Autosomal and sex chromosomal polymorphisms with multiple rearrangements and a new karyotype in the genus *Rhipidomys* (Sigmodontinae, Rodentia)

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Climbing rats, genus *Rhipidomys* (Sigmodontinae, Rodentia), occur in forest, gardens and plantations and also invade rural camps and houses throughout South America and Panama (Emmons and Feer 1997). According to Cabrera (1961), the genus comprised five species, four of which were polytypic; Honacki et al. (1982) and Nowak (1991) recognized seven species; Reig (1986) identified nine species; Musser and Carleton (1993) 14, and Tribe (1996) recognized 18 species. No recent critical synopses of valid species have been published and a broad systematic revision is needed.

Four of the 14 species accepted by Musser and Carleton (1993) are found in Brazil: *Rhipidomys leucodactylus* (synonyms: aratayae, bovalii, equatoris, lucullus, rex, sclateri) in the north; *R. mastacalis* (synonyms: cearamus, emiliae, macrurus, maculipes, yuruanus) in central and eastern Brazil; *R. nitela* (synonyms: fervidos, milleri, tobagi) in north-central Brazil; and *R. maccornelli* (synonym: subnubis) perhaps in the northernmost region of the country. On the other hand, Tribe (1996) considered that there are nine species and one incertae sedis in Brazilian territory: *R. leucodactylus*, *R. maccornelli*, *R. nitela*, *R. emiliae*, *R. macrurus*, *R. mastacalis*, *R. cf. macrurus*, *R. sp. 4*, *R. sp. 2*, *R. inc. sedis*.

Heretofore, cytogenetic studies of *Rhipidomys* specimens performed by Gardner and Patton (1976), Zanchin et al. (1992), Svartman and Almeida (1993), and Corrêa and Pessoa (1996) revealed *2n = 44* as the common diploid number found in one locality from Colombia and eight sites from Brazil (Fig. 1). Although the chromosome number was constant, variation occurred in the fundamental number (FN).

Herein we present five karyomorphs (two from Amazonas, one from Bahia, and two from Mato Grosso), including CBG, GTG, and RBG-banding, and Ag-NOR analysis in ten specimens of *Rhipidomys* as well as fluorescent in situ hybridization (FISH) with a telomeric probe.

**MATERIALS AND METHODS**

**Specimens**

Specimens identified preliminarily during fieldwork as *Rhipidomys cf. mastacalis* were captured in the Amazonian rain forest of the state of Mato Grosso: two males and four females at Vila Rica (9°54′28″S, 51°12′13″W) and one female at Aripuanã (10°10′S, 59°27′W). One female of *Rhipidomys* sp. A was
trapped at Morro do Chapéu (11°36'S, 41°08'W), state of Bahia at an altitude of about 1000 m in the rocky habitats called "campos rupestres". Two males of *Rhipidomys* sp. B were collected in the Amazonian rain forest near Manaus (2°25'S, 59°50'W), state of Amazonas. Fig. 1 includes these data.

Skulls and skins of the specimens from Mato Grosso and Bahia were deposited in the collection of the Museu de Zoologia, Universidade de São Paulo (MZUSP), Brazil.

**Chromosome preparation and banding**

Chromosome preparations were obtained from bone marrow and spleen after in vivo colchicine treatment. Fibroblast cultures from ear and tail biopsies from five individuals were established in the laboratory using Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 20% fetal bovine serum. CBG, GTG, and RBG-banding patterns, as well as Ag-NOR staining, were carried out using routine cytogenetic techniques, according to *Sumner* (1972), *Seabright* (1971), *Dutrillaux* and *Couturier* (1981), and *Howell* and *Black* (1980), respectively.

**Fluorescence in situ hybridization (FISH)**

Oligonucleotides (T<sub>1</sub>AGJ, in both sense and anti-sense orientation (GIBCO BRL) were used as probes for FISH, after 3'-end labeling with biotin-11-dUTP (Boehringer) for localization of telomeric sequences. Hybridization was performed overnight at 37°C and the signals were detected by incubation with fluorescein isothiocyanate (FITC)-labeled anti-biotin. FISH was also performed with the commercially available digoxigenin labeled “all human telomeres probe” (P5097-DG5, Oncor). The slides were counterstained with propidium iodide and mounted with an antifade solution. A total of 40 metaphases from six specimens was analyzed using a Zeiss Axioshot microscope equipped with a FITC filter and photographed with Ektachrome 400 (Kodak).

