A New Report of Multiple Sex Chromosome System in the Order Gymnotiformes (Pisces)

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Summary An X₁X₂Y sex chromosome system is reported for the first time in Gymnotus sp. The chromosome number observed was 2n=40 (14 M-SM+26 ST-A) in females and 2n=39 (15 M-SM+24 ST-A) in males, with the same fundamental number in both sexes (FN=54). The multiple sex chromosome system might have been originated by a Robertsonian translocation of an ancestral acrocentric Y-chromosome with an acrocentric autosome, resulting in a metacentric neo-Y chromosome observed in males. Single NORs were detected on the short arm of a middle-sized acrocentric chromosome pair. Constitutive heterochromatin was observed in the pericentromeric regions of several chromosome pairs, including the neo-Y chromosome and the NOR carrier chromosomes. The DAPI/CMA₃ stain revealed that all the pericentromeric heterochromatin are A/T rich whereas the NORs were associated with G+C rich base composition. The possible ancestral condition characterized by an undifferentiated Y-chromosome from all the Gymnotiformes fishes is discussed.

Key words X₁X₂Y sex chromosomes, C-NOR band, CMA₃-DAPI stain, Gymnotus, Fishes.

Multiple sex chromosome systems are known only for 7 neotropical fish species representing near 1% species cytogenetically analyzed already (Almeida-Toledo and Foresti 2001). This group is composed of species that are not closely related taxonomic units (different orders or families), indicating a polyphyletic origin for multiple sex chromosomes in fishes (Almeida-Toledo et al. 2000b, c).

Thus, in the neotropical fish fauna the X₁X₂X₁X₂/X₁X₂Y sex chromosome system has been described for 5 species. From these, in 2 Mexican species of Cyprinodontiformes (unnamed species, Uyeno and Miller 1971, 1972) and in 2 species of Gymnotiformes (Eigenmannia sp.2, Almeida-Toledo et al. 1984 and Brachyhypopomus pinnicudatus, Almeida-Toledo et al. 2000c), Robertsonian translocations could have involved in the origin of the neo-Y chromosome. In the remaining, one Characiformes species (Hoplias malabaricus), pericentric inversions and translocations are considered to be involved in the sex chromosome differentiation (Bertollo et al. 1987). Exceptionally, in Hoplias sp. was described a XX/XY₁Y₂ system, when the Y-chromosomes would be result of a centric fission from the ancestral Y-chromosome (Bertollo et al. 1983). A single species with female heterogamety presenting the multiple system ZZ/ZW₁W₂ was reported for Apareiodon affinis (Characiformes) (Moreira-Filho et al. 1980). The occurrence of a centric fission from a member of the pair 1 followed by pericentric inversions, was proposed to explain the origin and morphology of the female submetacentric W₁ and W₂ chromosomes (Jesus et al. 1999).

Studies carried out on several Gymnotus species showed a wide heterogeneity of diploid chromosome numbers. They range from 2n=40 in G. sylvius to 2n=54 in G. carapo, including species with 2n=42, 46, 48 and 52. In any cases has been identified sex chromosomes (Fernandez-Matioli et al. 1998).

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In the present work, we described the first occurrence of sex chromosome system in Gymnotus and the third occurrence of a \(X_1X_1X_2X_2/X_1X_2Y\) system for Gymnotiformes from a sample of Gymnotus sp. The hypothetical evolution of the sex determination in the order Gymnotiformes is also discussed.

Materials and methods

Twenty specimens of Gymnotus sp. (9 females and 11 males) from Corrientes Province were collected in ponds near to Riachuelo River, a tributary of Paraná River, Argentina (27°32′S; 58°17′W). The chromosome preparations were obtained from kidney cells using direct methods (Foresti et al. 1993). The final suspension was dropped on to the slides for diploid number determinations, C (Sumner 1972) and NOR (Howell and Black 1980) banding and Chromomicin/4′,6-diamidino-2-phenylindole (CMA3/DAPI) staining (Christian et al. 1998).

