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Research Article

A remarkable autosomal heteromorphism in *Pseudoryzomys simplex* 2n = 56; FN = 54-55 (Rodentia, Sigmodontinae)

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Abstract

Pseudoryzomys simplex, the false rice rat, is a monotypic genus of the Oryzomyini tribe (Sigmodontinae) distributed in part of Bolivia, Paraguay, Argentina and Brazil. Its diploid number has been described as 56 acrocentric chromosomes decreasing in size and no karyotype figure has been depicted. Herein, we present karyotypic data on *P. simplex*, including chromosome banding and molecular fluorescent *in situ* hybridization using telomeric sequences and the whole X-chromosome of its sister clade *Holochilus brasiliensis* (HBR) as probes. A case of remarkable autosomal heteromorphism due to the presence of a whole heterochromatic arm leading to the variability of FN is reported, as well as the occurrence of regions of homology between the X and Y chromosomes (pseudoautosomal regions) after chromosome painting with the HBR X probe on *P. simplex* metaphases.

Keywords: cytogenetics, fluorescent *in situ* hybridization, heterochromatin, Oryzomyini.

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Introduction

The subfamily Sigmodontinae (Family Cricetidae), which comprises about 84 genera and is predominantly distributed in South America (D'Elía *et al.*, 2007), is one of the most diverse and complex groups of the Neotropical mammals. Within Sigmodontinae, nine tribes are recognized, from which Oryzomyini is the most specious, with 33 genera and about 140 valid species (D'Elía *et al.*, 2007; Percequillo *et al.*, 2011; Pine *et al.*, 2012). In addition, Oryzomyini displays an exceptional range of diploid and fundamental numbers, ranging from 2n = 16-17 and FN = 25-29 in *Nectomys palmipes* to 2n = 80 and FN = 140 in *Oecomys bicolor,* autosomes and sex chromosomes polymorphisms and supernumerary elements (Gardner and Patton, 1976; Almeida and Yonenaga-Yassuda, 1991; Barros *et al.*, 1992; Silva and Yonenaga-Yassuda, 1998; Patton *et al.*, 2000; Weksler, 2006). *Pseudoryzomys*, the false rice rat, is a poorly known monotypic genus of Oryzomyini, represented by *P. simplex*. This species has been found from eastern Bolivia, western Paraguay and northeastern Argentina to eastern Brazil, in the states of Mato Grosso, Goiás, Tocantins, Minas Gerais, São Paulo, Bahia and into the northeastern Alagoas and Pernambuco States (Voss and Myers, 1991; Musser and Carleton, 2005; Bonvicino *et al.*, 2008; Brancalion and Percequillo, 2009). Phylogenetic analyses using morphological and molecular data supported a close relationship among *Pseudoryzomys*, *Holochilus* and *Lundomys*, three genera that share several morphological characters, including specializations towards a semiaquatic lifestyle, such as the presence of interdigital membranes (Weksler, 2006).

Cytogenetic data of *Pseudoryzomys simplex* is restricted to the description of a diploid number (2n) of 56 chromosomes and a fundamental number (FN) of 54, with exclusively acrocentric chromosomes that gradually decrease in size (Voss and Myers, 1991; Bonvicino *et al.*, 2005). No karyotype has ever been presented and cytogenetic data from this genus are still scarce.

In this work, the karyotype of *Pseudoryzomys simplex* (PSI) was studied using conventional staining, CBG- and GTG-banding, Ag-NOR staining and fluorescent *in situ* hybridization (FISH) with telomeric sequences and with the whole X chromosome of *Holochilus brasiliensis* (HBR) as probes. A comparison of the GTG-banding patterns of *P. simplex* and of its closely related species *H. brasiliensis* (2n = 56; FN = 56) is also presented.

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Material and Methods

Seven specimens (one male and six females) from four Brazilian states (Goiás, Mato Grosso, São Paulo and Tocantins) were cytogenetically analyzed (Table 1, Figure 1). Chromosome preparations were obtained from bone marrow (Ford and Hamerton, 1956) or from fibroblasts cultured in Dulbecco's modified Eagle's medium, supplemented with 20% fetal bovine serum (Freshney, 1986).