**RESULTS**

*Rhipidomys* *cf.* *mastacalis*

*Rhipidomys* *cf.* *mastacalis* from the two localities in the state of Mato Grosso presented a karyotype with 2<sub>n</sub> = 44, FN = 52 (Fig. 2a) with 16 uniarmed and 5 biarmed pairs of autosomes. Despite the similarities in diploid and fundamental numbers, two different karyotypes were detected on the basis of variations in the biarmed pairs. In the sample from Vila Rica (Fig. 2a) pair 4 and three small pairs (19 to 21) were biarmed, while in the Aripuanã specimen (Fig. 3a) pair 4 was uniarmed and four small pairs were biarmed (18 to 21). Pair 10 was biarmed in both karyomorphs. The X chromosome was acrocentric (Xa); the Y chromosome was acrocentric (Ya) and was the same size as pair 21.

The CBG-banding pattern revealed very small amounts of constitutive heterochromatin in the pericentromeric region of autosomes (Fig. 3a). The Xa

![Fig. 1. Localities, diploid and fundamental numbers of *Rhipidomys*. □ GARDNER and PATTON (1976); □ ZANCHIN et al. (1992); ● SVARTMAN and ALMEIDA (1993); ○ CORRÊA and PESSOA (1996) ▲ Present work. (*) Although the fundamental number is the same (FN = 52) in the specimens from MT, the biarmed homologues are different in the two karyotypes.](image-url)
Chromosomal polymorphism in Rhipidomys

Fig. 2. a–d. Conventionally stained karyotypes of Rhipidomys (bar = 10 μm). a Male of R. cf. mastacalis from Vila Rica, 2n = 44 (FN = 52) and XaYa. Inset: XaXa. b Female of R. sp. A, 2n = 44 (FN = 61) with nine heteromorphic pairs and XaXb. c Male of R. sp. B, 2n = 50 (FN = 71, heteromorphic for n°8) and XcYb. d Male of R. sp. B, 2n = 50 (FN = 72) and XcYc.

and Ya chromosomes also exhibited small pericentromeric C-bands. RBG-banding revealed the late replicating X chromosome and allowed precise identification of Xa (Fig. 4a).

The Ag-NOR analysis of 60 metaphases from the Vila Rica sample revealed NORs in the short arms of one large pair (no. 3) and three small pairs—probably pairs 12, 15 and 19 (Fig. 5a). The NORs ranged from 4 to 8 per cell, with a mean and mode of 7 NORs. In the specimen from Aripuanã, 11 metaphases showed variation from 7 to 12 Ag-NORs, with a mean and mode of 8 NORs, located in the short arms of one large pair and the short arms of some small autosomes. Additionally, one homologue of the smallest chromosome, the biarmed pair 21, was Ag-stained (Fig. 5a). Associations involving NOR-bearing chromosomes were observed in both kinds of karyotypes.

Rhipidomys sp. A

Rhipidomys sp. A from Bahia had 2n = 44 and FN = 61. The karyotype comprised seven heteromorphic pairs (1 to 5, 8 and 13) composed of one biarmed and one uniarmed homologue; pair 13 was also heteromorphic in size; pairs 7 and 9 were heteromorphic with subtelocentric and submetacentric homologues in the former and submetacentric and metacentric homologues in the latter (Fig. 2b). Pairs 6 and 10 to 12 were biarmed. Pair 14, the largest autosome pair
in the complement, and pairs 15 to 21 were acrocentric. The X chromosomes were heteromorphic: one was acrocentric (Xa) and the other was submetacentric (Xb). Xa had a very small short arm, which distinguished it from the autosomal acrocentrics. All autosomes could be identified not by conventional staining but based on banding patterns.

CBG-banding showed that constitutive heterochromatin was almost absent in the largest pairs but was present in the pericentromeric regions of certain small autosomes (Fig. 3b). Xa and Xb had conspicuous blocks in their pericentromeric regions. The heterochromatin in the short arm of Xb stained less than the pericentromeric heterochromatin. RBG and GTG-banding allowed identification of all the autosomal pairs. Incorporation of BrdU in fibroblast cultures showed that Xb was inactivated in all the 22 metaphases analyzed (Fig. 4b).