All fishes were classified by Dr. Jorge Casciotta and Dr. Adriana Almirón and deposited in the fish collection of the Instituto de Ictiología del Nordeste, Universidad Nacional del Nordeste, Corrientes, Argentina.

Results

The diploid number of all specimens of Gymnotus sp. analyzed was determined after the recount of 30 metaphase plates per individual. The chromosome modal number was \(2n=40\) for females and \(2n=39\) for males, with a karyotypic formulae of 14 M-SM+26 ST-A and 15 M-SM+24 ST-A, respectively, resulting in a fundamental number (FN) equal to 54 for all the fishes (Fig. 1). This difference in chromosome number is due to a Robertsonian translocation between 2 medium acrocentric chromosomes, becoming a major metacentric chromosome in males, the neo-Y. The \(X_1\) and \(X_2\) chromosomes were identified preliminarily as members of the chromosome pairs 13 and 18.

The nucleolus organizer regions were detected by silver staining in the short arm in the fifteenth ST-A chromosome pair (Fig. 2). Constitutive heterochromatic regions were detected in small sized blocks of the pericentromeric regions in several chromosome pairs (including the neo-Y chromosome) and adjacent to the NORs (Fig. 2). CMA3/DAPI stain applied to male preparations revealed small regions DAPI+ CMA3− occurring with the pericentromeric C-positive bands, while DAPI− CMA3+ bands were detected associated with NORs (Fig. 3a, b).

Discussion

The multiple sex chromosome systems are relatively unusual in Neotropical fishes. They have been described for not closely related taxonomic groups, which indicates a polyphyletic origin (Almeida-Toledo et al. 2000a, b, c).

Three different mechanisms were proposed by White (1973) to explain the origin of multiple sex chromosomes. The first, involves a centric fusion and was proposed for the origin of the neo-Y chromosome from two Mexican unnamed species (Uyeno and Miller 1971, 1972) and for the Gymnotiformes Eigenmannia sp. 2 and Brachyhypopomus pinnicaudatus (Almeida-Toledo et al. 2000c). The second is based on reciprocal translocations that could have been occurred in the neo-Y origin of Hoplias malabaricus (Bertollo et al. 1997). Finally, the third way could be either a chromosome dissociation or a centric fission and would explain the \(Y_1Y_2\) chromosomes observed in Hoplias sp. (Bertollo et al. 1983) as well as the \(W_1W_2\) chromosomes described in Apareidon affinis (Moreira-Filho et al. 1980).

The differences detected between male and female karyotypes of Gymnotus sp. could be originated by a Robertsonian translocation involving an ancestral acrocentric Y chromosome with an
acrocentric autosome, originating a neo Y-chromosome observed in the males. This chromosome, the major sized in the male karyotype, could have one arm homologue to the original X chromosome (now X₁ chromosome) and the other arm corresponding to autosome involved in the Robert-sonnian translocation (now X₂ chromosome) (Fig. 4). A similar mechanism was postulated for the origin of neo-Y chromosomes observed in the Gymnotiformes *Eigenmannia* sp. 2 and *Brachyhypo-popomus pinnicaudatus* (Almeida-Toledo et al. 2000a, b, c).

In a recent study, Almeida-Toledo et al. (2002) report the occurrence of female heterogamety in 3 isolated populations of *E. virescens*. This constitutes the first case of both XX/XY and ZZ/ZW sex chromosome system within a Gymnotiformes species. They could have derived from one morphologically undifferentiated acrocentric chromosome pair. It has been proposed that the variation of the sex chromosome determination could be result of a high plasticity of the sex-determining region. This along with crossing-over and heterochromatinization events would explain the change from male to female heterogamety (Almeida-Toledo et al. 2002). A similar situation was suggested to interpret the sex chromosome variability observed in *Xiphophorus maculatus* (Volff and Schartl 2001).

