Comparative cytogenetics of two of the smallest Amazonian fishes: Fluviphylax simplex Costa, 1996 and Fluviphylax zonatus Costa, 1996 (Cyprinodontiformes, Poeciliidae)

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Abstract
The genus Fluviphylax Whitley, 1965 is comprised of five valid species (F. pygmaeus Myers et Carvalho, 1955, F. zonatus, F. simplex, F. obscurus Costa, 1996, and F. palikur Costa et Le Bail, 1999), which are endemic to the Amazon region. These fishes are the smallest known South American vertebrates and among the smallest known vertebrates on Earth. All species but the type F. pygmaeus have been described in late 1990’s, and much remains unknown about the biology, taxonomy and systematics of this group of fishes. The aims of the present study were to establish the diploid and haploid number of F. zonatus and F. simplex, and to find species-specific markers for the discrimination of taxa. The diploid number for both species was 48 chromosomes, with no sex chromosome heteromorphism. Fluviphylax zonatus exhibited the karyotypic formula 4m+8sm+22st+14a and FN=82, and F. simplex exhibited 4m+16sm+18st+10a and FN=86. The determination of the total mean length of the chromosomes and their grouping into five size classes demonstrated different chromosome composition of the two species. This difference was further supported by the distribution of constitutive heterochromatin. The meiotic analysis revealed 24 bivalents in both species, but F. zonatus exhibited chromosomes with late pairing of the telomeric portions in the pachytene. These data reveal that cytogenetic characterization is useful and important for the discrimination of these species. Our study further indicates that this method could be employed in the analysis of other species of small fishes that are difficult to distinguish using traditional morphological traits or are morphologically cryptic.
Introduction

The Amazonian region has the most diverse freshwater fish fauna in the world, which, although only imperfectly known (Santos and Ferreira 1999). In general, cytogenetic studies of freshwater Neotropical fishes have resulted in the analysis of approximately 1040 species, of which more than 70% correspond to the orders Characiformes and Siluriformes (Oliveira et al. 1988, 2007, 2009). Cyprinodontiform fishes comprise approximately 850 species, mostly Neotropical fishes, of which only 67 neotropical species have cytogenetic information (Costa 1998, Oliveira et al. 2007, 2009). This dearth of information from cytogenetic data is mainly due to the low commercial importance and the small size of specimens that make up the cyprinodontiforms, limiting our understanding of their chromosomal organization and karyotype evolution.

The cyprinodontiforms are a large and diverse group of teleostean fishes comprising the family Poeciliidae. The fishes of the family Poeciliidae are small and laterally compressed, widely distributed in American and African continent. The Poeciliidae include the subfamilies Poeciliinae, Aplocheilichthyinae and Procatopodinae, a group composed of the South-American Fluviphylax Whitley, 1965 and the African procatopodines (Ghedotti 2000, Reis et al. 2003). The cyprinodontiform genus Fluviphylax comprises five species: F. pygmaeus Myers et Carvalho, 1955, F. zonatus Costa, 1996, F. simplex Costa, 1996, F. obscurus Costa, 1996 and F. palikur Costa et Le Bail, 1999 (Myers 1955, Costa 1996, Costa and Le Bail 1999). These fishes are commonly known as killifish and are the smallest South American vertebrates, reaching a maximal size of 22 mm. The genus is endemic to the basins of the Amazon and Orinoco Rivers and Atlantic drainages of the state of Amapá, Brazil (Myers 1955, Costa 1996, Costa and Le Bail 1999, Arrington and Winemiller 2003, Hoeinghaus et al. 2004, Lasso et al. 2004). Species F. simplex and F. zonatus are endemic to the central portion of the Amazon River Basin. The geographic distribution of F. simplex is in the Amazonian floodplain (Várzea) from the Amanã Reserve to the city of Santarém, whereas F. zonatus is restricted to the lower Negro River (Costa 1996, Souza 2008).

The taxonomic history of the genus Fluviphylax is relatively recent. The first species was discovered and scientifically described by Myers and Carvalho in 1955. The genus Fluviphylax has paucity of systematic, taxonomic and genetic information, with our knowledge being almost entirely restricted to the information published in the original description. Therefore, cytogenetic studies significantly expand our knowledge base of this group, especially in the realm of understanding of chromosome evolution of Fluviphylax.