From a total of 35 metaphases, 29 presented five Ag-NORs and 6 showed four AgNORs, which were located at the end of one homologue of pair 9, in the long arm of the acrocentric homologue of pair 13, and in the short arms of three medium-sized acrocentric pairs, probably nos. 16 and 18 (Fig. 5b).
**Fig. 4.** a–c. Partial metaphases of *Rhipidomys* after RBG-banding. a Female of *R. cf. mastacalis* from Vila Rica, 2n = 44 (FN = 52), XaXa. Asterisk indicates the late replicating Xa. b Female of *R. sp. A* with 2n = 44 (FN = 61) showing the late replicating Xb c Male of *R. sp. B* with 2n = 50 (FN = 71) exhibiting late replicating Yb and short arm of Xc.

**Rhipidomys** *sp. B*

Two males of *Rhipidomys* sp. B from Manaus had 2n = 50, but two different fundamental numbers, FN = 71 and 72 (Fig. 2c, d). The specimen with FN = 71 had 11 biarmed pairs (1 to 7 and 9 to 12), 12 uniarmed pairs (13 to 24) and a heteromorphic pair 8 (one biarmed and one uniarmed). The specimen with FN = 72 showed 12 biarmed pairs (1 to 12) and 12 uniarmed pairs of autosomes (13 to 24). The X chromosome was a large submetacentric (Xc) in both individuals, and the Y displayed two morphologies: a small acrocentric (Yb) in the specimen with FN = 71 and a medium-sized acrocentric (Yc) with a very small short arm in the other individual.

CBG-banding revealed the presence of weakly stained heterochromatin in the pericentromeric region of some autosomes and in the short arm of one homologue of the heteromorphic pair 8 in the specimen with FN = 71 (Fig. 3c). This pair is probably not equivalent to pair 8 in the specimen with FN = 72, which did not exhibit heterochromatin in its short arm. The short arm of Xc was heterochromatic and its long arm had two C-positive interstitial bands; Yb was heterochromatic in its pericentromeric region and the distal part of its long arm; and Yc was entirely heterochromatic (Fig. 3c). RBG-banding revealed late replication of the Y and of the short arm of the X chromosome (Fig. 4c).

Ag-NORs were analyzed in 50 metaphases of the specimen with FN = 71 and ranged from 4 to 6, with a mean and mode of 5 NORs. The NOR-bearing chromosomes were one homologue of pair 5 and one of 16, and both homologues of pairs 22 and 24. Associations were observed (Fig. 5c). In the specimen with FN = 72, analysis of 27 metaphases evinced from 3 to 6 Ag-NORs, with a mean and mode of 4 NORs. The NOR bearing chromosomes were one homologue of pair 5, one of pair 16, and both homologues of pairs 10 and 24 (Fig. 5d). Pair 5 exhibited one secondary constriction in the short arm, in which the NORs were located. Associations were not detected. Comparison of the Ag-NOR-bearing chromosomes in the FN = 71 and 72 karyotypes revealed that the difference between them was due to the presence of ribosomal cistrons in the short arms of pairs 10 and 22, respectively.

All these data together led us to suppose that pairs 8, 9, 10 and 11 could not correspond to the homologous with the same numbers in both karyotypes (FN = 71 and 72). Probably pair 8 from the karyotype with FN = 71 belongs to the acrocentric group in the FN = 72, and pair 10, which is a metacentric in the FN = 71, is different from the subtelocentric pair 10 of FN = 72 karyotype (Fig. 2c and 2d).

**Comparative GTG-banding pattern**

A detailed karyological comparison of GTG-banded chromosomes revealed total homology between the 2n = 44 karyotypes of *Rhipidomys cf. mastacalis* from Vila Rica and *R. sp. A* from Bahia, implying that their differences were the result of pericentric inversions in 14 autosome pairs (Fig. 6).

Comparison between the karyotypes of *R. cf. mastacalis* and *R. sp. B* with 2n = 50, FN = 72 (Fig. 6) showed the homology of 10 pairs of autosomes and
revealed five pericentric inversion events. Chromosome pairs 7, 9, 11, and 14 to 24 of the 2n = 50 karyotype could not be identified in the 2n = 44 karyotypes. The X chromosomes presented two characteristic dark bands in their long arms after G-banding. The Yb showed two dark bands, one pericentromeric and one distal, after G-banding and the Yc had the same pattern and also a positively stained short arm.