The presence of an heterochromatic segment DAPI⁺ was observed in the pericentromeric region of the neo-Y chromosome in *Brachyhypopomus pinnicaudatus* and *Eigenmannia* sp. 2, indicating a possible association of this A+T rich region with the translocated acrocentric chromosomes involved in the neo-Y origin (Almeida-Toledo et al. 2000a). The small bright segment in the peri-

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Fig. 1.  Giemsa stained karyotypes of *Gymnotus* sp.: a Female; b Male.

Fig. 2.  C-banding karyotype and the Ag-NOR bearing pair sequentially stained from a male of *Gymnotus* sp.
centromeric regions of the neo-Y chromosome after the DAPI staining observed here in Gymnotus sp., could indicate a similar base composition in the pericentromeric region of metacentrics neo-Y of the three species mentioned above.

Studies carried out on other Gymnotiformes species, Eigenmannia virescens (presenting XX/XY sex chromosome system), reveal that the distal region of the acrocentric X chromosome presents an additional block of heterochromatin G+C rich (CMA3+), without a corresponding segment in the Y-chromosome, suggesting that this heterochromatic block could be related with sex in Eigenmannia (Almeida-Toledo and Foresti 2001). In Eigenmannia sp. 2 (with X1X2Y sex chromosome system), an additional CMA3+ segment was also observed in females, which present eight large acrocentric chromosomes with CMA3+ blocks, while in the males only 7 bands have been detected. The authors interpreted this situation as a loss of the fragment G+C rich in the Robertsonian translocation responsible of neo-Y origin (Almeida-Toledo et al. 2000b).

The CMA3/DAPI staining made on Gymnotus sp. did not reveal CMA3+ regions associated with some chromosome pair excepting to the NORs carrier chromosomes, which present a banding pattern similar to other fish species (Mayr et al. 1986, Galetti Jr. et al. 1995, Affonso et al. 2001, among others). Thus, the CMA3+ region observed in the X-chromosome of Eigenmannia species could be restricted in this genus, while the A+T rich region associated with the Y-chromosome could be considered as an ancestral character of the order. Differences between sex chromosomes originated by heterochromatinization processes have been described for Poecilia sphenops (Haaf and Schmid 1984), Semaprochilodus taeniurus (Feldberg et al. 1987) and for several species of the genera Leporinus (Galetti and Foresti 1986) and Triportheus (Artoni et al. 2001).

It is interesting to emphasize that Gymnotiformes species with X1X2Y sex chromosome system belongs to 3 different families, the Hypopomidae (Brachyhypopomus pinicaudatus), Sternopygidae (Eigenmannia sp. 2) and Gymnotidae (Gymnotus sp.). In a recent study about phylogenetic relationships among several Gymnotiformes species, the families mentioned above were
classified into 3 different phyllogenetic lines (Albert and Campos-da-Paz 1998). These observations reinforce the hypothetical independent origin of the multiple sex chromosome systems within the order Gymnotiformes proposed by Almeida Toledo et al. (2000b, c).

According to the following evidence, we suggest that the ancestral condition of the order Gymnotiformes is the presence of genes responsible for male determination in an undifferentiated chromosome (ancestral Y-chromosome): i) the early XX/XY sex chromosome differentiation observed in *Eigenmannia virescens*; ii) the independent origin of multiple X<sub>1</sub>X<sub>2</sub>Y sex chromosome system in three not closely related taxonomic species; iii) the difficulty to recognize the X-chromosomes in all the species with sex chromosomes.

Thus, the occurrence of Robertsonian events between the hypothetical undifferentiated Y chromosome and a single autosome might have resulted in a multiple sex chromosome system in species belonging to genera without sex chromosome heteromorphism.

To prove this hypothesis, molecular studies are necessary to identify the undifferentiated sex chromosome pair that could be present in the remaining species of the order Gymnotiformes.

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