Cytogenetic studies have contributed significantly to the identification of fishes (Nakayama et al. 2001, 2008, Teixeira et al. 2006) as well as the understanding of
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However, such studies have been restricted to larger species that are more easily handled. The present study reports a cytological characterization of two species of Fluviphylax, obtained by modifications of the technique described by Moreira-Filho and Bertollo (1990) and karyotype comparison with other species Poeciliidae.

**Materials and methods**

Specimens of *F. simplex* were collected from Lua Beach (3°07′31.7″S, 60°10′38.9″W) near the confluence of the Negro and Solimões Rivers, Amazonas, Brazil. Specimens of *F. zonatus* were collected from a small lake (3°00′19.2″S/60°03′22.6″W) located near Manaus that gathers water from the Tarumã River, which is a tributary of the Negro River (Figs 1, 2). We analyzed 24 specimens of the *F. simplex* and 37 of the *F. zonatus*. The gender determination was made only for adults specimens of each species being 6 males, 8 females and 10 indeterminated for *F. simplex*, and 7 males, 6 females and 24 interminated for *F. zonatus*. Collections were performed under a license from the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA n. 11325-1/2007). Following the chromosome preparation, some specimens were fixed in 95% alcohol. Voucher specimens were deposited in the Fish Collection at the Instituto Nacional de Pesquisas da Amazônia (INPA) in Manaus, State of Amazonas, Brazil (number 25527), and in the Animal Genetics Tissue Collection of the Laboratory of Animal Evolution and Genetics of the Institute of Biological Sciences of the Universidade Federal do Amazonas (Brazil).

Due to the small size of the specimens (less than 20 mm in total length), the cell preparations were obtained through the maceration of the each individual in a cuvette containing 6 ml of hypotonic KCl solution with the aid of two pairs of tweezers. Eyes and intestines were removed prior to maceration. The cell suspension was infused with 0.3 ml of 0.0125% colchicine solution. This preparation was incubated for 40 minutes at 37°C. The subsequent fixation of cells was carried out following the method of Moreira-Filho and Bertollo (1990). C-banding was used to characterize the constitutive heterochromatin distribution (Sumner 1972).

The chromosome preparations were analyzed under an optical microscope with an immersion objective. Selected cells were photographed with a Canon Power Shot A650 IS digital camera. The mounting of karyotypes was carried out with mitotic metaphase chromosomes, which were cut out and tentatively paired. The chromosomes were measured using the free ImageJ program and organized in decreasing order of size. Chromosome morphology was determined taking into account the position of the centromere, based on the method proposed by Levan et al. (1964). Chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a) (Levan et al. 1964). The fundamental number (FN) was determined based on the number of chromosome arms, considering metacentric, submetacentric...
Figure 1. Sampling locations circle and star indicate sampling points for *F. zonatus* and *F. simplex*, respectively.

Figure 2. a *Fluviphylax zonatus* with 20.0 mm SL b *Fluviphylax simplex* with 18.4 mm SL.
and subtelocentric chromosomes as having two arms and acrocentric chromosomes as having only one arm. Using the data of the mitotic chromosome measurements, of all karyotype for both species, a comparative analysis between *F. simplex* and *F. zonatus* was performed based on the length frequency of chromosome pairs by size class. Sturges’ formula was used for determining the ideal number of classes: \( n = 1 + 3.32 \times \log N \), in which “n” is the number of classes and “N” is the number of chromosomes in the haploid complement (Fonseca and Martins 1982).

**Results**

In this study we analyzed 428 cells of the *F. zonatus*, 16% corresponded to mitotic metaphase cells and the others were to meiotic cells, of which 46% leptotene/zygotene, 24% pachytene, 24% diplotene/diakinesis/metaphase I and 6% in metaphase II. For *F. simplex* were obtained 384 cells corresponded to 36% mitotic cells in metaphase and the others were to meiosis cells, of which 12% leptotene/zygotene, 67% pachytene, 16% diplotene/diakinesis/metaphase I and 5% in metaphase II.