**FISH**

Hybridization signals were observed in the telomeres of all chromosomes of *Rhipidomys* cf. *mastacalis*, *R*. sp. A and *R*. sp. B. No interstitial telomeric sites (ITS) were seen (Fig. 7).

**DISCUSSION**

Cytogenetic and taxonomic research on the genus *Rhipidomys* is almost absent in the literature and few cytogenetic studies have been performed on Brazilian populations. Karyological information is available for around 21 specimens, ten of which are described herein. Our studies reveal a high level of polymorphism with five different karyomorphs, including two karyotypes with 2n = 50 and three with 2n = 44.

The karyotypes of *R*. sp. B represent a previously unreported diploid number for the genus, and the difference between the fundamental numbers found in this species is due to two different chromosomal rearrangements: pericentric inversion and addition/

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**Fig. 5.** a–d. Ag-NO₃ stained karyotypes of *Rhipidomys*. a *R*. cf. *mastacalis* from Vila Rica (2n = 44, FN = 52) with 6 Ag-NORs. Inset: pair 21 of the specimen from Aripuanã. b *R*. sp. A (2n = 44, FN = 61) with 5 Ag-NORs. c *R*. sp. B (2n = 50, FN = 71) with 6 Ag-NORs. d *R*. sp. B (2n = 50, FN = 72) with 5 Ag-NORs. Arrows indicate associations involving pairs 3 and 12 in (a) and pairs 22 and 24 in (c).
deletion of constitutive heterochromatin. The former mechanism probably involves pair 10 from the FN = 72 karyotype and its counterpart, pair 22, from the FN = 71 complement. This hypothesis could be corroborated by the observation that the NOR-bearing chromosomes are the same in both karyotypes, except for pairs 10 and 22. It led us to hypothesize that pair 10 of the FN = 71 karyotype is equivalent to pair 22 of the FN = 72 karyotype, and that a pericentric inversion would be involved in the differentiation of this pair.

The other mechanism responsible for the variability in fundamental numbers is addition/deletion of constitutive heterochromatin in the short arm of one homologue of pair 8 from the specimen with FN = 71. Since there is no similar heterochromatic pattern in the FN = 72 specimen, this pair probably corresponds to an acrocentric pair in the latter karyotype.

Fundamental number variability in *Rhipidomys cf. mastacalis* resulted from pericentric inversions: the six individuals from Vila Rica presented a biarmed homomorphic pair 4, whereas this pair was homomorphic and uniarmed in the specimen from Aripuanã; a heteromorphic pair 4 was not observed in our sample. A pericentric inversion involving one small pair in the specimen from Aripuanã was also detected and resulted in a karyotype with four small biarmed pairs, whereas the karyotype of the sample from Vila Rica included only three small biarmed pairs. Pair 10 was biarmed in all these specimens from the state of Mato Grosso. A heteromorphism of pair 10 in individuals from São Paulo and Brasilia was reported by SVARTMAN and ALMEIDA (1993).

Variability in the number of Ag-NORs between the Vila Rica and Aripuanã samples could reflect either a real difference in the number of nucleolar organizer regions or differential activity of ribosomal cistrons. This could also be the case with the smallest stained metacentric in pair 21 of the Aripuanã specimen.

Among the specimens with 44 chromosomes, *Rhipidomys* sp. A presented an unexpected and exceptional karyotype with nine heteromorphic autosomal pairs and heteromorphic X chromosomes. Considering the high number of heteromorphic pairs, we hypothesize that: (1) the *Rhipidomys* sp. A individual could be from a population in which these hetero-

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**Fig. 6.** Comparison of GTG-banded haploid sets of *Rhipidomys*. From left to right in each triplet: @) *R* sp. B (2n = 50, FN = 72); (2) *R*. cf. *mastacalis* from Aripuanã (2n = 44, FN = 52); (3) *R*. sp. A (2n = 44, FN = 61). Inset: Yc and Yb of *R*. sp. B.
morphic pairs were established as a polymorphism; or (2) the specimen could represent a hybrid between an individual with a karyotype predominantly composed of biarmed chromosomes, quite similar to that of *R. mastacalis* (FN = 74) from Bahia (Zanchin et al. 1992), and an individual with a karyotype formed of mainly uniarmed chromosomes, as described in a number of specimens of the genus.