Metaphases were analyzed after Giemsa staining, CBG- and GTG-banding and Ag-NOR staining, performed according to routine techniques. For FISH with telomeric probes, the Dako Telomere PNA FISH Kit/FITC (code number 5325) was used following the recommended protocol.

In order to precisely identify the X chromosome, we used a whole X-chromosome painting probe obtained from the Oryzomyini species *Holochilus brasiliensis* (HBR). The probe was generated from flow-sorted chromosomes from HBR cells at the Cambridge Resource Centre for Comparative Genomics (Department of Veterinary Medicine, University of Cambridge, UK) and amplified by degenerate oligonucleotide-primed PCR (DOP-PCR). Hybridization of the whole HBR X probe was performed according to Yang *et al.* (1995). The cross-species hybridization was performed for 24 h at 37 °C. Post-hybridization washes included two 5-min incubations in 50% formamide/2xSSC at 42 °C followed by two 5 min incubations in 2xSSC and a 4 min in 4xT (100 mL 20xSSC + 400 mL H_2O + 250 µL Triton X-100). The biotin-labeled probe was visualized with streptavidin-Cy3.

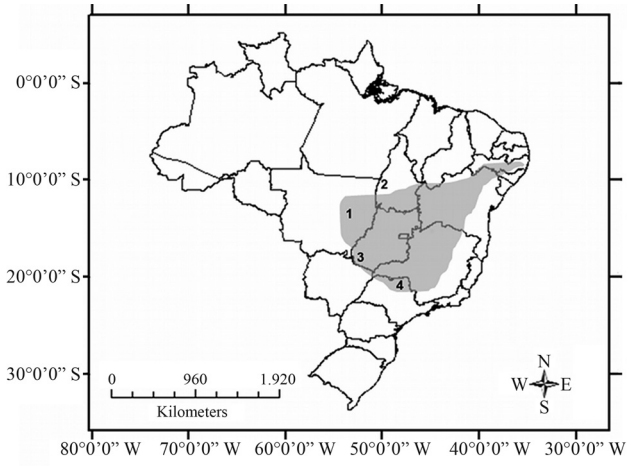

Figure 1 - Map of Brazil with the collecting localities of *Pseudoryzomys simplex*. 1. Gaúcha do Norte, Mato Grosso; 2. Parque Nacional do Araguaia, Tocantins; 3. Parque Nacional das Emas, Goiás; and 4. Guará, São Paulo. In gray, the distribution of the genus in Brazil, according to Bonvicino *et al.* (2008).

Results and Discussion

Pseudoryzomys simplex presented 2n = 56 and FN = 54-55. The individuals with FN = 54 presented only acrocentric autosomes decreasing in size. Two specimens (one male from Parque Nacional do Araguaia, TO, and one female from Guará, SP) showed FN = 55 due to the presence of one heteromorphic pair (17) with one metacentric and one acrocentric element (Table 1, Figure 2A). The X chromosome was a large acrocentric and the Y chromosome, a medium acrocentric.

The CBG-banded metaphases exhibited blocks of constitutive heterochromatin in the pericentromeric region of all autosomes. When pair 17 was heteromorphic, the metacentric element exhibited one entirely heterochromatic arm. The X chromosome presented pericentromeric heterochromatic blocks and an interstitial lightly stained CBG band on its long arm. The Y chromosome presented two conspicuous interstitial heterochromatic bands on its long arm, in addition to the pericentromeric heterochromatin (Figure 2B).

Oryzomyini species usually present easily recognizable sex chromosomes after CBG-banding. Large heterochromatic blocks at Xp or at the pericentromeric regions of acrocentric X chromosomes and almost entirely heterochromatic Y chromosomes have been described for

Table 1 - Sex, diploid (2n) and fundamental numbers (FN), and collection localities of *Pseudoryzomys simplex* from Brazil.