In the mitotic analysis, both species had a diploid number of 48 chromosomes, with symmetrical karyotypes and no sex chromosome heteromorphism. *Fluviphylax zonatus* karyotype consists of 2n=4m+8sm+22st+14a (Fig. 3a), and *F. simplex* 2n=4m+16sm+18st+10a (Fig. 3f). Constitutive heterochromatin was detected in the pericentromeric region in the majority of chromosomes in the two species (Figs 3b, g). However, in *F. zonatus* the constitutive heterochromatin occupied entire short arms of all chromosomes, with the exception of 1st and 6th pairs. Also, in the 1st pair the constitutive heterochromatin was bitelomeric and in the 6th additional marks was found in long arms (Fig. 3b). In *F. simplex* the heterochromatin blocks were less evident and in the 20th pair were found additional interstitial marks on the long arms (Fig. 3g). The mean total length of the chromosomes ranged from 1.47 to 3.06 \( \mu m \) in *F. zonatus* and from 1.46 and 3.28 \( \mu m \) in *F. simplex* (Table 1). The grouping of chromosomes into five size classes, also revealed the different length chromosome composition between the two species (Fig. 4). In *F. zonatus*, there was a greater frequency of chromosomes in Class III, which encompasses pairs ranging in size from 2.21 to 2.57 \( \mu m \), and heterogeneity in chromosomal frequencies among other classes. Moreover *F. simplex* also had greater frequency of chromosomes in Class III, however, the distribution of chromosomal frequencies among other classes were homogeneous.

Gonadal cells of *F. zonatus* and *F. simplex* at interphase and prophase I had no heteropicnotic regions that indicated the presence of sex chromatin (Fig. 3c, h). The chromosomal behavior in some meiotic phases of both species was similar, but differences were detected. In pachytene cells, both species had 2n=24 bivalents, but *F. zonatus* showed chromosomes with late pairing in the telomeric portions.
(Fig. 3d), which did not occur in *F. simplex* (Fig. 3i). The analysis of diplotene cells also revealed 2n=24 bivalents in both species, but *F. zonatus* had 10 bivalents with a terminal chiasma and 14 with an interstitial chiasma (Fig. 3e), and *F. simplex* had 12 bivalents with a terminal chiasma and 12 with an interstitial chiasma (Fig. 3j). For both species, metaphases I had 2n=24 bivalents and metaphases II had n=24 chromosomes (data not shown).
Table 1. Average chromosome measurements (μm) and classifications in *F. zonatus* and *F. simplex* (Ch. Pair: Chromosome Pair; LA: Long arm; SA: Short arm; TL: Total length; AR: Arm ratio; CT: Chromosome type; m: metacentric; sm: submetacentric; st: subtelocentric; a: acrocentric). The LA, SA, TL are average values obtained from the measure of all karyotypes analyzed.

