaculture. 2011;**322**:47-50

[146] Oyeleye OO, Ogundeji ST, Ola SI, Omitogun OG. Basics of animal cell culture: Foundation for modern science. Biotechnology and Molecular Biology Reviews. 2016;**11**(2): 6-16. DOI: 10.5897/BMBR2016.0261

[147] Paim FG. Desenvolvimento de cultura de células aderentes em peixes neotropicais e sua aplicação em estudos citogenéticos [thesis]. Botucatu, SP, Brasil: Universidade Estadual Júlio Mesquita Filho; 2018

[148] Rabova M, Monteiro R, Collares-Pereira MJ, Ráb P. Rapid fibroblast culture for teleost fish karyotyping. In: Ozouf-Costaz C, Pisano E, Foresti F, FA TL, editors. Fish Cytogenetic Techniques: Ray-Fin Fishes and Chondrichthyans. CRC Press; 2015. pp. 66-73

[149] Amemiya CT, John WB, John RG. A cell culture technique for chromosome preparation in cyprinid Fishes. Copeia. 1984;**1984**(1):232-235

[150] Zhang Q, Cooper RK, Wolters WR, Tiersch TR. Isolation, culture and characterization of a primary fibroblast cell line from channel catfish. Cytotechnology. 1998;**26**(2):83-90

[151] Wang X, Yang J, Chen X, Pan X. Establishment and characterization of a fibroblast-like cell line from *Anabarilius grahami* (Cypriniformes: Cyprinidae). Zoological Research. 2012;**33**(E5-6):E89-E97

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