Specimen number	Sex	2n	FN	Locality	Geographic coordinates	
CIT 603	F	56	54	Gaúcha do Norte, MT	13º11'00" S	53º15'23" W
CIT 1152	M	56	55	Parque Nacional do Araguaia, TO	9°50'59" S	49°56'59" W
CIT 1159	F	56	54	Parque Nacional do Araguaia, TO	9°50'59" S	49°56'59" W
CIT 1168	F	56	54	Parque Nacional do Araguaia, TO	9°50'59" S	49°56'59" W
CIT 1219	F	56	54	Parque Nacional do Araguaia, TO	9°50'59" S	49°56'59" W
CIT 1333	F	56	54	Parque Nacional das Emas, GO	18°6'23" S	52°55'40" W
ROD 286	F	56	55	Guará, SP	20°48'55" S	47°48'59" W

Brazilian states: MT = Mato Grosso; TO = Tocantins; GO = Goiás, SP = São Paulo.
F: female. M: male.

Holochilus brasiliensis, *Nectomys squamipes, Euryoryzomys russatus* and *Oligoryzomys nigripes* (Yonenaga-Yassuda *et al.*, 1987; Andrades-Miranda *et al.*, 2000; Paresque *et al.*, 2007). In *P. simplex*, the X chromosome is recognizable by the tenuous heterochromatic block in its long arm and the Y chromosome by the two interstitial CBG bands in Yq.

GTG-banding allowed the correct identification of all homologues, including the acrocentric and the metacentric elements of the heteromorphic pair 17 and the sex chromosomes (Figure 2C). Comparative analysis of the GTG-banded complements of PSI (2n = 56, FN = 54-55) and HBR (2n = 56, FN = 56) allowed the inference of partial homologies between both karyotypes. A complete homology was verified between 26 autosomes of PSI and HBR, but only partial homology could be inferred between PSI 23 and HBR 13. Besides, the metacentric HBR 27 is similar in size and GTG-banding pattern to the acrocentric PSI 22,