| Ch. Pair | LA | SA | TL | AR | CT | Ch. Pair | LA | SA | TL | AR | CT |
|----------|----|----|----|----|----|----------|----|----|----|----|----|
| 1        | 1.41 | 1.08 | 2.55 | 1.30 | M | 1        | 1.64 | 1.08 | 2.91 | 1.52 | M |
| 2        | 0.89 | 0.56 | 1.68 | 1.58 | M | 2        | 1.01 | 0.63 | 1.63 | 1.61 | M |
| 3        | 2.04 | 0.74 | 2.79 | 2.76 | SM | 3        | 2.14 | 0.82 | 3.13 | 2.61 | SM |
| 4        | 1.55 | 0.53 | 2.40 | 2.92 | SM | 4        | 2.12 | 0.71 | 2.84 | 2.97 | SM |
| 5        | 1.49 | 0.57 | 2.15 | 2.63 | SM | 5        | 1.79 | 0.79 | 2.54 | 2.26 | SM |
| 6        | 1.22 | 0.51 | 1.75 | 2.40 | SM | 6        | 1.68 | 0.68 | 2.33 | 2.46 | SM |
| 7        | 2.33 | 0.64 | 3.06 | 3.65 | ST | 7        | 1.57 | 0.66 | 2.31 | 2.39 | SM |
| 8        | 2.32 | 0.55 | 1.66 | 4.23 | ST | 8        | 1.30 | 0.53 | 1.93 | 2.43 | SM |
| 9        | 1.95 | 0.54 | 2.57 | 3.58 | ST | 9        | 1.12 | 0.44 | 1.65 | 2.54 | SM |
| 10       | 1.75 | 0.46 | 2.39 | 3.79 | ST | 10       | 0.72 | 0.31 | 1.46 | 2.33 | SM |
| 11       | 1.62 | 0.40 | 2.22 | 4.04 | ST | 11       | 2.57 | 0.49 | 3.28 | 5.20 | ST |
| 12       | 2.34 | 0.52 | 2.98 | 2.53 | ST | 12       | 2.66 | 0.49 | 3.21 | 5.38 | ST |
| 13       | 2.32 | 0.38 | 2.72 | 6.05 | ST | 13       | 2.26 | 0.57 | 2.98 | 3.93 | ST |
| 14       | 2.05 | 0.33 | 2.55 | 6.24 | ST | 14       | 2.29 | 0.60 | 2.92 | 3.80 | ST |
| 15       | 2.15 | 0.62 | 2.77 | 3.47 | ST | 15       | 2.44 | 0.45 | 3.08 | 5.40 | ST |
| 16       | 1.82 | 0.58 | 2.51 | 3.12 | ST | 16       | 1.99 | 0.61 | 2.72 | 3.27 | ST |
| 17       | 1.80 | 0.47 | 2.37 | 3.85 | ST | 17       | 2.13 | 0.47 | 2.68 | 4.57 | ST |
| 18       | 2.22 | 0.14 | 2.32 | 16.20 | A | 18       | 1.93 | 0.51 | 2.53 | 3.81 | ST |
| 19       | 2.28 | 0.15 | 2.50 | 15.16 | A | 19       | 1.78 | 0.55 | 2.34 | 3.26 | ST |
| 20       | 1.97 | 0.13 | 2.26 | 15.32 | A | 20       | 2.24 | 0.09 | 2.62 | 25.77 | A |
| 21       | 2.04 | 0.21 | 2.25 | 9.82 | A | 21       | 2.00 | 0.18 | 2.35 | 11.21 | A |
| 22       | 1.78 | 0.12 | 2.07 | 14.89 | A | 22       | 1.74 | 0.17 | 2.09 | 10.51 | A |
| 23       | 1.79 | 0.09 | 2.11 | 20.34 | A | 23       | 2.06 | 0.27 | 2.37 | 7.58 | A |
| 24       | 1.25 | 0.07 | 1.47 | 17.04 | A | 24       | 1.41 | 0.14 | 1.71 | 10.32 | A |

Figure 4. Analysis of chromosome size in *F. zonatus* and *F. simplex*; Y axis gives frequency of chromosomes with pair sizes in classes informed on X axis.
Discussion

The Procatopodinae and their sister sub-family Poeciliinae belong to the family Poeciliidae within order Cyprinodontiformes (Ghedotti 2000). Most of the Neotropical Poeciliidae species are diploid with 48 chromosomes (Ohno and Atkin 1966, Ojima et al. 1976, Ráb 1984, Oliveira et al. 2007). This diploid number has been found in around 51% of the species currently described and it is considered modal number for the order Cyprinodontiformes (Scheel 1972, Oliveira et al. 1988, García et al. 2001). However, variations at the ploidia level have been reported, especially in the genus Poecilia (Oliveira et al. 1988, Sola et al. 1990, Galetti Jr and Rasch 1993, Arkhipchuk 1999). Phylogenetic and biogeographic studies of the poeciliid fishes (Hrbek et al. 2007) report Fluviphylax as basal group for Poeciliidae family, corroborating the modal diploid number found in this study.

Comparative analysis of chromosome size between F. zonatus and F. simplex revealed differences in the organization of the genome, that is reflected in difference of karyotype formulae, due occurrence of pericentric inversion rearrangements, which alter the karyotype formula without altering the diploid number.

Chromosomal rearrangements are considered an important mechanism of karyotypic differentiation in Aplocheiloidei and Cyprinodontiformes in general (Scheel 1972) and the fixation of chromosomal rearrangements can occur by different processes, such as genetic drift or meiotic drive (Völker et al. 2006). Currently, it is widely accepted that diversity in the size and organization of genomes is influenced by non-coding repetitive DNA, such as pseudogenes, retrottransposons, transposons and satellite DNA, the most part found in the heterochromatin. The characteristics of an actual genome of an organism is determined by differential epigenetic activity of mechanisms that cause either an increase or decrease in the amount of DNA in response to the surrounding environment (Leitch 2007). Fluviphylax zonatus and F. simplex inhabit waters with different physiochemical characteristics, which in theory can influence via epigenetic mechanisms the organization of the karyotype. Fluviphylax zonatus is found in black waters from the Guiana Shield while F. simplex occurs in white-water rivers (Costa 1996, Souza 2008). Geological and ecological differences between the habitats of the species analyzed may have driven their speciation, as they are subjected to different types of selective pressure, which may have allowed the fixation of rearrangements that resulted in the different karyotype formulae and specie-specific pattern of heterochromatin distribution.