Comparative cytogenetics has been a powerful tool in establishing interspecific homologies through the comparison of banding patterns in vertebrates. A detailed comparison of banding patterns suggests that pericentric inversions, addition/deletion of constitutive heterochromatin and Robertsonian rearrangements were involved in the differentiation of 2n = 50 and 2n = 44 karyotypes of *Rhipidomys*. Moreover, some chromosome pairs of the 2n = 44 karyotypes—-for example, pairs 2, 4, 6, and 10 of *R. cf. mastacalis* and pairs 1, 3, 5, and 8 of *R. sp. A* (Fig. 6) — may be homologous with chromosomes in the 2n = 50 karyotype (*R. sp. B*). Besides, it would also be necessary to assume that at least one Robertsonian fission/fusion involving pair 4 of *R. sp. B* had occurred (Fig. 6).

Although a remarkable number of chromosomal rearrangements were detected in *Rhipidomys*, our FISH data revealed no ITS. Absence of ITS after chromosomal rearrangements has also been reported in feral mouse populations (Garagna et al. 1995; NANDA et al. 1995) and in species of *Oligoryzomys* (Silva and Yonenaga-Yussuda 1997) and *Nectomys* (Silva and Yonenaga-Yussuda 1998).

**Fig. 7.** a–c FISH with telomeric *(T\_AG\_)_\_ probes. Telomeric signals are evident in all chromosome telomeres. Interstitial telomeric sequences (ITS) are not observed. a *Rhipidomys* cf. *mastacalis*, 2n = 44 (FN = 52). b *R. sp. A*, 2n = 44 (FN = 61). c *R. sp. B*, 2n = 50 (FN = 71).
The X and Y chromosomes in our sample were variable in size and morphology (Xa, Xb and Xc and Ya, Yb and Yc) because of additions/deletions of constitutive heterochromatin. Our Xa, Xb and Ya were similar, respectively, to the Xa, Xb and Y described by Svartman and Almeida (1993). However, the short arm of our Xc was larger than the short arm of Xb+ described by the same authors.

All specimens of Rhipidomys for which karyotypes have so far been published (Fig. 1) have had the same diploid number of 2n = 44, with variable fundamental numbers due to the occurrence of pericentric inversions. Although skull and skin data show a clear difference between the samples, the karyotype of R leucodactylus from Rondônia (FN = 48; uniarmed pair 4 and 10) (Zanchin et al. 1992), is similar to that of a female Rhipidomys sp. from Brasilia (FN = 48) (Svartman and Almeida 1993) and a specimen from Mocambinho (FN = 48) (Corrêa and Pessoa 1996), except in its sex chromosome morphology. A Rhipidomys sp. from São Paulo has an autosomal set similar to that of a male from Brasilia (2n = 44, FN = 49 and pair 10 heteromorphic) (Svartman and Almeida 1993). These karyotypes differ from that of Rhipidomys cf. mastacalis from Vila Rica only in the morphology of pairs 4 and 10, which are biarmed in the latter species. All the karyotypes mentioned above differ from the complement found in a specimen from the state of Espírito Santo (Zanchin et al. 1992), which presented four biarmed chromosome pairs, and from the karyotype of the Aripuanã specimen, which had five biarmed pairs.

Tribe (1996) identified the Rhipidomys specimens from São Paulo and Espírito Santo as R. cf. macrurus, and those from Brasilia and Mocambinho as R. macrurus. He considered our R. sp. B preliminarily to be R. nitela. A detailed systematic study of the different forms of Rhipidomys, including our samples, seems urgent.

Our cytotogenetic studies suggest that our R. sp. B represents a distinctive species, separate from R cf. mastacalis and R sp. A. Moreover, there are two sympatric species in Mato Grosso state: the smaller R. nitela (Musser and Carleton 1993; Tribe 1996) and the larger R. emiliae (Tribe 1996) or R. mastacalis (Musser and Carleton 1993). Morphological studies should be performed on our samples preliminarily identified as R. cf. mastacalis in order to clarify this point. More Rhipidomys specimens should also be studied from the “campos rupestres” of Bahia, a habitat different from the Atlantic and Amazonian rain forests, in order to ascertain whether our R. sp. A might represent a hybrid in the genus.

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