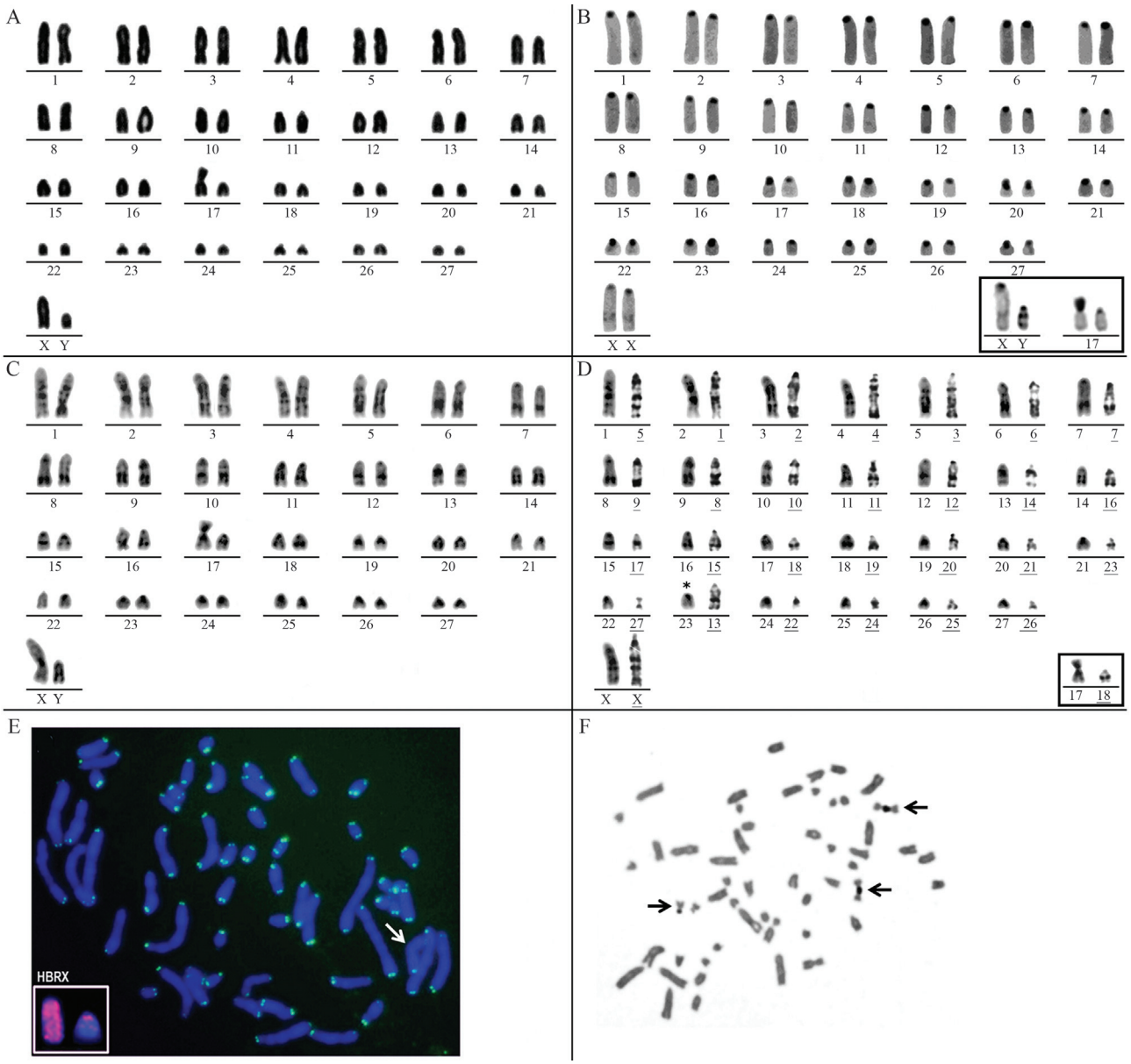

Figure 2 - Karyotype analysis of *Pseudoryzomys simplex* and comparisons with other species. (A-C) Karyotypes of *Pseudoryzomys simplex* with 2n = 56. (A) Conventionally stained karyotype of a male with FN = 55; (B) CBG-banded karyotype of a female with FN = 54; inset: sex chromosomes of a male and heteromorphic pair 17; (C) GTG-banded karyotype of a male with FN = 55. (D) Comparison between the GTG-banded chromosomes of *P. simplex* with 2n = 56 (left) and *H. brasiliensis* with 2n = 56 (right with underlined numbers). (E-F) Metaphases of *P. simplex* with 2n = 56. (E) Male metaphase with FN = 55 after telomeric FISH; the arrow shows the biarmed element of the heteromorphic pair 17. Inset: The X and Y chromosomes of *P. simplex* after chromosome painting with the HBR X probe. * Partial homology detected. (F) After Ag-NOR staining. Five Ag-NORs-bearing chromosomes of PSI are indicated. Note the two chromosome associations.

suggesting that these chromosomes differ due to a pericentric inversion. Despite the morphological differences of the X chromosomes (subtelocentric in HBR and acrocentric in PSI), their GTG-banding patterns were similar, supporting the idea that the mammalian X chromosome is conserved (Figure 2D). The GTG-banded Y chromosomes could not be compared because they did not present a definite banding pattern.

The high similarity of GTG-banding patterns between the autosomes of *P. simplex* and *H. brasiliensis* is in accordance to their close phylogenetic relationship (Weksler, 2006). However, comparative analyses based on GTG-banding may be ineffective because some homologous regions escape detection. For instance, GTG-banding comparisons between *Akodon* sp. (2n = 10) and *A. cursor* (2n = 16) (Silva *et al.*, 2006) did not allow the disclosure of complete homology or of the occurrence of the high complex rearrangements between both karyotypes, which could only be detected after cross-species chromosome painting (Ventura *et al.*, 2009).