Although not commonly performed, meiotic analyses are an extreme powerful tool for chromosomal characterization (Gross et al. 2009). Analysis of meiotic chromosomes was also of fundamental importance in the study of the species of Fluviphylax, as it resulted in more a thorough characterization of their chromosomes. The success of the meiotic analysis was likely due to the fact that the species analyzed reproduce throughout a large portion of their life cycle and thus are continuously producing gametes. Continuous reproduction was also reported by Roberts (1970) when analyzing populations of F. pygmaeus. Moreover, late pairing was observed in the telomeric portions of some chromosomes in the pachytene stage in F. zonatus. Late pairing is
a species-specific marker in meiotic analyses and may either occur randomly or as a result of epigenetic mechanisms (Grewal and Jia 2007). As the species did not exhibit heterochromatin in the telomeric portions, this type of chromosome behavior is likely the result of gene regulation.

Some species of Poeciliidae have visible sex chromosome, such as the ZW/ZZ sex determining system in Gambusia puncticulata (Ráb 1984), Poecilia latipinna (Sola et al. 1990), P. formosa (Sola et al. 1993) and P. sphenops (Haaf and Schmid 1984) and the XX/XY system in P. reticulata (Feichtinger 1988), or both systems in Xiphophorus maculatus (Gordon 1950). However, the two species of Fluviphylax analyzed here did not exhibit differentiated sex chromosomes.

Organisms with differentiated sex chromosomes generally display positive heteropycnotic corpuscles in the early stages of prophase I and differentiated meiotic behavior in these chromosomes (John 1990). In F. zonatus and F. simplex, the lack of atypical chromosome behavior in both confirms the absence of sex chromosomes. This lack of differentiated sex chromosomes is found in approximately 95% of Neotropical teleosts. The most striking characteristic with respect to the occurrence of sex chromosomes in fishes is their apparent random distribution across the phylogenetic tree of fishes, as different systems are found in closely related species of the same genus or even in different populations of the same species (Almeida-Toledo and Foresti 2001).

As evident from our and other studies (Benzaquem et al. 2008, Gross et al. 2009, Oliveira et al. 2009), chromosomal characterization is an important tool for taxonomic and karyotype evolution studies in fishes. Moreover, the comparative description of karyotype characteristics in F. zonatus and F. simplex, which are among the smallest known vertebrates, may be considered an innovative, pioneering approach for fishes of the Amazon region. The methodology employed in the present study could be used in the analysis of other species of small Amazonian fishes which abound in the Negro River basin, many of which are miniaturized and with unclear taxonomic boundaries, as well as assist in the understanding of karyotype evolution.

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References

Almeida-Toledo LF, Foresti F (2001) Morphologically differentiated sex chromosomes in neotropical freshwater fish. Genetica 111: 91–100. doi: 10.1023/A:1013768104422
Arkhipchuk VV (1999) Chromosome database. Database of Dr. Victor Arkhipchuk. Available at: http://www.fishbase.org [last accessed 10 February 2010].

Arrington DA, Winemiller KO (2003) Diet changeover in sandbank fish assemblages in a Neotropical floodplain river. Journal of Fish Biology 63: 442–459. doi: 10.1046/j.1095-8649.2003.00167.x

Benzaquem DC, E Feldberg, JIR Porto, MC Gross, Zuanon JAS (2008) Cytotaxonomy and karyoevolution of the genus Crenicichla (Perciformes, Cichlidae). Genetics and Molecular Biology 31: 259–255. doi: 10.1590/S1415-47572008000200016

Costa WJEM (1996) Relationships, monophyly and three new species of the Neotropical miniature poeciliid genus Fluviphylax (Cyprinodontiformes: Cyprinodontoidei). Ichthyological Exploration of Freshwaters 7: 111–130.

Costa WJEM (1998) Phylogeny and classification of the Cyprinodontiformes (Euteleostei: Atherinomorpha): A reappraisal. In: Malabarba LR, RE Reis, RP Vari, ZMS Lucena, CAS Lucena (Eds) Phylogeny and Classification of Neotropical Fishes. Edipucrs, Porto Alegre, 537–560.

Costa WJEM, Le Bail PY (1999) Fluviphylax palikur: A new poeciliid from the Rio Oiapoque basin, northern Brazil (Cyprinodontiformes: Cyprinodontoidei), with comments on miniaturization in Fluviphylax and other neotropical freshwater fishes. Copeia 1999: 1027–1034. doi: 10.2307/1447977

Feichtinger W (1988) Cytogenetic investigation in livebearing toothcarps (Pisces, Poeciliidae). In: Oliveira C, LF Almeida-Toledo, F Forest, HA Britski, AS Toledo-Filho (1988) Chromosome formulae of neotropical freshwater fishes. Revista Brasileira de Genética 11: 577–624.

Fonseca JS, Martins GA (1982) Curso de estatística. Atlas, São Paulo, 1–286.

Galetti Jr PM, Rasch EM (1993) Chromosome studies in Poecilia latipunctata with NOR polymorphism as shown by silver nitrate and chromomycin A3 staining. Brazilian Journal Genetics 16: 927–938.

García G, Lalanne AI, Aguirre G, Cappetta M (2001) Chromosome evolution in the annual killifish genus Cynolebias and mitochondrial phylogenetic analysis. Chromosome Research 9: 437–448. doi: 10.1023/A:1011664009509

Ghedotti MJ (2000) Phylogenetic analysis and taxonomy of the poecilioid fishes (Teleostei: Cyprinodontiformes). Zoological Journal of the Linnean Society 130: 1–53. doi: 10.1006/zzls.1999.0213

Gordon M (1950) Fishes as laboratory animals. In: Farris EJ (Ed) The Care and Breeding of Laboratory Animals. New York, 345–449.

Grewal SIS, Jia S (2007) Heterochromatin revised. Nature Review 8: 35–46. doi: 10.1038/nrg2008

Gross MC, Feldberg E, Cella DM, Schneider MC, Schneider CH, Porto JIR, Martins C (2009) Intriguing evidence of translocations in Discus fish (Symphysodon, Cichlidae) and a report of the largest meiotic chromosomal chain observed in vertebrates. Heredity 102: 435–441. doi: 10.1038/hdy.2009.3
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Haaf T, Schmid M (1984) An early stage of ZW/ZZ sex chromosome differentiation in Poecilia sphenops var. melanistica (Poeciliidae, Cyprinodontiformes). Chromosoma 89: 37–41. doi: 10.1007/BF00302348

Hoeinghaus DJ, Winemiller KO, Taphorn DC (2004) Compositional change in fish assemblages along the Andean piedmont – Llanos floodplain gradient of the río Portuguesa, Venezuela. Neotropical Ichthyology 2: 85–92. doi: 10.1590/S1679-62252004000200005

Hrbek T, Seckinger J, Meyer A (2007) A phylogenetic and biogeographic perspective on the evolution of poeciliid fishes. Molecular Phylogenetics and Evolution 43: 986–998. doi: 10.1016/j.ympev.2006.06.009

John B (1990) Meiosis. Cambridge University Press, New York, 401pp.

Lasso CA, Mojica JI, Usma JS, Maldonado C, Nascimento C, Taphorn DC, Provenzano F, Lasso-Acalá OM, Galvis G, Vásquez L, Lugo M, Machado-Allison A, Royero R, Suárez C, Ortega-Lara A (2004) Peces de la cuenca del río Orinoco. Parte I: Lista de especies y distribución por subcuencas. Biota Colombiana 5: 95–157. http://www.siac.net.co/biota/123456789/172

Leitch IJ (2007) Genome sizes through the ages. Heredity 99: 121–122. doi: 10.1038/sj.hdy.6800981

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

Moreira-Filho O, Bertollo LAC (1990) Asyana x scabripinis (Pisces, Characidae): A species complex. Revista Brasileira de Genética 14: 331–347.

Myers GS (1955) Notes on the classification and names of cyprinodont fishes. Tropical Fish Magazine 4: 7.