FISH with telomeric sequences revealed signals exclusively at the ends of all chromosome arms and no interstitial signals were observed (Figure 2E). Interstitial telomeric signals co-localized with regions of constitutive heterochromatin have been described in autosomes and supernumerary chromosomes of some marsupials and rodent species, probably due to amplification of $(TTAGGG)_n$ sequences in these regions (Silva and Yonenaga-Yassuda, 1998; Pagnozzi *et al.*, 2002; Ventura *et al.*, 2006). Nevertheless, an amplified non-telomeric heterochromatin (Multani *et al.*, 2001) is found in the heteromorphic pair 17 of *P. simplex*.

The hybridization of the HBR X painting probe on *P. simplex* metaphases confirmed the acrocentric form and large size of the X chromosome. The HBR X paint hybridized on the whole PSI X corroborating the conservation of the mammalian X chromosome. The same probe also hybridized to the pericentromeric region of the PSI Y and produced a weak interstitial signal on Yq (Figure 2E, inset), evidencing the pseudoautosomal region (PAR) of the PSI Y. A similar case has been reported for the akodontine genus *Oxymycterus,* although in that case chromosome painting using an *Akodon paranaensis* Y chromosome probe allowed the detection of the PAR on both sex chromosomes (whole Xp and Yq) (Ventura *et al.*, 2012). Multiple Ag-NORs, varying from five to eight were localized at the telomeric regions of the short arms of small autosomes. Associations involving two and three chromosomes were frequent (Figure 2F).

Pseudoryzomys simplex has been collected in localities in eastern and central Brazil (Voss and Myers, 1991; Musser and Carleton, 2005; Bonvicino *et al.*, 2008). The specimens collected in Parque Nacional do Araguaia, Tocantins State, extend the northernmost distribution of the species in this state and the specimen from Guará represents the third record of the genus in the State of São Paulo (Brancalion and Percequillo, 2009).

The cytogenetic data presented herein for five specimens of *P. simplex* with 2n = 56 and FN = 54 is in accordance with previous reports (Voss and Myers, 1991; Bonvicino *et al.*, 2005). In addition, a new karyotype with 2n = 56 and FN = 55 is being described for two individuals, one male from Tocantins and one female from São Paulo. The difference in FN resulted from a heteromorphism due to the presence of a single biarmed element in this karyotype. The GTG-banding patterns allowed the identification of this chromosome as a homologue of pair 17 and the CBG-banding evidenced the addition/amplification of constitutive heterochromatin, as one chromosome arm of the metacentric homologue is entirely heterochromatic. The presence of the heteromorphic pair 17 in two individuals collected in different localities, one in São Paulo and another in Tocantins, may indicate the occurrence of chromosome polymorphisms in *Pseudoryzomys simplex*.

Among the deer mice *Peromyscus* spp., heterochromatic whole-arm additions to an otherwise mostly acrocentric chromosomes karyotype has been reported for a few species and for groups of species that exhibit short heterochromatic arms on different chromosomes, which are frequently polymorphic for this character (Greenbaum *et al.*, 1994).

A similar case of chromosome heteromorphism has been recently described by Bezerra *et al.* (2012) for the spiny rat *Clyomys laticeps*. The authors described a new karyotype for the species with 2n = 32; FN = 54, as well as the presence of a heteromorphic pair in which only one homologue presented a large heterochromatic block. However, differently from what was observed in *P. simplex*, the heteromorphic heterochromatic block in *Clyomys* was reported in an acrocentric pair, causing a difference in size between both homologues, but no variation in FN. A similar case has also been observed in the akodontine *Thaptomys* (Ventura *et al.*, 2004).

An increase in sampling efforts and the application of molecular cytogenetic techniques as chromosome painting using the microdissected heterochromatic arm of the metacentric element of PSI 17 as probe will help in establishing the complete homology between PSI and its sister clade HBR, to better characterize this unusual heteromorphism and to understand the nature of the amplified heterochromatic region.

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