Nakayama CM, Jégu M, Porto JIR, Feldberg E (2001) Karyological evidence for a cryptic species of piranha within Serrasalmus rhombues (Characidae, Serrasalmidae) in the Amazon. Copeia 3: 866–869. doi: 10.1643/0045-8511(2001)001[0866:KEFACS]2..0.C0;2

Nakayama CM, Feldberg E, Bertollo LAC (2008) Mapping of ribosomal genes and chromosomal markers in three piranha species of the genus Serrasalmus (Characidae, Serrasalmini) from the Amazon basin. Genetics and Molecular Biology 31: 868–873. doi: 10.1590/S1415-475720080005000018

Ohno S, Atkin NB (1966) Comparative DNA values and chromosome complements in eight species of fishes. Chromosoma 18: 455–456. doi:

Ojima Y, Ueno K, Hayashi M (1976) A review of the chromosome numbers in fishes. La kromosome, 2: 19–47.

Oliveira C, Almeida-Toledo LF, Forest F, Britski HA, Toledo-Filho AS (1988) Chromosome forumulae of neotropical freshwater fishes. Revista Brasileira de Genética, 11: 577–624.

Oliveira C, Almedia-Toledo LF, Foresti F (2007) Karyotypic evolution in Neotropical fishes. In: Pisano E, Ozouf-Costaz C, Foresti F (Eds) Fish cytogenetics. Science Publishers, Enfield, USA, 421–453.

Oliveira C, Foresti F, Hilsdorf AWS (2009) Genetics of neotropical fish: from chromosomes to populations. Fish Physiology Biochemistry, 35: 81–100. doi: 10.1007/s10695-008-9250-1

Ráb P (1984) Chromosome study of four poeciliid fishes from Cuba. Folia Zoologica, 33: 183–185.
Reis RE, Kullander SO, Ferraris CJ (2003) Check List of the Freshwater Fishes of South and Central America. Edipucrs, Porto Alegre, 555–581.

Roberts TR (1970) Description, Osteology and relationships of the amazonian cyprinodont fish Fluviphylax pygmaeus (Myers and Carvalho). Breviora, 347: 1–28.

Santos GM, Ferreira EJG (1999) Peixes da bacia Amazônica. In: Lowe-Mcconnell RH (Ed). Estudos ecológicos de comunidades de peixes tropicais. São Paulo, Editora da Universidade de São Paulo, 345–373.

Schedl JJ (1972) Rivulinae karyotypes and their evolution (Rivulinae, Cyprinodontidae, Pisces). Journal of Zoological Systematic and Evolution Research 10: 180–209. doi: 10.1111/j.1439-0469.1972.tb00797.x

Sola L, Mônaco PJ, Rasch EM (1990) Cytogenetics of bisexual/unisexual Poecilia. I. C-bands, AgNOR polymorphisms and sex chromosomes in three populations of Poecilia latipinna. Cytogenetics and Cell Genetics 53: 148–154.

Sola L, Rossi AR, Bressanello S, Rasch EM, Monaco PJ (1993) Cytogenetics of bisexual/unisexual species of Poecilia. IV. Sex chromosomes, sex chromatin compositions and Ag-NOR polymorphisms in Poecilia latipinna: a population from Mexico. Heredity 70: 67–71. doi: 10.1038/hdy.1993.9

Souza ER (2008) Filogeografia do gênero neotropical Fluviphylax (Cyprinodontiformes: Poeciliidae) das bacias do Amazonas e do Orinoco. Masters Thesis, Manaus, Brazil: Instituto Nacional de Pesquisas da Amazônia, 106 pp.

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research 75: 304–306. doi: 10.1016/0014-4827(72)90558-7

Teixeira AS, Nakayama CM, Porto JIR, Feldberg E (2006) Esterase-D and chromosome patterns in Central Amazon piranha (Serrasalmus rhombeus Linnaeus, 1766) from lake Catalão. Genetics and Molecular Biology 29: 498–502. doi: 10.1590/S1415-47572006000300018

Völker M, Sonnenberg R, Ráb P, Kullmann H (2006) Karyotype differentiation in Chromaphyoseminon killifishes (Cyprinodontiformes, Nothobranchiidae). II: Cytogenetic and mitochondrial DNA analyses demonstrate karyotype differentiation and its evolutionary direction in C. riggenbachi. Cytogenetic Genome and Research 115: 70–83. doi: 10.1159/000094803